Materials Science and Engineering C 33 (2013) 3986–3993

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Systematic approach to treat chronic osteomyelitis through localized drug delivery system: Bench to bed side

Rupnarayan Bhattacharya [a], Biswanath Kundu [b,*], Samit Kumar Nandi [c,**], Debabrata Basu [b,1]

[a] Department of Plastic Surgery, R. G. Kar Medical College and Hospital (RGKMCH), Kolkata 700004, India
[b] Biocermics and Coating Division, CSIR — Central Glass and Ceramic Research Institute (CSIR-CGCRI), Kolkata 700032, India
[c] Department of Veterinary Surgery and Radiology, West Bengal University of Animal and Fishery Sciences (WBUAFS), Kolkata 700037, India

ARTICLE INFO

Article history:
Received 8 September 2012
Received in revised form 20 March 2013
Accepted 16 May 2013
Available online 25 May 2013

Keywords:
Chronic osteomyelitis
Porous hydroxyapatite
Antibiotic
In vivo animal trial
Human application

ABSTRACT

Chronic osteomyelitis is a challenging setback to the orthopedic surgeons in deciding an optimal therapeutic strategy. Conversely, patients feel frustrated of the therapeutic outcomes and development of adverse drug effects, if any. Present investigation deals with extensive approach incorporating in vivo animal experimentation and human application to treat chronic osteomyelitis, using antibiotic loaded porous hydroxyapatite scaffolds. Micro- to macro-porous hydroxyapatite scaffolds impregnated with antibiotic ceftriaxone–sulbactam sodium (CFS) were fabricated and subsequently evaluated by in vivo animal model after developing osteomyelitis in rabbit tibia. Finally 10 nos. of human osteomyelitis patients involving long bone and mandible were studied for histopathology, radiology, pus culture, 3D CT etc. up to 8–18 months post-operatively. It was established up to animal trial stage that 50N50H samples [with 50–55% porosity, average pore size 110 μm, higher interconnectivity (10–100 μm), and moderately high drug adsorption efficiency (50%)] showed efficient drug release up to 42 days than parenteral group based on infection eradication and new bone formation. In vivo human bone showed gradual evidence of new bone formation and fracture union with organized callus without recurrence of infection even after 8 months. This may be a new, alternative, cost effective and ideal therapeutic strategy for chronic osteomyelitis treatment in human patients.

1. Introduction

Chronic osteomyelitis is an osseous infection that has progressed to bone necrosis and sequestrum formation [1–3] and is a burning problem in the field of orthopedic and reconstructive surgery. Surgical debridement and prolonged administration (about 4–6 weeks) of antibacterial agents are the common therapeutic approach for orthopedic infections [1]. In spite of the availability of countless of antibiotics and striking advances in surgical treatment, the long-term recurrence rate remains shocking at a rate of 20% to 30% [2]. Very high concentrations of antibiotics are of paramount prerequisite at the target site but could hardly be attained by conventional routes of drug administration without aggravating serious side effects because of paucity of blood supply to the local area [3]. Therefore, conventional therapy poses severe challenges to the clinician in deciding an optimal treatment strategy for osteomyelitic patients. Conversely, patients feel frustrated and disappointed in optimal therapeutic outcomes and development of adverse drug effects. Systemically administered antibiotics cannot reach the osteomyelitic sites in sufficient concentration due to lack of blood vessels, sclerosis of bone periphery of the osteomyelitic area and presence of sequestrum in the osteomyelitic cavity, which acts as a continuous source of infection [4,5]. Hence, the development of new therapeutic strategies for treating chronic osteomyelitis is important for future advances.

Local drug delivery systems have the advantage of releasing the drug locally for prolonged periods [6] and at concentrations generally higher than those achieved by conventional drug delivery strategies [7]. Better healing rate, low cost therapy and insignificant chances for development of side effects or adverse drug effects are some striking advantages of local drug delivery. A large number of biomaterials either bioinert or biodegradable are in vogue as local drug delivery systems of which antibiotic impregnated polymethyl methacrylate (PMMA) beads are commonly used in resistant cases of osteomyelitis for several decades [8]. However, PMMA is bioinert, damages the drug during incorporation by heat generation, has poor antibiotic elution properties and needs revision surgery for removal of beads which is costly and painful [4,9]. Although certain bioactive materials like tricalcium phosphate (TCP), bioactive glass convert to hydroxyapatite and bond directly with bone and soft tissue [6,10–12] but degradation rate of TCP is too slow to match the bone regeneration rate, requiring biological increase of degradation rate and osteoinductive capabilities in vivo [13]. Therefore, nowadays much attention has been focused on ceramic based local drug delivery system for treating osteomyelitis.

* Corresponding author. Tel.: +91 9831772081; fax: +91 33 24730957.
** Corresponding author. Tel.: +91 9433111065; fax: +91 33 25571986.
E-mail addresses: biswa_kundu@rediffmail.com (B. Kundu), samitnandi1967@gmail.com (S.K. Nandi).
[1] Deceased 10 May 2012.

0928-4931/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2013.05.036

Porous hydroxyapatite (HAp) is considered to be superior to acrylic bone cement because of its biocompatibility, bioactivity, osteoconductivity and high binding affinity for a variety of bioactive molecules [14,15] superseding the disadvantages mentioned above. Hydroxyapatite, however, is typically not absorbable at all within <3 years depending on its actual composition (stoichiometric or non-stoichiometric). Micro- and/or macro-porous structures of the implant allow slow and sustained release of the drug at the local site maintaining the desired antibiotic level at the target area [16,17]. However, influence of pores in the scaffold is very much pertinent as blood flow and vascularization in in vivo environment plays vital role in drug release and further bone tissue in growth. Pore size also influences migration and proliferation of osteoblasts, mesenchymal cells and matrix deposition in the unfiled space of scaffold along with drug diffusion [18,19]. Pore interconnection offers the channel network for efficient cell distribution, migration and consequent well-organized blood vessel formation [20,21]. These importance variables like pore size, amount, interconnectivity and drug release were not well reported from porous HAp scaffolds. Further, mostly the bacteria involved in chronic osteomyelitis produce β-lactamase, an enzyme that inactivates β-lactam antibiotics. To counteract β-lactamase-producing strains, drug combinations that include an irreversible β-lactamase inhibitor with β-lactam antibiotics demonstrate to be an ideal approach [22,23] which reduce the minimum inhibitory concentrations (MICs) of hydrolyzed β-lactams to normal [24] and increase the antimicrobial spectrum to include previously β-lactam resistant microorganisms [25]. Application of such antibiotic combinations in treating chronic osteomyelitis either in animal or human subject through local delivery system is very rare. Many other bioceramic materials [like β-tri calcium phosphate (β-TCP), bioactive glass, etc.] have also been tried by the authors' group with different antibiotics to compare the results in vivo using animal models [26–30]. To the best of our knowledge a complete cycle starting from material development (that does not include mere raw antibiotic powdered filled in HAp porous scaffold) to post-operative studies on human species via animal trial stage is not known for the possible treatment modality of human subjects with severe chronic osteomyelitis.

The purpose of this paper is to present our early clinical experience with highly interconnected porous HAp scaffold impregnated with third generation drug ceftriaxone sodium and sulbactam sodium (2:1 ratio) as a local antibiotic delivery system for treating chronic osteomyelitis in human patients. Infection control and bone repair will be reported in a chronological fashion.

2. Materials and methods

2.1. Development of hydroxyapatite based porous scaffolds with or without drug impregnation

Pure hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] was synthesized in the laboratory via a simple economical wet-chemical precipitation method utilizing calcium hydroxide and ortho-phosphoric acid in 1.67 molar ratios of Ca/P. Sintered (1250 °C) porous scaffolds were fabricated by first very slow drying and subsequently firing the green compacts premixed with required fugitive materials (scintillation grade naphthalene with required fraction and size distribution was used in this case). Two different percentages of the scaffold formulation premixed with 50 and 60%, (hereinafter designated as 50N50H and 60N40H) of fugitive materials were employed to obtain different percentages of micro- (10–50 μm) to macro-pores (>50 μm) [31]. An irreversible β-lactamase inhibitor with β-lactam antibiotics (CFS) composed of sulbactam sodium (SUL) and ceftriaxone sodium (CFT) respectively in 2:1 ratio was used further for impregnation purpose into the said porous scaffolds (both the drugs were procured in raw form from Akorn India, Himachal Pradesh, India). High vacuum (10 mm Hg for 30 min) and later freeze drying (−80 °C at 15 Pa pressure) were employed for infiltration of 500 mg/mL concentration of CFS inside porous scaffolds. After thorough material characterization of powders and with or without CFS loaded porous HAp scaffolds, 50N50H and 60N40H samples were studied for in vitro drug elution studies both in contact with PBS (phosphate buffer saline) and SBF (simulated body fluid) at pH 7.4 and 37 °C up to 42 days where the concentrations of drugs were estimated by HPLC methods.

2.2. In vivo animal study

In vivo animal studies were 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 (Permission No. 137 E, dated 23 July 2008) using the 50N50H samples having an adsorption efficiency of ~48% (on an average). The parameter was expressed by the change in weight of HAp scaffold before and after drug impregnation divided by the weight before drug loading. The samples were selected after assessing elution kinetics of drug release up to 42 days in contact with SBF and PBS and studying the bioactivity and percent yield. Percent yield of CFS was expressed as the total amount of released antibiotic after 42 days divided by the amount of CFS held in the samples before the start of elution in PBS and SBF separately. After induction of osteomyelitis with *Staphylococcus aureus* suspension in the right tibia of 24 New Zealand white rabbits (1.5–2 kg body weight, aged about 8 months to 1 year) as per model of Norden [32], proximal part of the tibia was exposed anteriorly after sedation with xylazine hydrochloride (1 mg/kg body weight; Indian Immunologicals, India) and anesthesia with ketamine hydrochloride (11 mg/kg body weight; Ketalar®, Parke-Davis, India). A hole was drilled through the cortex into the medullary cavity using a 1.2 mm diameter dental burr. One mL of *S. aureus* suspension containing approximately 3×10^6 CFU/mL was injected into the drilled medullary cavity and the hole was sealed with bone wax to avoid bacterial leakage into the surrounding soft tissues. Each animal was examined after surgery and received standard analgesics (carprofen; 4 mg/kg of body weight) for 3 days. After three weeks of development of osteomyelitis, CFS-impregnated HAp scaffolds were implanted in the infected bone after a second surgery following the same anesthetic and aseptic protocol. Animals were divided into three groups [group I: no implants, no treatment, group II: CFS injection parenterally (15 mg/kg bid) twice daily for 6 weeks and group III: CFS impregnated HAp scaffolds] and studied up to 42 days. Histological examinations, radiography, estimation of antibiotics both in bone and serum and scanning electron microscopic (SEM) studies on the extracted bone were carried out post-operatively. For histological examinations, antibiotic impregnated HAp implants with bone were initially fixed in 10% formalin for 7 days, decalcified and subsequently stained with hematoxylin and eosin. Radiological examinations of the subjected bones were carried out under direct radiographic magnification. For estimation of antibiotics (CFT and SUL) both in bone and serum, pulverized, homogenized, centrifuged supernatant fluid from cortico-cancellous portion of tibia (removing bone marrow) and blood samples were used and measured by high performance liquid chromatography (HPLC) techniques (Shimadzu, SPD-MIOA, Japan). The results were expressed as means ± standard deviations (detection wavelengths for CFT and SUL were 254 and 313 nm respectively). For SEM, implants with bone were first fixed with 5% glutaraldehyde phosphate solution, washed twice for 30 min with PBS (pH 7.4) and distilled water, dehydrated in a series of graded ethanol followed by final drying with hexamethyldisilizane (HMDS). The samples were gold coated by ion sputtering (JEOL ion sputter, Model JFC 1100, Japan) at 7–10 mA and 1–2 kV for 5 min and examined under SEM (JEOL JSM 5200 model, Japan) after proper alignment.

The swab specimen was collected from the infected site of bone after 3rd week of post inoculation from animals of all groups and was streaked on mannitol 10% salt agar slant and incubated at 37 °C for overnight. Characteristic bacterial growth was observed (Fig. 1). From single colony, bacterial growth was collected and stained by Gram's