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In vitro and *in vivo* release of cefuroxime axetil from bioactive glass as an implantable delivery system in experimental osteomyelitis

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Abstract

The aim of this study was to evaluate the characterisation, *in vitro* and *in vivo* biocompatibility and antimicrobial activity of bioactive glass (BG) impregnated with an antibiotic. The BG was prepared by normal glass melting procedures as a controlled release device to treat experimental osteomyelitis. The study design was for prospective *in vivo* experimental study. Two sets of porous bioactive glass ceramic blocks (9 mm × 4 mm × 4 mm and 20 mm × 9 mm × 9 mm) were fabricated using bioactive glass powder and subsequently antibiotic cefuroxime axetil (CFA) (55 and 125 mg on an average) was impregnated in these two sets of blocks, respectively. Osteomyelitis was produced in the right tibia of the rabbits according to the model of Norden. After thorough *in vitro* characterization of the porous blocks [including X-ray diffraction (XRD), Fourier-transformed infra-red spectroscopy (FTIR), thorough chemical analysis by inductively coupled plasma-atomic emission spectra (ICP-AES) and field-emission scanning electron microscopy (FESEM)] and *in vitro* elution of the said drug, *in vivo* test was carried out with rabbit species split into two groups: (a) animals treated with CFA impregnated bioactive glass and (b) parenteral [intra muscular (IM)] administration of CFA. Histological, radiological and drug concentration in bone and serum (measured by HPLC) in both groups were carried out. HPLC technique was used for determination of concentration both *in vitro* and *in vivo*. Fabricated porous struts showed amorphous microstructure without formation of any crystallite. The elution of said drug was stopped after 6 days *in vitro*. Histological studies at 3 and 6 weeks revealed formation of well-developed lamellar bone and havarsian canal. Radiological evaluation pointed out disappearance of sequestrum and existence of newly formed bony specules. Concentration of cefuroxime axetil in bone and serum showed highest value on day 21 which reduced marginally by day 42 and these values were higher than minimum inhibitory concentration (MIC) against *Staphylococcus aureus* (known pathogen for chronic osteomyelitis). It could be concluded that the biodegradable antibiotic carrier system developed in this study proved to be an effective therapeutic approach toward an experimental model of osteomyelitis. Based particularly on the *in vivo* results of the study, this cefuroxime axetil incorporated bioactive glass blocks can be successfully used in clinical cases of osteomyelitis in veterinary as well as human orthopaedic surgery.

Keywords: Osteomyelitis; Sustained release; Bioactive glass; *In vitro*; *In vivo*

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]. The current aggressive treatment protocols for chronic osteomyelitis constitute both prolonged IM (intramuscular) antimicrobial therapy and radical surgical debridement of all dead bone [1,2]. However, these strategies still carry

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significant relapse rates. Even if all necrotic tissues have been thoroughly surgically debrided; the bone bed has to be considered contaminated with the pathogens [3]. Obviously, systemic antibiotic therapy has a limited efficacy in this poorly perfused bone bed and alternative strategies of antimicrobial delivery should be explored. Even after successful eradication of the infection, there is a remaining problem related to the reconstitution of bone continuity. Radical debridement leaves inevitably a major bony defect, which generally requires bone grafting or some other bone reconstruction procedure at a second stage [4].

Antibiotic-impregnated poly-methyl methacrylate (PMMA) beads, the drug delivery systems which have been used successfully [5], provided a simple method to treat such chronic infection 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 effects [6]. 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 [7]. 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 [7]. Impregnation of antimicrobial agents within osteoconductive biomaterials like calcium sulphate, different calcium phosphates like hydroxyapatite or tri-calcium phosphate have been proposed for local treatment of osteomyelitis and to aid dead space management [8,9]. As a common feature, these implants show a rapid release of the antibiotic in a more or less controlled manner [10].

It has been known that bioactive glass (BG) as a synthetic bone graft material prove to be promising [11,12]. For example, BG may be superior to other graft materials to regenerate osseous tissue loss from periodontal disease and maintenance of the alveolar ridge after dental extraction. It is assumed that 'bioactive nature' of BG helps bone formation [13] and osteoblasts exploit the BG as an osteoconductive template. Moreover, bone forms within the BG matrix concurrently with BG biodegradation. Bonding to bone was first demonstrated for a certain compositional range of these bioactive glasses, which contained SiO_2, Na_2O, CaO and P_2O_5 in specific proportions [14,15]. However, the following three key compositional features are essential to impose bioactivity within the material: (i) <60 mol% SiO_2; (ii) high Na_2O and CaO content; and (iii) high CaO/P_2O_5 ratio. These compositional features make the surface highly reactive when exposed to a respective aqueous medium.

Owing to its ability to bond to bone strongly, we now report the development of an antibiotic cefuroxime axetil impregnated bioactive glass composite by simple vacuum infiltration technique and describe its release profile *in vitro* and *in vivo* and outline its ability to repair osteomyelitis at the site of the tibia of rabbits. In this regard, it may be noted that cefuroxime axetil has broad spectrum antimicrobial activity against Gram negative and Gram positive organisms. Most of the major causative bacteria of osteomyelitis are sensitive to cefuroxime axetil. In a preliminary study, it has been observed that this drug impregnated cement was proved to be effective in the prevention of early to intermediate deep infection after primary total knee arthroplasty [16].

2. Materials and methods

2.1. Bacterial isolate

Clinical isolate of *Staphylococcus aureus* (coagulase positive) from the abscess of a rabbit 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. The swab specimens for culture were taken from cavity of infected bone in order to confirm the clinical success of the induction of *Staphylococcus aureus* based on Manitol salt agar test.

2.2. Preparation and characterization of a bioactive glass composition

The bioactive glass was prepared through a conventional glass melting procedure [17]. The appropriate amounts of reagents/raw materials silica (SiO_2), calcium carbonate ($CaCO_3$), dry soda ash (Na_2CO_3), decahydrated borax ($Na_2B_4O_7{\cdot}10H_2O$), TiO_2, di-ammonium hydrogen ortho-phosphate (all chemicals were analytical grade from M/s S.D. Fine-Chem Limited, India) were mixed homogeneously in water. The batch composition of the glass is shown in Table 1, while Fig. 1 shows the schematic representation of the procedures followed to prepare the final glass composition.

X-ray diffraction (XRD) analysis (X'Pert Pro, Phillips Analytical, Netherlands) using 35 milliamps, and 40 kV current, with a monochromatic Cu Kα1 radiation ($\lambda = 1.5406$ Å) with scanning range from $2\theta = 10$–$80°$, was performed on the formed powders to check the compositional nature, Fourier-transformed infra-red (FTIR) spectroscopy studied for confirmation of the functional groups present. These were measured at room temperature (~20 °C) in the wavenumber range of 4000–400 cm^{-1} at resolution 2 cm^{-1} using Spectrum 100 (PerkinElmer Instruments, USA). The samples were pulverized into fine powder, and then mixed with potassium bromide powder, a weight ratio of 1:100 (0.002 g:0.2 g, samples:KBr, respectively). The mixture was subjected to a load of 15 T cm^{-2} in an evacuable die for 5 min to produce clear homogenous discs. The spectra were measured