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Isoniazid conjugated poly(lactide-co-glycolide): Long-term controlled drug release and tissue regeneration for bone tuberculosis therapy

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ABSTRACT

Bone tuberculosis (TB) is one of the most common extrapulmonary TB. Effective integration of chemotherapy and bone regeneration is an optimal solution for bone TB therapy. Herein, we produce a composite scaffold drug delivery system fabricated with an isoniazid conjugated star poly(lactide-co-glycolide) (PLGA-INH_4) and β-TCP. The cytological assay indicated the composite system possesses good biocompatibility. The *in vitro* and *in vivo* drug release evaluations showed that the composite system can intactly release the pristine INH and maintain effective INH concentration in a controlled manner for more than 100 days, and achieve high localized drug concentration and low systemic drug concentration. The rabbit radius repair experiment testified the scaffold has good bone regeneration capacity. Our work demonstrate the composite system can simultaneously achieve localized long-term drug controlled release and bone regeneration, which provides a promising route for improved bone TB treatment.

1. Introduction

Tuberculosis (TB), a chronic infectious disease which once outbreak in the global scope, is a serious threat to human health [1–3]. According to the report of World Health Organization (WHO), there are estimated 8.6 million newly infected TB cases and 1.3 million died in 2012 [4]. Bone TB is one of the most common extrapulmonary TB, and its cases are approximately 19–38 million in the world [5–7].

A large number of clinical cases in treating serious bone TB suggest the effectively therapeutic strategy should combine taking anti-TB drugs for an appropriate period and surgical intervention [8,9]. Based on WHO guideline, long-term enough dosage of given anti-TB multi-drug is necessary for bone TB treatment [10]. It was generally suggested that the drug treatment should continue for at least 9 and 12 months for adults and children, respectively [11]. Garrido G and coworkers even put forward that medication time should last 18 months for adults [12]. Currently, systemic drug chemotherapy including oral administration and intravenous injection is the most popular approach in clinics. However, it requires high dosage to reach effective treatment concentration at nidus sites because of low permeability and metabolism of the anti-TB drugs. This long-term high dosage chemotherapy causes severe toxic side effects [13–16].

Implantable anti-TB drug carriers with targeted drug release may be advantageous since they can achieve high drug concentration at focus site while low drug concentration in the whole body, thereby significantly reduce toxic side effects. Jianlin Shi and coworkers reported an implantable drug carrier fabricated by coating mesoporous silica nanoparticles and bioactive glass on β-TCP bioceramic scaffold [17]. The scaffold carrier can realize co-release of isoniazid and rifampicin and maintain their effective drug concentrations to kill mycobacterium tuberculosis for 42 days,

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but the sustained release time is not long enough for bone TB treatment. In addition, an initial burst release, ~30% of the loaded drugs was released during the first day, is observed from this drug delivery system. The burst release not only shortens the drug delivery duration but also results in local high drug concentration that may cause toxic side effects. To enhance chemotherapeutic safety and relieve patient's suffering, it is highly desirable to develop novel implantable carriers with long-term controlled release of anti-TB drug to match with therapeutic period of bone TB.

Isoniazid (INH), one of the most efficacious anti-TB drugs recommended by WHO, possesses many advantages such as high selectivity to mycobacterium tuberculosis, excellent bacteriostatic capacity (minimum inhibitory concentration (MIC) is about 0.05–0.1 μg/mL), low price and good patient compliance [18–20]. Physical encapsulation is a mostly adopted method to fabricate INH delivery system. Since INH is highly aqueous soluble, the approach inevitably results in some drawbacks including low encapsulation efficiency, burst release and short drug delivery duration [17,21–23]. For example, Baoqiang Li and coworkers designed a concentric multi-layered chitosan hydrogel that can enhance loading efficiency of INH to be 14.6%, but all the INH completely released from the hydrogel after 4 h [22]. Kaur and coworkers reported INH-solid lipid nanoparticles with high entrapment efficiency (~69%), however, the drug release duration is only 24 h [23]. Therefore, physical encapsulation approach is not applicable to develop long-term INH delivery system. INH-conjugated polymers linked by a covalently cleavable bond, which elegantly combine stability and stimuli triggered release, would be a promising alternative solution.

Surgical intervention is another necessary strategy for treating serious bone TB [24,25]. Debridement of the niduses that can extremely remove known source of infection is a generally preferable choice, but the surgical debridement inevitably causes bone defects [26,27]. In order to avoid common bacterial infection and rapid propagation of the residual TB germs in the residual cavity, bone repair to fill the cavity is indispensable. Therefore, effective integration of chemotherapy and bone regeneration should be an optimal therapeutic method for bone TB. Tissue engineering scaffold has demonstrated its success in bone regeneration [28–32]. However, to the best of our knowledge, there is no report that any scaffold drug delivery system can simultaneously realize long-term controlled drug release and bone regeneration.

Polymer/ceramics composite scaffolds taking into account both mechanical stability and tissue interactions are of the most promising success for bone repair [33–36]. FDA-approved biocompatible poly(lactide-co-glycolide) (PLGA) with suitable mechanical properties and adjustable degradation time, has been widely used to fabricate tissue engineering scaffolds [37–39]. Calcium phosphate ceramics such as β-tricalcium phosphate (β-TCP) with excellent biocompatibility and osteoconductivity has been examined in terms of suitability as a bone substitute in the clinical setting [33,40,41]. However, pure β-TCP scaffolds are brittle and exhibit slow degradation rates that do not match the growth rate of new bone tissues.

Herein, we reported a composite scaffold drug delivery system constructed by compositing a hydrazone-linked isoniazid conjugated 4-arm poly(lactide-co-glycolide) (PLGA-INH_4) and β-TCP. Since hydrazone bond is acid-labile, pristine INH can be released intactly in weakly acidic surrounding environment resulted from PLGA/β-TCP scaffold degradation [42]. In accordance with the long degradation period of PLGA, INH release is long-term controlled from the scaffold. INH sustained concentrations both in foci and blood are effective to kill mycobacterium tuberculosis, and the INH concentration is higher in foci than in blood as we expect. The INH-loaded scaffold also shows good bone regeneration capacity, as well as the PLGA-OH_4/β-TCP scaffold. Our work demonstrated the INH-loaded composite scaffold combines the merits of localized long-term drug release and bioactivity for bone regeneration, which is a tremendous potential for improved bone TB treatment.

2. Materials and methods

2.1. Materials

Lactide and glycolide were purchased from Purac (Netherlands) and purified by recrystallization from ethyl acetate twice. Stannous octoate (analytical grade) and isoniazid (INH, 99%) were purchased from Sigma–Aldrich. Pentaerythritol (PET, 98%), dicyclohexyl carbodiimide (DCC, 99%), 4-(dimethylamino) pyridine (DMAP, 99%) and 4-carboxybenzaldehyde (97%) were purchased from J&K Scientific Ltd. β-tricalcium phosphate (β-TCP) powders were purchased from the Forth Reagent Factory of Shanghai, China. Solvents and other compounds were obtained from Beijing Chemical Reagents Company, China. All reagents were used as received unless otherwise noted.

2.2. Synthesis and characterization of PLGA-INH_4

PLGA-INH_4 was synthesized following a synthetic route as shown in Fig. 1. Firstly, 4-arm poly(lactide-co-glycolide) (PLGA-OH_4) was obtained via a two-step approach [43,44]. At the first step, a 4-arm PLGA oligomer (O-PLGA-OH_4) was yielded in a typical procedure. Rigorously dried PET (0.31 g, 2.3 mmol), lactide (7.42 g, 51.5 mmol), glycolide (2.56 g, 22.0 mmol) and stannous octoate (0.05 wt% to lactide and glycolide) were introduced into a polymerization tube. After three cycles of freeze–pump–thaw, the tube was sealed under vacuum. Then the tube was immersed and kept in an oil bath thermostated at 170 °C for 20 h. The raw product was dissolved in chloroform, then precipitated into alcohol. The obtained pale O-PLGA-OH_4 was dried under vacuum at 40 °C to constant weight. Yield: 8.42 g (84.2%). Then PLGA-OH_4 was synthesized in a similar way by replacing PET with O-PLGA-OH_4 and the feeding ratio of lactide and glycolide to O-PLGA-OH_4 was 700 to 1. Yield: 9.26 g (92.6%).

Then aldehyde group functionalized PLGA (PLGA-CHO_4) was synthesized as follows. PLGA-OH_4 (10.0 g, 0.1 mmol), 4-carboxybenzaldehyde (1.2 g, 8 mmol), and DMAP (0.24 g, 2 mmol) were dissolved in 200 mL of dichloromethane (DCM) in a 500 mL flask. DCC (1.648 g, 8 mmol) in 50 mL of DCM was added to this flask one time. The mixture was stirred for 24 h at room temperature before it was filtered. The filtrate was precipitated into 1 L of alcohol, then the precipitate was collected and dried under vacuum at 40 °C to yield the PLGA-CHO_4. Yield: 9.21 g (92.1%).

PLGA-INH_4 was synthesized according to the following procedure. PLGA-CHO_4 (4.0 g, 0.04 mmol), INH (0.22 g, 1.6 mmol) were dissolved in 100 mL of *N*,*N*′-Dimethylformamide (DMF) in a 250 mL flask. After purged with argon for 10 min, the flask was immersed and kept in an oil bath thermostated at 80 °C for 5 h. Then the mixture was cooled to room temperature and precipitated into 500 mL of alcohol. The precipitate was collected and dried under vacuum at 40 °C to obtain the PLGA-INH_4. Yield: 3.57 g, (89.3%).

Nuclear magnetic resonance (NMR) spectra of these products were recorded using a Bruker 400 MHz spectrometer. The number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (PDI) of the polymers were determined by gel permeation chromatography (GPC) at 50 °C using an SSI pump connected to Wyatt Optilab DSP and Wyatt DAWN EOS light scattering detectors with THF as the eluent at a flow rate of 1.0 mL/min and a linear PS as standard.

2.3. Fabrication and characterization of the composite scaffolds

The PLGA-INH_4/β-TCP composite scaffolds were fabricated by a particle leaching in combination with phase separation technique [45]. Sieved NaCl particles and β-TCP were added in 9 wt% PLGA-INH_4 solution in dioxane to form slurry with 20/1/1 weight ratio of NaCl/β-TCP/PLGA-INH_4. Then the slurry was cast into a mould, frozen under −20 °C, and lyophilized for 48 h. The porous PLGA-INH_4/β-TCP composite scaffolds were obtained after the NaCl particles as porogen were washed away completely by distilled water and lyophilized for 24 h. In a similar procedure, porous PLGA-OH_4/β-TCP composite scaffolds were fabricated for a control. All the scaffolds were sealed and preserved at 4 °C for use.

Drug loading amounts of INH in the PLGA-INH_4 were investigated firstly. After weighted exactly, PLGA-INH_4 was dissolved in DMF and then pH of the solution was adjusted to 3 using HCl. After the solution was vibrated for 3 h to make the INH release completely, the concentration of INH was determined by measuring the maximum absorbance at the wavelength of 262 nm on a UV-Vis spectrophotometer (Shimadzu UV-1601PC). A calibration curve, the absorption intensity at 262 nm as a function of INH content in DMF in the linear range, was used as the multipoint working curve. Drug loading amounts could be calculated according to the concentration.

The morphology of PLGA-INH_4/β-TCP and PLGA-OH_4/β-TCP composite scaffolds was examined by scanning electron microscopy (SEM; JEOL, JSM-6700F) after the scaffold was coated with gold using a sputter coater (Desk-II; Denton Vacuum Inc.). The averaged diameters of the micropores were measured based on 100 micropores