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Antibiotic-loaded poly-ε-caprolactone and porous β-tricalcium phosphate composite for treating osteomyelitis

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Abstract

A composite of poly-ε-caprolactone (PCL) loaded with gatifloxacine (GFLX), an antibiotic, and a β-tricalcium phosphate (βTCP) porous ceramic body was prepared by a solvent-free process in which no toxic solvent was used. GFLX mostly retained its bactericidal property after the processing. The composite of GFLX-loaded PCL and βTCP ceramic released GFLX for 4 weeks in Hanks' balanced solution, and had sustained bactericidal activity against *Streptococcus milleri* and *Bacteroides fragilis* for at least 1 week. The composite of the GFLX-loaded PCL and βTCP ceramic was implanted in an osteomyelitis lesion induced by *S. milleri* and *B. fragilis* in the rabbit mandible. The osteomyelitis lesion expanded in the mesial–distal direction when no composite was implanted or when the lesion was treated with debridement only. The composite of GFLX-loaded PCL and βTCP showed efficacy in controlling infection at the bone defect formed by debridement, and supported bone tissue reconstruction at the bone defect. Twelve and 50 weeks after the implantation, the inflammation even disappeared. New bone formation was observed on the surface of the composite after 4 weeks. After 50 weeks, ingrowth of bone tissues with vascular channels was observed along the PCL and βTCP interface, which indicated degradation of PCL and/or βTCP ceramic at the ceramic/polymer interface followed by replacement by bone tissues. The GFLX concentrations in the serum and soft tissues were very low. Therefore, the composite of GFLX-loaded PCL and βTCP ceramic would help arrest osteomyelitis when it is used in addition to intravenous antibiotic administration, and help new bone formation and osteoconduction.

Keywords: Polycaprolactone; Drug delivery; Calcium phosphate; Antimicrobial; Osteoconduction; Animal model

1. Introduction

Chronic osteomyelitis is an intractable inflammation of the bone caused by pathogenic microorganisms, and is associated with the destruction of bone tissues and vascular channels [1–3]. The inflammation is characterized by a predominant presence of leukocytes and macrophages, which contribute to the destruction of bone tissues. Vascular channels are compressed and obliterated by the inflammatory process. The destruction of vascular channels leaves a portion of dead and infected bone (sequestrum) that is detached from the adjoining healthy bone and surrounded by avascular soft tissue. Owing to the impaired vascularity, antibiotics may not be delivered adequately to the lesion by the intravenous route.

The local delivery of antibiotics is an effective option for treating chronic osteomyelitis [4]. In general, chronic osteomyelitis cannot be eradicated without surgical elimination of the sequestrum accompanied by antibiotic therapy administered by the intravenous route [1]. The elimination of the sequestrum leaves a large dead space. This dead space is a problem because it is poorly vascularized, which is a predisposing condition for the

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persistence of infection. To sterilize and maintain a dead space, antibiotic-impregnated polymers can be used, while to sterilize the remaining bone tissue, antibiotic is delivered by the intravenous route. Among the antibiotic-impregnated polymers, polymethylmethacrylate (PMMA) beads are the most common delivery carrier [5–9]. However, PMMA beads require a second operation for removal. Another class of antibiotic delivery carrier is biodegradable polymers. The effectiveness of local antibiotics delivered using biodegradable polymers has been substantiated by in vitro and in vivo studies. The biodegradable polymers that have been used include poly(D,L-lactic acid) [10,11], polylactide-*co*-glycolide [4,12–14], copolymers of L-lactide and D,L-lactide [15], polyanhydrides of bis-carboxyphenoxypropane and sebacic acid [16], polycaprolactone [17–19], and polyhydroxyalkanoate [20]. These polymers release antibiotics for several hours to 40 weeks in vitro, and are effective for several weeks in vivo [21,22].

These antibiotic-impregnated polymers, however, are deficient in terms of providing support for bone tissue reconstruction at the dead space. Since biodegradable polymers show impaired osteoconduction and sometimes provoke an adverse tissue response, calcium phosphate ceramics are combined with biodegradable polymers to improve bone–tissue interaction [23–26]. In this context, a composite of a biodegradable antibiotic polymer and β-tricalcium phosphate (βTCP) would be better than the biodegradable antibiotic polymer alone for use as the local antibiotic delivery carrier. βTCP is osteoconductive and serves as a resorbable scaffold in bone tissue. The role of βTCP in osteoconduction is not passive but participatory, which differs from that of biodegradable polymers [27]. Therefore, a composite of a biodegradable antibiotic polymer and βTCP could provide a sustained release of antibiotics from the polymer for sterilizing the dead space, support bone tissue reconstruction, and finally, be totally resorbed and replaced by new bone tissue.

In the present study, we attempted to develop a composite material composed of a porous βTCP ceramic and biodegradable polymers loaded with gatifloxacine (GFLX) for treating chronic osteomyelitis, particularly that in the mandible. The biodegradable polymers used were poly-ε-caprolactone (PCL) and polyethylene glycol. GFLX is a fluoroquinolone that has bactericidal property for both anaerobic and aerobic bacteria. The composite was fabricated using a solvent-free process in which no toxic solvent was used, and characterized in terms of its in vitro antibiotic release efficiency, bactericidal property as well as in vivo effectiveness and safety.

2. Materials and methods

2.1. Preparation of GFLX- loaded biodegradable polymer

One gram of commercially available PCL with a molecular weight of 10,000 (PCL10; Wako Pure Chemical Industries, Ltd., Japan), and polyethylene glycol with molecular weights of 4000 and 8000 (PEG4 and PEG8, respectively; Wako Pure Chemical Industries, Ltd., Japan) were melted at 120 °C in polypropylene tubes using an aluminum block heater. 10 mg of gatifloxacine hydrate (GFLX; $C_{19}H_{22}FN_3O_4 \cdot 1.5H_2O$; LKT Laboratories, Inc., USA) was added to the molten polymer and stirred vigorously. The temperature used for the melting (120 °C) was 67 °C lower than the decomposition temperature of GFLX (187 °C).

2.2. Preparation of GFLX- loaded biodegradable polymer disks

The GFLX-containing molten polymer was solidified in a disk shape 6.35 mm in diameter and 1.2 mm in thickness using a metal mold. Since the weight of the disk was 60 mg, each disk contained 600 μg of GFLX.

2.3. Preparation of GFLX- loaded PCL10 and porous βTCP composite

A three-dimensionally perforated porous βTCP ceramic 4.75 mm in diameter and 2.0 mm in thickness (Fig. 1) was prepared by the method described elsewhere [28]. Briefly, βTCP slurry was prepared by mixing a 1% methyl cellulose aqueous solution and βTCP powder under 75 μm in particle size at a solution-to-powder weight ratio of 6:4. Needlelike male dies made of stainless steel 0.5 mm in diameter were arranged in a layer and parallel to each other at intervals of 0.3 mm in a mold, and other

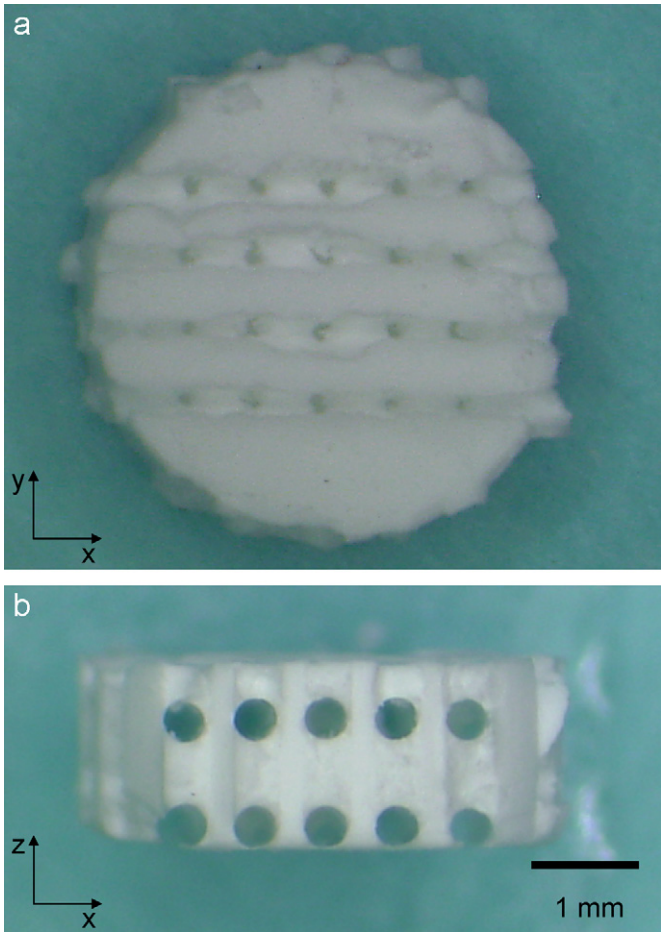

Fig. 1. Three-dimensionally perforated porous βTCP ceramic 4.75 mm in diameter and 2.0 mm in thickness used in the present study. Linearly penetrating pores 380 μm in diameter run in the x and y directions alternately at different z coordinates. The pores running in the x direction and those running in the y direction are in contact with each other at many points. The contacting points form pores 50–200 μm in diameter running in the z direction.