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Cefuroxime-impregnated calcium phosphates as an implantable delivery system in experimental osteomyelitis

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Abstract

The study aimed to investigate the effectiveness of porous calcium phosphates viz., hydroxyapatite (HAp) and a bi-phasic calcium phosphate (BCP) with predominately β-tricalcium phosphate (β-TCP) prepared by aqueous solution combustion method impregnated with cefuroxime axetil for the treatment of experimental osteomyelitis and compared with parenteral treatment. *In vitro* release of the drug was tested for its sustained elution characteristics for 21 days in PBS (pH 7.2) and measured by HPLC. In the *in vivo* study, bone infection was induced in tibia of rabbits by inoculation of 1 ml (3×10^6) CFU *Staphylococcus aureus*. On the 21st day, after the development of osteomyelitis, six animals were treated by filling the cavity with cefuroxime-impregnated HAp blocks (Group II), six animals with the same drug-impregnated β-TCP (Group III) and in six others, only cefuroxime (15 mg/kg twice daily) was injected parenterally 6 weeks (Group IV). Group I with six animals was kept untreated. Histologically, the signs of infection were found to subside by 3 and 6 weeks. Radiological evaluation with cefuroxime-impregnated HAp and β-TCP pointed out the disappearance of sequestrum and existence of newly formed bony specules. Concentration of cefuroxime in bone and serum as estimated by HPLC showed highest value on day 21 itself which reduced marginally by day 42 in both the groups and these values were higher than minimum inhibitory concentration (MIC) against *S. aureus*. Our findings suggest that bi-phasic calcium phosphates with predominately β-TCP content is a very efficient carrier material for antibiotic compounds even for refractory infections by *S. aureus*.

Keywords: Osteomyelitis; Sustained release; Local anti-infective agents; Hydroxyapatite; β-Tricalcium phosphate

1. Introduction

Despite continuous advances in the surgical and antimicrobial armamentarium, the treatment of osteomyelitis poses a significant challenge even in specialized centres with a team approach involving clinicians of different subspecialties. Osteomyelitis is refractory because of the characteristics of bone. The soft tissues of bone are surrounded by hard walls, and inflammation of the contained tissues cause circulatory disturbances which can readily lead to necrosis of various parts of the bone. These anatomical features provide an environment suited for the localization and colonization by bacteria [1]. Surgical debridement of necrotic tissue and administration of antibiotics are the principal methods of treatment [2,3]. Because antibiotics cannot be delivered to the infected bone at a sufficiently high concentration intravenously without producing systemic toxic effects, local administration such as closed irrigation and suction [4], local injection [5] and implantable pumps [6] have been used but are clinically inconvenient. Antibiotic-impregnated poly-methyl methacrylate beads, the drug delivery systems which have been used successfully [7,8], provided a simple method but the disadvantages include low biocompatibility, a very low release ratio and possible thermal damage to the antibiotics. Attention has therefore, been focused on biodegradable antibiotic delivery systems which provide high, effective concentrations at the site of infection with no systemic side effects [9].

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To attain the desired therapeutic effect without the side effects, it is necessary that (i) initial release of an active drug should exceed the minimum effective concentration (MEC) in the systemic circulation but should be less than the minimum toxic concentration (MTC), (ii) after that, the drug release should be at a constant or near-constant rate, according to zero-order kinetics, resulting in a constant, non-fluctuating plasma drug concentration in the prescribed therapeutic range and (iii) the duration of drug release should be prolonged e.g. 12 h to 1 year. Osteomyelitis could be treated following the above parameters using a bioceramic based drug delivery system since compatibility with the body fluids and the physical characteristics of them are well suited to the body environments. Moreover, these are porous, biocompatible, non-immunogenic and eventually biodegradable [10]. Now, for slow release of antibiotic, porous hydroxyapatite (HAp) is considered to be superior to acrylic bone cement because there is no risk of thermal damage to the antibiotic. In addition, HAp has excellent biocompatibility and does not need to be removed [11,12]. Further, the antibiotic loaded HAp block is useful not only as an antibiotic carrier to the infected site but also act as bone substitutes. Porous β-tricalcium phosphate (β-TCP), on the other hand, is one of most biocompatible, resorbable synthetic hard tissue implant material that can be directly bonded to bone via natural mechanisms to stabilize traumatized bone fractures [13]. β-TCP also has also been studied as a drug delivery system individually [14]. But, the efficacy of bi-phasic calcium phosphate (BCP) has not been fully understood for its clinical efficacy to combat the chronic osteomyelitis in the long term. To our knowledge, if it is possible to vary the composition of bioceramic materials (composed of HAp and β-TCP) with its inherent porosity, could be a solution owing to the faster resorption of this BCP together with the sustained release of the antibiotic. In the present investigation, hence, we have developed drug cefuroxime axetil-impregnated calcium hydroxyapatite only and a BCP [90% β-TCP + 10% HAp], powder prepared by a single step of synthesis termed aqueous solution combustion method, and evaluated the sustained release of drug both *in vitro* and *in vivo* for the treatment of experimental osteomyelitis in rabbits and further compared with routine antibiotic therapy. The influence of antibiotic and bioceramic type on effectiveness of treatment was the basic parameter tested to design the best therapeutic system. Further, as the BCP composed mainly of β-TCP, henceforth to distinguish, the BCP will be mentioned as β-TCP only in the text.

This processing technique of the solution combustion technique is often adapted for the rapid preparation of variety of oxide ceramic powders [15]. The process involves an exothermic, usually very rapid and self-sustaining chemical reaction between the desired metal salts (oxidizer), preferably nitrates, and a suitable organic fuel, such as urea [16], glycine [16], carbohydrazide [17] and citric acid [18] in an aqueous solution. The reaction initiated at a fairly low temperature [19] followed by rapid cooling and this in turn leads to nucleation of crystallites without much growth [20]. The reaction between oxidizer and fuel releases large amount of reaction heat that is utilized to synthesize the desired materials *in situ* and the large volume of gas evolved disintegrates the high purity products to friable agglomerates of very fine particulates.

2. Experimental procedure

2.1. Synthesis of HAp and β-TCP powders

In this study, calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) (S.D. Fine-Chem Ltd., India), and di-ammonium hydrogen ortho-phosphate (DAP, $(NH_4)_2HPO_4$) (S.D. Fine-Chem Ltd., India) were used as the starting raw materials. Glycine ($C_2H_5NO_2$) (Glaxo, Qualigens, India) was used as fuel. Aqueous stock solutions of calcium nitrate tetrahydrate and DAP were mixed in the ratio of 1.67 and 1.50, which are required for formation of hydroxyapatite and β-TCP. A few drops of concentrated nitric acid (Merck, India) were added to dissolve the resulting white precipitate to make a clear homogeneous solution. A predetermined amount of fuel was added to the clear solution, and the mixture was homogenized by stirring with a magnetic stirrer for 30 min at room temperature. The solution was taken in a glass–ceramic coated mild steel container, which was introduced into a muffle furnace preheated to the desired temperature. Fig. 1 shows the schematic of the process by which the combustion was being carried out. A stainless steel wire mesh was put on the reaction container to prevent loss through aerosol formation and maintain uniformity of temperature. When placed in the furnace, the mixed solution soon started to boil, underwent dehydration and decomposition and a large volume of gases containing oxide of nitrogen and traces of ammonia evolved. During the process an incandescent flame appeared and the mass frothed and swelled to yield foam. The entire process was complete within less than 10 min with flame duration of nearly one minute. The as-prepared products were typically voluminous, fluffy foam-like mass of soft-agglomerated powders, which were readily ground manually in an agate mortar/pestle into fine powder for further characterization, X-ray diffraction (XRD) (Philips Analytical, X'Pert, 1830, Netherlands) patterns of the as-prepared powders were recorded to analyze the phase formation while Infrared spectra of the powder samples were

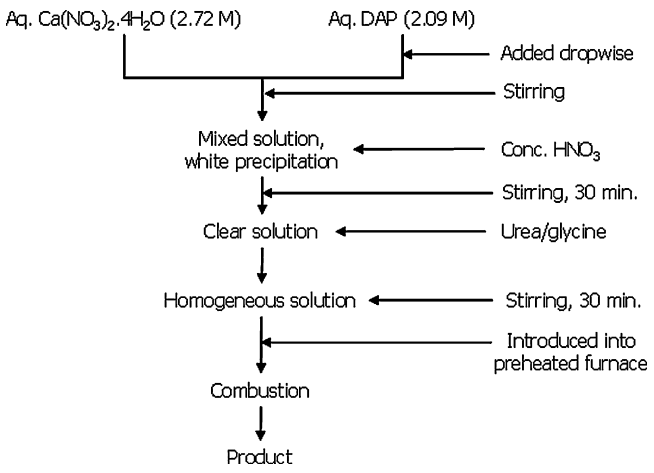

Fig. 1. Schematic flow chart of combustion synthesis process.

recorded in the mid-IR region (4000–400 cm^{-1}) by Fourier Transform Infra-Red (FTIR) spectrometer (Perkin-Elmer, Model 1615, USA) in the transmission mode for confirmations of the phases evolved.

2.2. Fabrication of porous HAp and β-TCP

Porous HAp and β-TCP blocks with dimensions $20 \times 9 \times 9\ mm^3$ and $9 \times 4 \times 4\ mm^3$ respectively were fabricated using β-naphthalene (<200 μm) and polyvinyl alcohol as combustible organic materials. For the purpose, respective powders were first ground separately with oleic acid surfactant and pre-calculated amount of β-naphthalene were properly mixed, rectangular blocks were uniaxially cold compacted with low pressure (40 MPa). The compacted samples were then cold iso-statically pressed at 100 MPa for homogeneous densification. The specimens were then very slowly dried in an air oven for complete removal of the naphthalene. Finally, HAp and β-TCP specimens were sintered at 1250 °C, held for 2 and 1 h at those temperatures for the two cases respectively. Mercury immersion densitometer was used to calculate the bulk density; apparent porosity of sintered specimens were calculated using the relation: [1 − (bulk density/true density)] × 100. True density of the HAp and β-TCP powders was taken to be 3.167 and 3.08 g/ml. X-ray diffraction was carried out for confirmation of the phases, microstructure and pore morphology were evaluated by SEM. Pore size and its distribution were analyzed by mercury intrusion porosimetry technique (Quantachrome Poremaster, version 4.01, USA). The porous struts, however, were initially pasteurized using distilled water and subsequently autoclaved at 121 °C for 30 min before further experimentation.

2.3. Preparation of antibiotic loaded HAp and β-TCP block

As the drug is insoluble in water, a solution of Cefuroxime axetil (M/s Zuventus Pharmaceuticals Limited, India) in acetone (S.D. Fine-Chem Ltd., India) was used to infiltrate the porous samples by vacuum infiltration method. Samples were kept in vacuum (10^{-3} Torr) for at least 30 min for each sample unless all the solution containing predetermined amount of drug went into scaffold. Smaller sized ($9 \times 4 \times 4\ mm^3$) struts were used for the *in vivo* trials while bigger ones ($20 \times 9 \times 9\ mm^3$) were used for the *in vitro* purpose. On an average, it has been found that there were 44 mg and 47 mg of antibiotic resided in case of HAp and β-TCP blocks for *in vivo* samples while *in vitro* samples contained about 105 mg and 96 mg respectively.

2.4. In vitro studies

To evaluate the elution ability of the porous blocks of HAp and β-TCP, each block of the compositions were placed in three test tubes separately with 3 ml of PBS (phosphate-buffered saline) (pH ∼7.2) and stored in a thermostat chamber at 37 °C. The PBS was replaced every 24 h and preserved at −20 °C until assayed. The beads were reinstated with fresh buffer for 21 consecutive days for latter determination of the cefuroxime concentration by employing high performance liquid chromatography (HPLC) technique (SPD-M10A, Shimadzu Company, Kyoto, Japan). The column RPC_{18} was used as a stationary phase. The mobile phase consisted of a mixture of 50:50 volumes of acetonytryl and 0.05 M ammonium di-hydrogen phosphate. The flow rate was 1 ml/min. The detection wavelength was 278 nm.

2.5. Bacterial isolate

A clinical isolate of *Staphylococcus aureus* (coagulase positive) from an animal with chronic osteomyelitis was used. Pure cultures of the bacteria were obtained on blood agar at 37 °C. Standardized suspensions (3×10^6 CFU/ml) were prepared in saline and kept on ice throughout the surgical procedure. The samples (1 ml) were directly delivered into the medullary cavity of rabbit tibiae.

2.6. In vivo studies

Osteomyelitis were produced in the right tibia of the 24 (twenty-four) numbers of New Zealand white rabbits (2.5–3 kg body weight) according to the model of Norden [21]. They were randomly distributed into four groups of six animals each. They were maintained under identical environment, management and balanced standard diet along with *ad libitum* supply of water. After anaesthesia with Nembutal 0.5 mg/kg IV, the proximal part of the tibia was exposed anteriorly and a hole was drilled through the cortex into the medullary cavity using a 1.2 mm diameter dental burr. About 1 ml of bacterial suspension containing approximately 3×10^6 CFU/ml of *S. aureus* were inserted into the medullary cavity. The hole was covered with bone wax to prevent bacterial leakage into the soft tissues. The skin was closed and the animals were then allowed to free move in their cages. Infection was allowed to develop for 3 weeks and thereafter, the animals underwent second-stage surgery. Now, animal experimentation was carried out following the procedures conforming to the standards of the Institutions Animal Ethical Committee of the West Bengal University of Animal and Fishery Sciences, India. Using the previous surgical approach, the area was exposed and bone defects were created. The swab specimens for culture were taken from each animal species to confirm the clinical development of *S. aureus*. Antibiotic-impregnated HAp and β-TCP blocks were then impacted into the medullary space of defect area to treat the infection. The wound was closed in layers. The animals were monitored after surgery. All the animals received standard postoperative pain medication (Carprofen; 4 mg/kg of body weight) for 3 days. All the 24 animals were divided into four groups viz. Groups I, II, III and IV. The details of the experimentation with these animals are tabulated (Table 1). Specimens for histology were fixed first in 10% formalin for 7 days and decalcified subsequently in Goodling and Stewarts' fluid. Four (4) micron sections were cut from the decalcified tissues and stained with hematoxylene and eosin. The stained sections were observed for status of the implants and cellular

Table 1
Design of experiment for *in vivo* animal experimentation.

Group	No. of animals	Implant	Days of experiment	Experiment
Group I	6	Not given	After 3 weeks	Six animals were sacrificed for histological, radiographic and microbiological examination to confirm development of osteomyelitis
Group II	6	Cefuroxime axetil injection parenterally (15 mg/kg, bid) twice daily for 6 weeks	After 3 weeks	Three animals were sacrificed for histological and estimation of drug concentration in bone and serum
			After 6 weeks	Three animals were sacrificed for histological and estimation of drug concentration in bone and serum
Group III	6	Cefuroxime axetil-impregnated HAp beads	After 3 weeks	Three animals were sacrificed for histological and estimation of drug concentration in bone and serum
			After 6 weeks	Three animals were sacrificed for histological, radiographic, and estimation of drug concentration in bone and serum
Group IV	6	Cefuroxime axetil-impregnated β-TCP beads	After 3 weeks	Three animals were sacrificed for histological and estimation of drug concentration in bone and serum
			After 6 weeks	Three animals were sacrificed for histological, radiological and estimation of drug concentration in bone and serum

response at the interface. X-rays from the infected bones were taken by direct radiographic magnification. Estimation of concentrations of antibiotic (Cefuroxime axetil) in bone samples and serum were made by High Performance Liquid Chromatography (HPLC) technique with the same instrument and condition stated previously. Samples from bone and serum were prepared by the following method: Blood samples were taken from the ear vein of rabbits for determination of the level of cefuroxime axetil in serum. After coagulation and centrifugation, the serum was stored at −20 °C until assay. In case of bone, after removal of soft tissue and bone marrow, the cancellous and cortical tibia bone was pulverized, homogenized with phosphate buffer and centrifuged. The supernatant fluid was collected and stored at −20 °C for evaluation of cefuroxime axetil. The results, further, were expressed as Means ± Standard Errors. Statistical analyses were performed using the simple one-way analysis of variance (ANOVA) method.

3. Results and discussion

3.1. XRD and FTIR of the as-prepared powders and porous struts

The XRD pattern of the synthesized as well as the fabricated HAp and β-TCP (shown in Fig. 2a, b and c, d) showed highly crystalline phase with confirmation of JCPDS file 09-432 and 09-169 for HAp and β-TCP respectively. The fabricated block of TCP, which was fired at 1250 °C, contained about 10% of the HAp phase as the secondary phase (Fig. 2a and b). By employing Scherrer equation [22], average crystallite sizes of the prepared powders were determined and found to be 49–

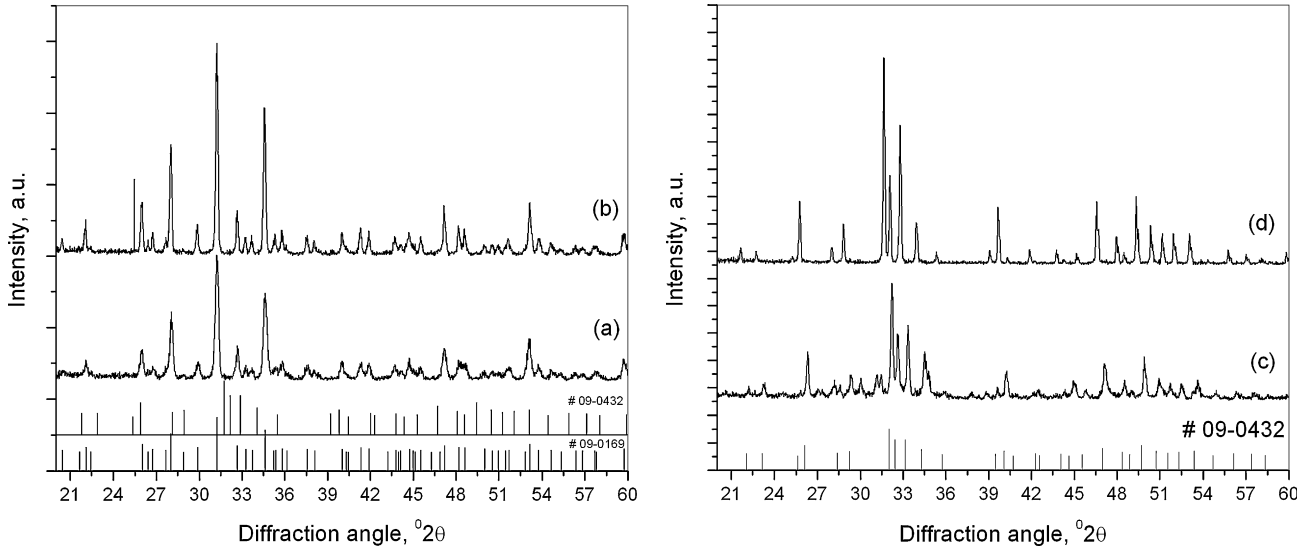

Fig. 2. X-ray diffraction of (a) combustion synthesized β-TCP powders, (b) same fabricated at 1250 °C/1 h, (c) combustion synthesized HAp and (d) same fabricated at 1250 °C/2 h.

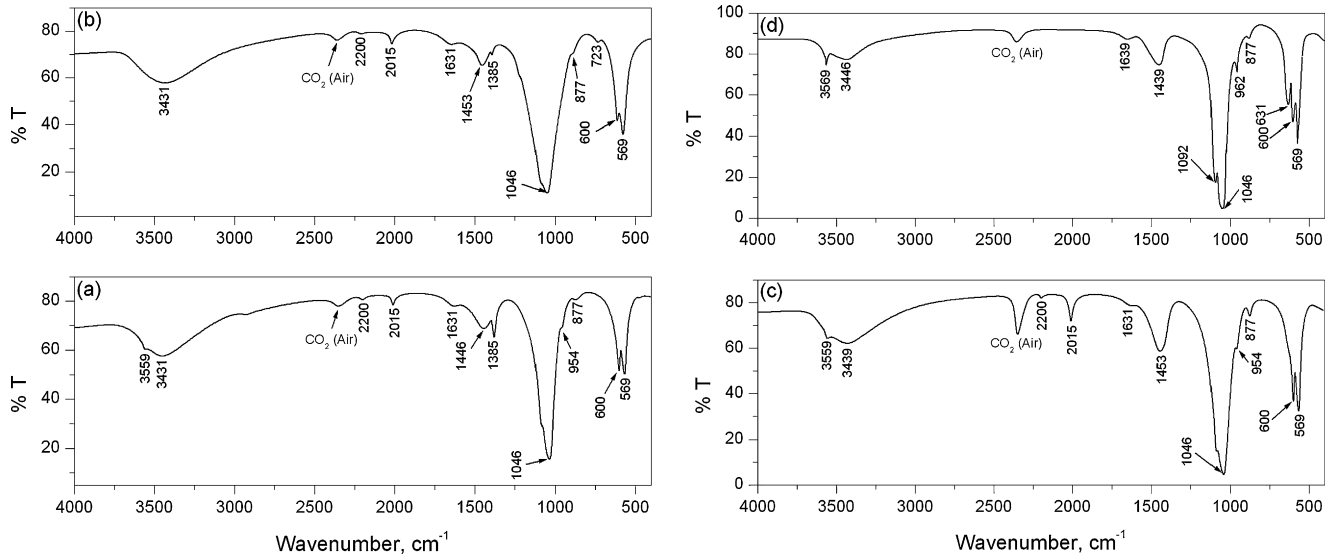

Fig. 3. FTIR spectra of combustion synthesized (a) β-TCP as-prepared powder, (b) the same fabricated at 1250 °C/1 h, (c) HAp as-prepared powder and (b) the same fabricated at 1250 °C/2 h.

61 nm. It confirmed that solution combustion method could yield nano-sized, crystalline calcium hydroxyapatite and of HAp/TCP composite powder. Variation in stoichiometry of calcium nitrate and DAP content in the reactants was observed to control the phase composition of the composite products. In the solution combustion process, it has been reported elsewhere that a calcium phosphate phase (HAp) was formed initially that underwent transformation to TCP and yielded the composite materials [23] depending on the Ca/P molar ratio (in the range of 1.45–1.67) of the reactants and eventual thermal stability of the calcium phosphate phase formed initially. After firing at high temperature, both HAp and β-TCP showed a little enhancement in crystallinity of the as-prepared powders together with no decomposition of the formed phase.

Fig. 3a, b and c, d shows the FTIR spectra of the as-prepared powders obtained from the batches containing different proportions of Ca-nitrate and DAP solutions with Ca/P molar ratios of 1.50 and 1.67 respectively. The characteristic features, namely, the PO_4 bands at 1046 cm^{-1}, 962 cm^{-1} and 600 cm^{-1} and OH-stretching mode at 3446 cm^{-1} are clearly present in all the as-prepared HAp and TCP powder as well as in their fabricated specimen. Since, the as-prepared TCP powder contained about 10% HAp phase, a small spike at around 3559 cm^{-1} for the OH-stretching band in TCP powder was also observed (Fig. 3a and b). Further absences of any peak at 2213–2034 cm^{-1} indicate that the batches were free from the nitrates group, which is desirable to develop biocompatibility in the materials. Bands at around 877 cm^{-1} indicate the presence of CO_3^{2-} group in both as-prepared structures, but, when fired at their respective sintering temperature to fabricate the porous body, the peaks were almost disappeared (Fig. 3). A small hump for the CO_2 was also observed in all the FTIR spectra probably due to CO_2 in the atmosphere.

3.2. Characterization of the porous blocks

In SEM, bigger size pores were observed in case of TCP compared to HAp (Fig. 4a and b). Pore sizes <10 μm were

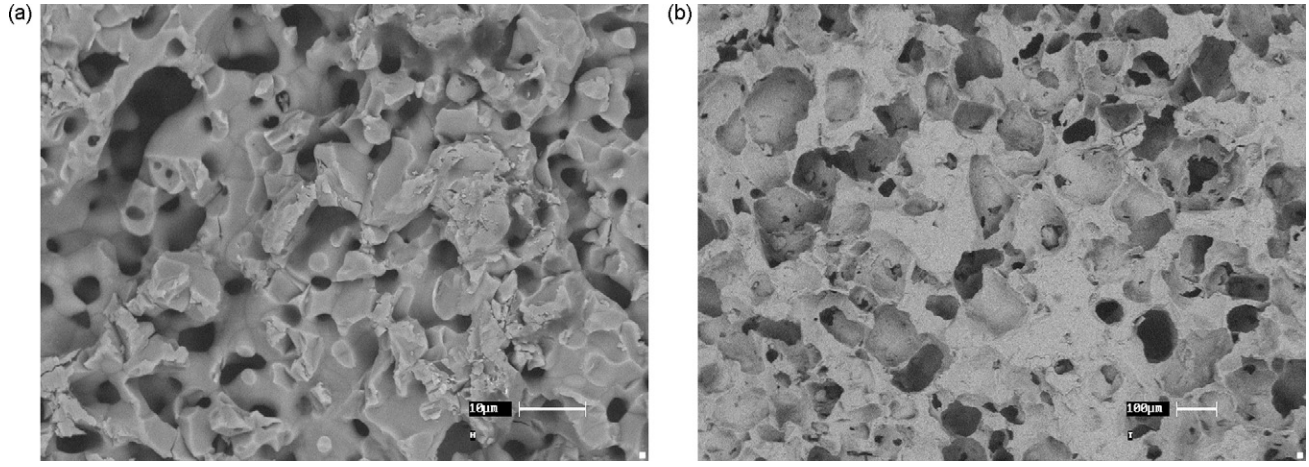

Fig. 4. SEM of porous specimens before impregnation of the drug for (a) HAp and (b) β-TCP.

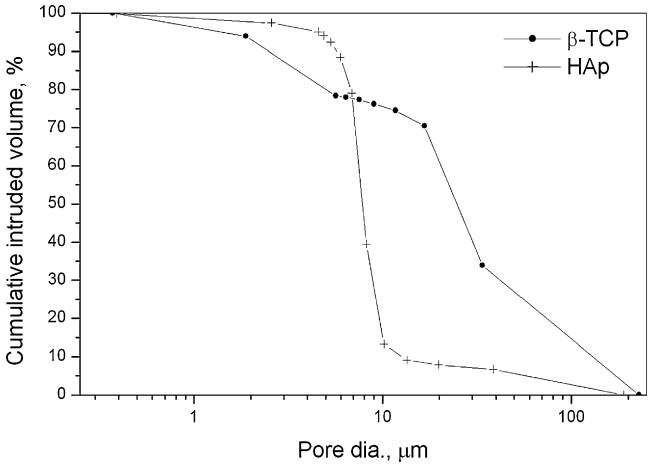

Fig. 5. MIP data plotted to show the pore size distribution patterns of HAp and β-TCP.

observed in case of HAp, which is again confirmed by the MIP plot. Pores for HAp specimens were basically micropores whereas the TCP had a range of pores having a size 30–155 μm. The open porosity % for the specimens, however, on an average, was found to be 55 and 58 respectively for HAp and TCP specimens. The plot (Fig. 5) of the mercury intrusion porosimetry results revealed the cumulative pore size distribution for all the specimens. It may be noted that although the average total pore were almost similar but their distribution was quite different. In HAp struts about 87 vol.% of the pore fell within the size range of 1–10 μm, very few large pores could be observed in both SEM as well as the cumulative volume intrusion plot of MIP. TCP although had about 30 vol.% pores fell within size range 1–16 μm, 36 vol.% within 17–34 μm and the rest were bigger pores. It is clear in Fig. 4 that larger pores were more in number in β-TCP compared to other specimens.

3.3. In vitro study

Cefuroxime release profiles from the hydroxyapatite and β-TCP implant containing the drugs are plotted in Fig. 6. The result

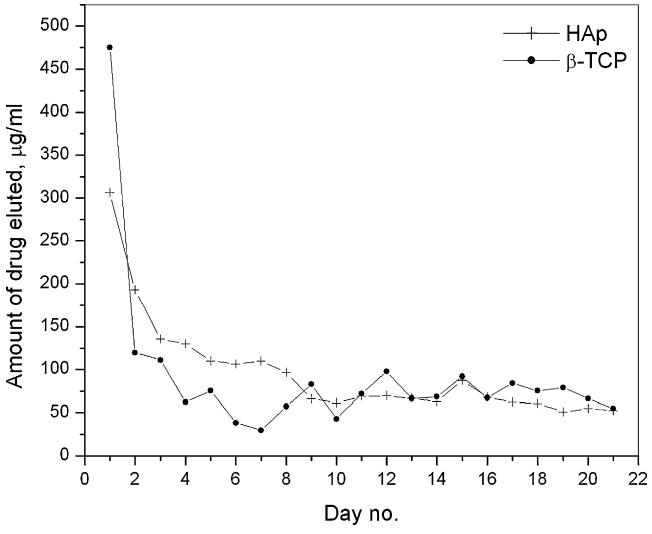

Fig. 6. Cefuroxime release profile from the HAp and β-TCP implant *in vitro*.

of this study indicated that a large proportion of this drug was released into the buffer during first 24 h, continued but diminished release found over the subsequent 24 h in β-TCP and 72 h in HAp. Thereafter the rate of release of the antibiotic was greatly reduced from the blocks although the concentration of the drug in the first 21 days remained many times higher than the minimum inhibitory concentration needed for *S. aureus* (0.5–2 μg/ml).

3.4. In vivo study

3.4.1. Histopathological examination

Histological observations provide more detailed knowledge about the cellular events during incorporation of different types of ceramic implants. Besides, best evidence of the efficiency of treatment of osteomyelitis could be observed in histopathological and microbiological findings [24].

The bone marrow showed degenerative changes of haemopoiesis centre, consisting of degeneration of fat cells (adipose tissue) along with mild fibrovascular proliferation of connective tissues. The peripheral region of the bone marrow showed infiltration with mononuclear cells, osteoclast etc. indicating development of osteomyelitis (Fig. 7). In this study, well-developed lamellar bone with haversian canal was formed on day 21 after local implantation of cefuroxime-impregnated hydroxyapatite and β-tricalcium phosphate in osteomyelitis of rabbit tibia (Figs. 8 and 9). Haversian canal also contained foreign unabsorbed material with well-developed vascularization. This might be due to the fact that cefuroxime was released at an adequate concentration in the osteomyelitis site and controlled the infection which subsequently helped in new bone formation. The presence of particles of unabsorbed ceramic materials in both the groups (Groups III and IV) suggested that the materials were not resorbed within this time. Parenteral treatment with cefuroxime even after day 42, however, showed moderate lamellar bone formation with haversian canal and marrow material (Fig. 10). Histological study on day 42 exhibited well-developed lamellar bone with haversian canal in cefuroxime-impregnated HAp and TCP beads (Figs. 11 and 12). These histological findings suggest that there was only a minimal reaction towards biomaterial and gradual new bone formation in

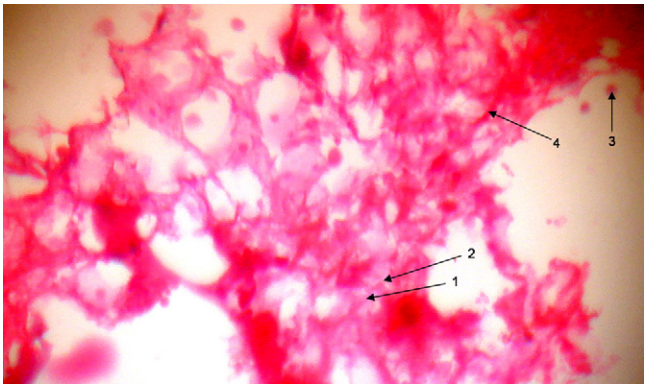

Fig. 7. Photomicrograph showing degeneration of osteoblast indicating osteomyelitis (HE × 10) after 21 days: 1. bony matrix, 2. osteocyte, 3. osteoclast, 4. immature bony osteoid.

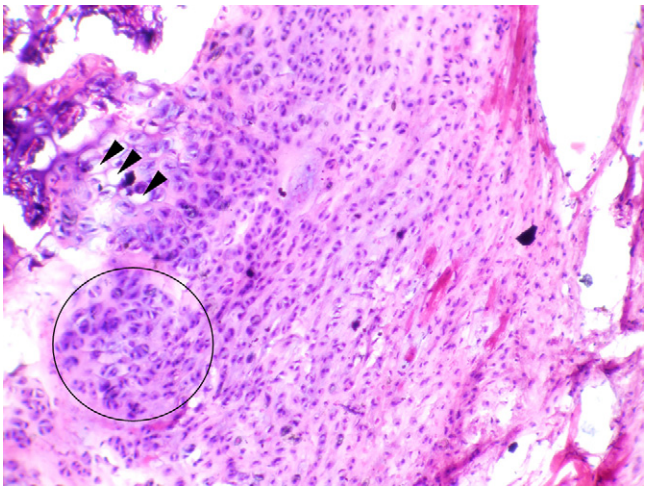

Fig. 8. Histopathology after 21 days for hydroxyapatite impregnated with the drug (Group III animal), showing well-developed haversian canals (rounded circle) and presence of HAp particles (HE × 10) (solid black arrow).

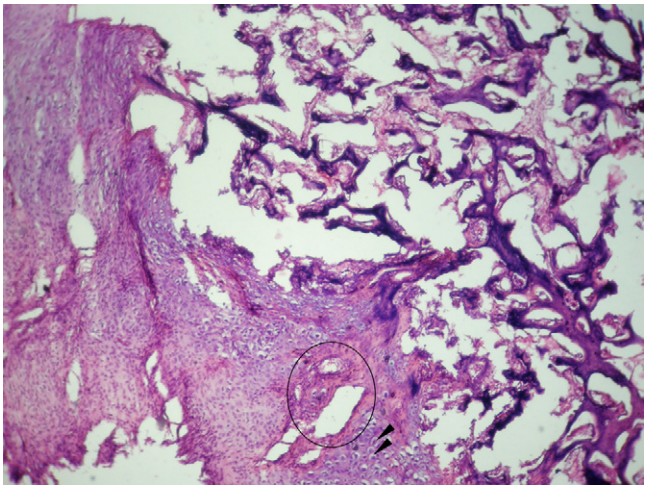

Fig. 9. Photomicrograph showing well-developed lamellar bone with presence of haversian canals and TCP particles (HE × 10) after 21 days in Group IV. Rounded circle: well-developed haversian canal and solid black arrow: cartilaginous tissue.

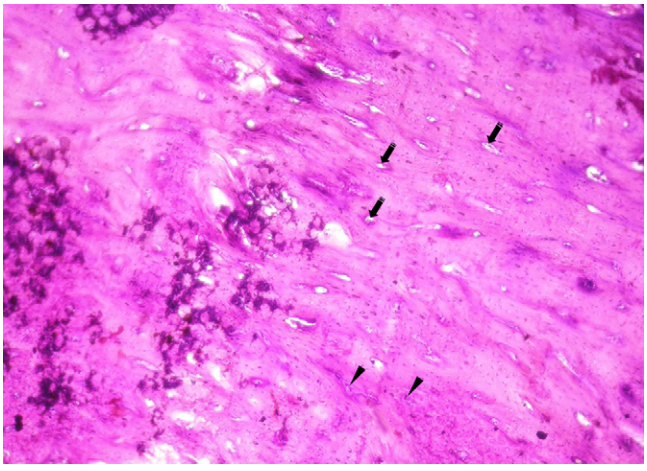

Fig. 10. Histopathology photograph after parenteral treatment with the drug (Group II animal) after 42 days. Broken black arrow: moderately developed haversian system and solid black arrow: scanty cartilaginous cells.

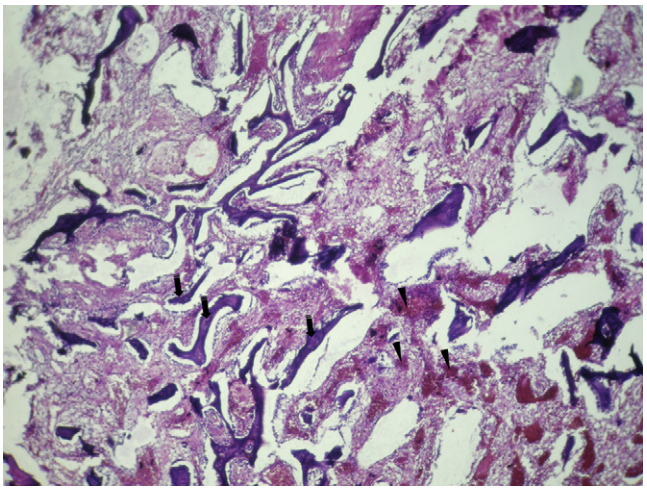

Fig. 11. Photomicrograph showing well-developed lamellar bone (broken black arrows) with presence of haversian spaces and angiogenic tissues (HE × 10) (solid black arrows) after day 42 in Group III (drug-impregnated HAp).

the area. In similar findings, Korkusuz et al. [25] observed that infection was subsided by 3 weeks and 6 weeks and inflammatory cells were replaced with bone forming cells upon treatment of osteomyelitis with antibiotic-impregnated biodegradable implants. Similar findings were also observed by Itokazu et al. [26] and Sanchez et al. [27] in osteomyelitis model.

3.4.2. Radiological examination

The treatment of osteomyelitis in orthopaedic surgery poses a great challenge due to improper and unsustained drug concentration at the site. Though the microbial organisms responsible for causing osteomyelitis are very much sensitive to different routinely used antibiotics *in vitro* but due to inherent characteristic of bony tissue, the success of treatment with such infections are very limited.

In this study, osteomyelitis was inducted successfully in animals of all groups by inoculating *S. aureus* as evidenced by

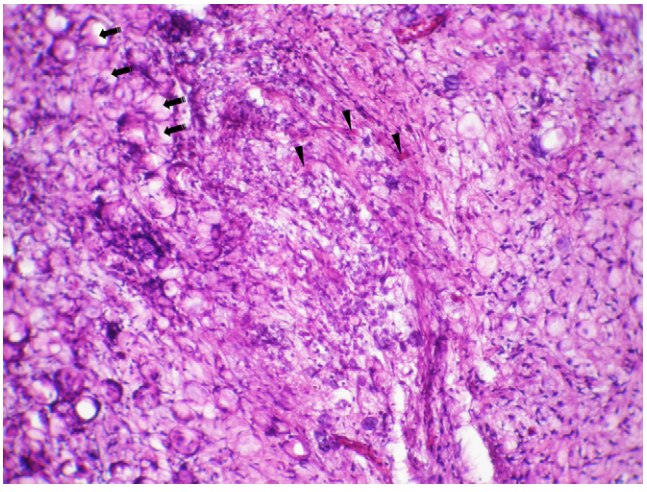

Fig. 12. Photomicrograph showing well-developed lamellar bone with presence of haversian spaces (HE × 10) after 42 days in Group IV animal (β-tri calcium phosphate impregnated with the drug). Broken black arrows: well-developed lamellar bone and solid black arrows: well-developed angiogenic tissues.

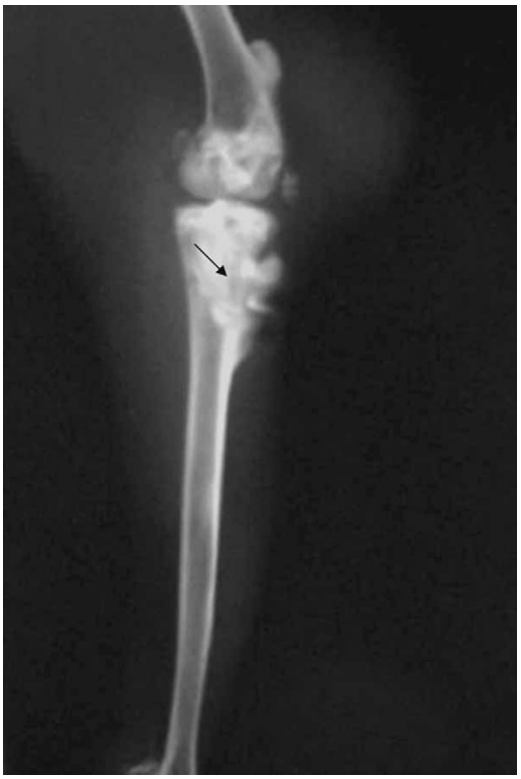

Fig. 13. Radiograph showing periosteal reaction in the epiphysis with presence of secondary sequestrum in the medullary cavity indicating development of osteomyelitis.

radiographic zone of cortical lysis, formation of sequestrum and radially oriented radiodense bony tissue at the site (Fig. 13). Inflammation with hyperemia is the squeale of bacterial contamination of bone, which ultimately leads to formation of abscess [28]. The abscess continues to expand, with further production of purulent exudates [29]. The exudate enters the cortical bone and spreads across it via haversian and volkman's canals and ultimately leading to necrosis of fragments of bone sequestrum and lyses [29,30].

In animals of Groups III and IV which were treated with antibiotic-impregnated HAp and β-TCP respectively showed more or less equally controlled infection (Fig. 14a and b). After a certain period, the lysis and sequestrum, so far observed in the pretreatment period, could not be ascertained to that extent due to effect of antibiotic in desirable concentration at the site, which might have controlled antimicrobial activity. Normal phenomenon of bone healing are formation of osteon in the phase of remodelling which aggregate in an acentric manner at any site of fracture area during the remodelling phase. In Groups III and IV, the desired level of antibiotic concentration at the site might have controlled the infection which in turn accelerated the bone healing and remodelling as evidenced by newly grown periosteal bone. In similar finding, Korkusuz et al. [25] also reported radiographically mild periosteal elevation, architectural deformation, widening of bone shaft, new bone formation and soft tissue deformation after 6 weeks of implantation with Duocid and Sulperazone-loaded poly-hydroxybutyrate-co-hydroxyvalerate (PHBV) rods in rabbits.

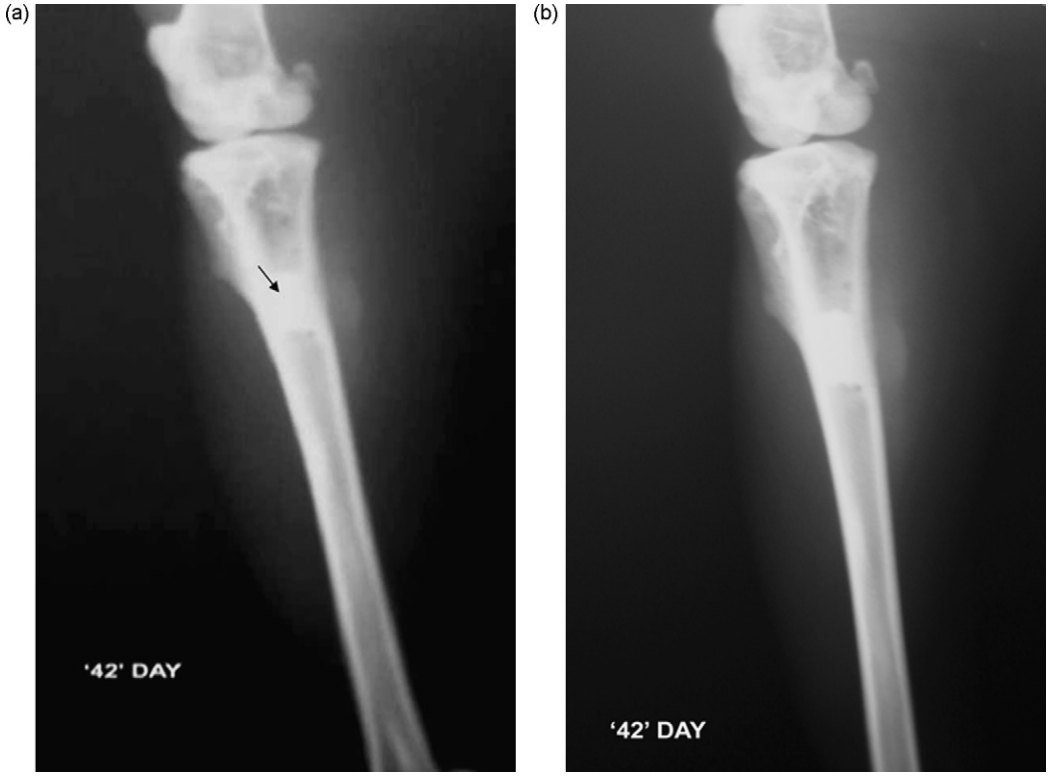

Fig. 14. Radiograph at day 42 with antibiotic incorporated (a) HAp and (b) β-TCP showing recovery from the osteomyelitis changes at the adjacent areas of respective implants.

Table 2
Concentration of cefuroxime (Mean ± S.E.) in serum and bone in parenteral injection (Group II animals).

Treatment	21 day		42 day	
	Bone (μg/gm)	Serum (μg/ml)	Bone (μg/gm)	Serum (μg/ml)
Cefuroxime axetil injection parenterally (15 mg/kg, bid) twice daily for 6 weeks	4.54 ± 0.6a	22.04 ± 0.58a	7.27 ± 0.42b	28.49 ± 0.83b

Means with different letters (a, b) within rows varied significantly ($P < 0.0001$).

In another studies, radiological observations also showed beneficial effects of antibiotic loaded porous hydroxyapatite implant in osteomyelitis [26] and well toleration and gradual new bone formation with biodegradable and osteointegrable gentamicin bone implants [27].

3.4.3. Drug concentration

Maximum concentration of cefuroxime in the bone and serum of animals (Group II) after intramuscular injection is presented in Table 2. Values for concentrations were expressed as micrograms of antibiotic per gram of bone (wet average). *In vivo*, the release of cefuroxime from the ceramic implants (HAp and β-TCP) in bone and serum is presented in Table 3. It has been observed from the table that there was an initial high release of cefuroxime in HAp implanted group followed by β-TCP implant in bone on day 21 and the same declining tendency was observed on day 42. Concentration of cefuroxime in serum reached maximum on day 21 in HAp followed by β-TCP whereas at the end of study (42 day) maximum concentration was observed in β-TCP followed by HAp. These observations were also in line with the *in vitro* studies where even after day 21 the concentration of cefuroxime in the eluted PBS, the concentration was higher in case of TCP than of HAp.

Chronic osteomyelitis is very difficult to treat and presents a challenge to clinicians. In addition to local debridement and systemic therapy with antimicrobial agents, antibiotic-impregnated poly-methyl methacrylate (PMMA)-beads applying high antibiotic concentrations have been locally used for therapy of osteomyelitis and deep soft-tissue infections. However, these beads and spacers are associated with certain disadvantages. Second surgical procedure is always required to remove the PMMA-beads and to insert bone graft instead. In addition, bone grafts do not always acceptable at the site. This study showed the efficacy of porous calcium phosphate struts as a biocompatible carrier material that sets *in vivo* and allows prolonged release of antibiotics. None of the infected animals treated with cefuroxime-loaded calcium phosphates showed signs of infection. In contrast, all rabbits in the control groups showed evidence of chronic infections. Greater success rate may be due to the fact that higher concentrations of cefuroxime were used to prepare the porous struts. Serum levels of the drug were also below the threshold required for systemic toxicity, so that no side effects were observed. Several biomaterials have been used as vehicles for the transport and sustained release of antibiotics. The pharmacokinetics of the composites *in vivo* showed that therapeutic concentration of antibiotic was maintained at the site of implantation, which was adequate to provide antimicrobial activity. The reason for the efficacy of HAp and β-TCP ceramics in the treatment of osteomyelitis is probably the advantageous pharmacokinetics at the site of infection. The minimum inhibitory concentration (MIC) of cefuroxime against *S. aureus* is 0.5–2 μg/ml [31]. Concentrations exceeding the MIC were obtained around the implant in all bone tissues. The above results suggest that the composites in this study fulfill this condition. The administration of antibiotics for 4–6 weeks is usually recommended for the treatment of chronic osteomyelitis [32,33]. The efficacy of systemically applied antibiotic for precluding osteomyelitis seems to be very poor duo to impermeability of this antibiotic in attaining desirable concentration at the target site due to blood–bone barrier [34]. Juxtaposely same phenomenon is also applicable for poor concentration of antibiotic in serum from bone tissue while applied by drug delivery system. The local antibiotic treatment may use the blood–bone barrier effectively as a protection of the body against a very high local antibiotic concentration without systemic side effects [35]. In this study also, wide difference of antibiotic concentration between bony tissue and serum irrespective of any group in corresponding days were noticed which might be due to presence of such blood–bone barrier. Further, porosity had a definite role to play *in vivo*, because HAp struts in this study were composed of mainly micropores with a few macropore inside the surface. After 21 days, the concentration of drug in bone was found to be higher than the porous TCP which was contradictory what observed *in vitro* in the eluted fluid. Interaction of biological fluid with the calcium phosphates might have some influence on the elution of the drug. We know that HAp and TCP both undergoes several stages of reaction with the body fluid that might also acted behind this anomaly result.

Table 3
Concentration of cefuroxime in bone (μg/gm) and serum (μg/ml) following implantation of antibiotic-impregnated ceramic beads on tibia.

Treatment	21 day		42 day	
	Bone (μg/gm)	Serum (μg/ml)	Bone (μg/gm)	Serum (μg/ml)
Hydroxyapatite (Group III)	37.65 ± 0.56a	13.72 ± 0.76a	24.64 ± 1.13a	10.44 ± 0.77b
β-Tricalcium phosphate (Group IV)	10.45 ± 1.07b	9.42 ± 0.51b	8.62 ± 0.51b	10.89 ± 0.20b

Means with different letters (a, b) within rows varied significantly ($P < 0.05$).

So, in this context, it is difficult to conclude about the suitable implant which could be used for the sustained release of the same drug, but, it is proved that a composite material having bi-phasic calcium phosphate content together with the drug might deliver prolonged timed dug release as evidenced from both *in vitro* and *in vivo* interpretation in the present investigation.

4. Conclusion

The novel biodegradable antibiotic carrier systems developed in this study proved to be an effective therapeutic approach towards an experimental model of osteomyelitis. Further studies on the prolongation of the drug release duration and more bone ingrowth into the pores are needed before clinical studies can be undertaken. Further, these porous calcium phosphates showed good biocompatibility. By adding antibiotic substances, this can be used as a carrier material for sustained antibiotic delivery. Because it releases high levels of antibiotics over a prolonged period, this system provides very effective local antimicrobial activity. Our findings suggest that bi-phasic calcium phosphates with predominately β-TCP content is a very efficient carrier material for antibiotic compounds even in refractory infections *S. aureus*.

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