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A bioactive implant in situ and long-term releases combined drugs for treatment of osteoarticular tuberculosis

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ABSTRACT

Anti-tuberculosis chemotherapy with a long duration and adequate dosing is the mainstay for treatment of osteoarticular tuberculosis (TB). However, it is difficult for systemic administration to reach adequate local drug concentrations and achieve effective treatment. Herein, a hydroxyapatite (HA) scaffold implant combined with a drug-releasing system was designed to achieve in situ and long-term anti-TB drug release and highly efficient therapeutic activity *in vitro* and *in vivo*. The clinical anti-TB drugs hydrophilic isoniazid (INH) and hydrophobic rifampicin (RFP) were molecularly dispersed into polyvinyl alcohol (PVA) through immersion-curing techniques and were steadily adhered onto the surfaces of HA scaffolds (HA-drug@PVA). The HA-drug@PVA scaffolds showed a long-term, sustained drug release profile and killed proliferating *Mycobacterium in vitro*. *In vivo* experimental results revealed that the HA-drug@PVA scaffolds provided over 10- and 100-fold higher concentrations in muscles and bones, respectively, as well as a much lower concentration (<0.025) in blood. Furthermore, the HA-drug@PVA scaffold implanted in an osteoarticular TB rabbit model showed obvious bone regeneration and fusion due to the inhibition of TB-associated inflammatory changes. The excellent therapeutic effects indicate that in situ implant materials combined with a long-term drug release system are promising for the treatment of osteoarticular TB and other osteoarticular infections.

1. Introduction

The World Health Organization reported that there were an estimated 10.4 million new tuberculosis (TB) cases worldwide in 2016 [1]. Osteoarticular TB is the most common form of extra-pulmonary TB, accounting for 3%–5% of all TB cases, and it affects mainly the vertebrae and weight-bearing bones and has aggressive behaviors of profound bone destruction, deformity, and neurological deficits [2]. A majority of the patients with this disease are from poor families in developing countries such as India, Indonesia, and China. Surgical intervention is usually necessary for osteoarticular TB patients with severe complications; however, complete removal of the *Mycobacterium TB* is always impossible. Post-operative recurrence can be induced, together with the emergence of drug resistant TB [3]. Therefore, long-term anti-TB chemotherapy by systemic oral administration is highly needed post-operatively.

It is still a challenge for oral administration of rifampicin (RFP) and isoniazid (INH), two clinical anti-TB drugs, to overcome the barriers of blood, tissue, *etc.*, in order reach adequate concentrations in the nidus bone [4,5] Currently, innovative antibacterial drug delivery biomaterials have tremendous potential for the treatment of osteoarticular infection, which could simultaneously

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increase local antimicrobial drug concentrations and achieve bone defect reconstruction [6–10]. To achieve high concentration in the nidus bone for the treatment of osteoarticular TB, herein, we designed and prepared a synthetic bone with combined drug releasing capability (HA-drug@PVA). Implanting the HA-drug@PVA into an osteoarticular TB rabbit model resulted in excellent therapeutic effects with bone regeneration and fusion due to the inhibition of TB-associated inflammatory changes by the in situ drug release, which resulted in over 10- and 100-fold higher concentrations in muscles and bones, respectively, as well as a much lower concentration (<0.025) in blood (Scheme 1). This study catered the needs of in situ and long-term drug release for osteoarticular TB together with surgery, and other osteoarticular infections.

2. Materials and methods

2.1. Materials

The HA powders were purchased from Si Chuan University, China. INH and RFP were purchased from Shanghai Macklin Biochemical Co., Ltd. PVA was purchased from Beijing Yili Fine Chemicals Co, Ltd. Sodium alginate, dimethylacetamide, lithium chloride and calcium chloride were purchased from Si Chuan Kelong Chemical Reagent Factory, China. Dimethyl sulfoxide (DMSO) was purchased from Beijing Chemical Works.

2.2. Fabrication and characterization of the porous HA scaffolds

2.2.1. Preparation of alginate hydrogel beads

The 2.0% (w/v) alginate solution was extruded dropwise through gauge needles into a calcium chloride ($CaCl_2$, 1 M) solution by a peristaltic pump. The alginate hydrogel beads were left to stand for 2 h to allow for adequate crosslinking, followed by washing 3 times with distilled water, and removal of the excess solution. Solvent exchange from water to ethanol was achieved by immersing the wet beads in ethanol (referred to as SA-Ethanol-bead).

2.2.2. Fabrication of HA scaffolds

An HA slurry was prepared by mixing HA powder (20 g) with an alginate solution (2%). Initially, SA-Ethanol-beads and the HA slurry were transferred into a cylindrical mold. Then, the mixture was uniaxial compressed with a plunger in order to fill the cavities among the SA-Ethanol-beads with the HA slurry, and connect SA-Ethanol-beads. The slurry-infiltrated template was put into a $CaCl_2$ solution in order to gel the HA slurry. Subsequently, the samples were removed from the solution, dried at 40 °C to obtain scaffolds in a 3D column shape, and sintered in atmospheric conditions at 700 °C for 2 h to remove the alginate and at 1200 °C for 2 h to form porous HA scaffolds.

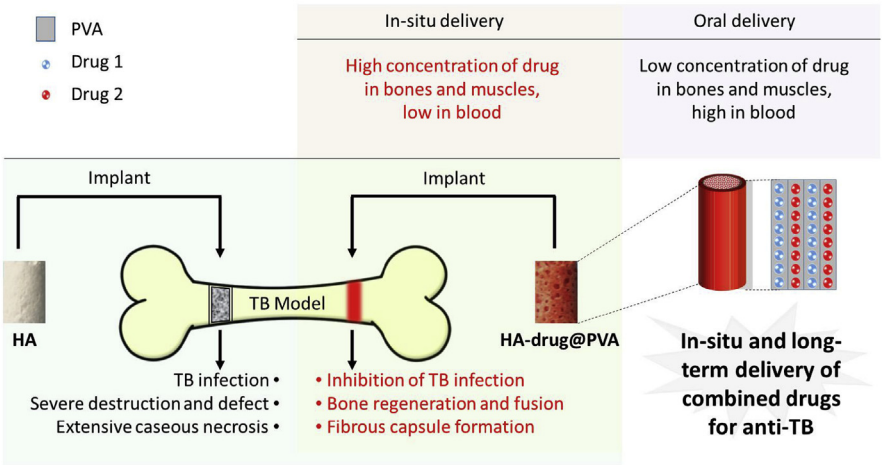
2.3. Fabrication of HA-drug@PVA scaffolds

Isoniazid (INH, 2 g) was dissolved in 50 mL of a PVA solution (4% w/w) with magnetic stirring for 2 h to obtain 50 mL of an INH@PVA solution (INH, 4% w/w). Rifampicin (RFP, 1.5 g) was added to 20 mL DMSO and was completely dissolved by magnetic stirring, and the solution was then mixed with 30 mL of a PVA (4% w/w) solution to obtain an RFP@PVA solution (RFP, 3% w/w).

HA scaffolds were added to the INH@PVA solution under 0.1 MPa negative pressure until there were no visible bubbles around the HA. The HA was removed, and the solution was centrifuged at 1000 r/min for 10 min to eliminate the excess drugs inside the pores and stored for 5 h inside an electrothermal blow drier until it was completely dried to obtain HA-INH@PVA. Then, the HA-INH@PVA was added to the RFP@PVA solution, and the step above was repeated. These processes were repeated twice. Finally, HA scaffolds with four coating layers, including 2 layers of INH and another 2 layers of RFP in the order of HA-INH-RFP-INH-RFP@PVA (HA-drug@PVA), were fabricated. In addition, another 3 HA scaffolds were fabricated to form 2-layer HA-INH-INH@PVA (HA-INH@PVA), HA-RFP-RFP@PVA (HA-RFP@PVA) and 4-layer HA-INH-RFP-INH-RFP (HA-drug) as control groups.

2.4. In vitro drug release of HA-drug@PVA scaffolds

The HA-drug@PVA scaffolds were added to a 2 mL PBS dialysis bag at 37 °C, soaked in a 5-mL PBS test tube and placed on a constant temperature shaker. At 0.25, 0.5, 1, 2, 3, 6, 9, 12, 15, 18, 25, 32, 39, and 46 days, 5 mL of the PBS in the tube was removed for testing, and another 5 mL of fresh PBS solution was simultaneously added. The drug concentration was calculated from the absorbance measured with an ultraviolet spectrophotometer at the wavelength of 263 nm for INH and 474 nm for RFP.



Scheme 1. The schematic illustration of in-situ and long-term drug delivery strategy based on implantation of HA-drug@PVA scaffolds against tuberculosis over oral administration and bare HA scaffolds.