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Multi-loaded ceramic beads/matrix scaffolds obtained by combining ionotropic and freeze gelation for sustained and tuneable vancomycin release

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ABSTRACT

For a targeted release against bacteria-associated bone diseases (osteomyelitis) ceramic beads with a high drug loading capacity, loaded with vancomycin as model antibiotic, are synthesized as drug carrier and successfully incorporated in an open porous hydroxyapatite matrix scaffold via freeze gelation to prevent bead migration at the implantation site and to extend drug release. We demonstrate that the quantity of loaded drug by the hydroxyapatite and β-tricalcium phosphate beads, produced by ionotropic gelation, as well as drug release can be tuned and controlled by the selected calcium phosphate powder, sintering temperature, and high initial vancomycin concentrations (100 mg/ml) used for loading. Bead pore volume up to 68 mm^3/g, with sufficiently large open pores (pore size of up to 650 nm with open porosity of 72%) and high surface area (91 m^2/g) account likewise for a maximum drug loading of 236 mg/g beads or 26 mg/sample. Multi-drug loading of the beads/matrix composite can further increase the maximum loadable amount of vancomycin to 37 mg/sample and prolong release and antibacterial activity on *Bacillus subtilis* up to 5 days. The results confirmed that our approach to incorporate ceramic beads as drug carrier for highly increased drug load in freeze-gelated matrix scaffolds is feasible and may lead to a sustained drug release and antibacterial activity.

1. Introduction

Bacteria-associated bone infections (osteomyelitis) are characterized by an acute or chronic inflammatory response and often lead to osseous necrosis, bone loss, vascular thrombosis, and joint destruction [1,2]. Osteomyelitis can be caused by nosocomial infections or superinfections following musculoskeletal injuries, trauma, nosocomial infection, or by orthopaedic operations, irradiation, or bisphosphonate-related osteonecrosis of the jaw (BRONJ). The infection can also spread outward from the bone and spread in nearby tissues. The treatment of bacteria-associated bone infections is complex and still considered a major problem in orthopaedics and cranio-maxillo-facial surgery. Bone infections can likely lead to implant failure, mortality, and thus associated with a high economic cost [3–6].

Traditional treatments often include surgical debridement, the removal of the injured tissues, and systemic intravenous antibiotic therapy [1,2]. A systemic administration requires large doses of antibiotics for long periods but remains often unsuccessful because many antibiotics have short half-life and can poorly penetrate in the tissues [3]. The limited blood circulation in the infected area implies that only a fraction of the administered antibiotic dose reaches the target site [4,5,7]. Additionally, prolonged or high dose systemic antibiotic administration presents serious side-effects such e.g. systemic toxicity [8,9]. An inappropriate or delayed infection treatment can also induce an antibiotic resistance and biofilm formation at the implantation site. This in turn leads to a formation of a devascularized surface that protects bacteria from antibiotics and makes their usage counterproductive [10–13].

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An alternative strategy to systemic treatment involves the localized delivery of antibiotics from carrier materials. Insertion of antibiotic containing carriers directly at the site of infection provides higher local antibiotic concentrations than by parenteral administration, and may reduce the risk of systemic toxicity and side effects by lowered systemic concentrations [14–17]. Currently, the clinical standard and most widely employed carrier material consist of poly(methyl methacrylate) (PMMA) in the form of beads or self-setting PMMA bone cements [18–20]. Numerous studies have proven that antibiotic-impregnated PMMA can be efficiently applied for the treatment and prophylaxis of osseous infections [21,22]. However, PMMA is not resorbable and must be removed in a second surgical procedure and the risk of reinfection and increasing patient morbidity are very likely [23,24]. Retention of antibiotic within PMMA is inevitable and only a small percentage of drug is delivered during the functional period of the PMMA implant [25]. Moreover, the monomers required for PMMA polymerization can induce systemic toxicity and the exothermic polymerization reaction can deactivate heat-sensitive antibiotics [26,27].

Alternative carrier materials are biodegradable materials like calcium sulfate and calcium phosphate which are used as resorbable drug delivery systems [14,28–31]. Both materials are resorbable and thus their removal after implantation is not required [31–33], but they feature some drawbacks as e.g. very high resorption rate, quick drug release, and low mechanical strength [26,31,34,35].

In our previous work [36] we show that biodegradable, open porous hydroxyapatite scaffolds can be used as antibiotic depot and release system. Such scaffolds are fabricated using a freeze gelation-based process and antibiotics can be incorporated in the scaffolds in a one-step process without denaturation. However, in these scaffolds only a limited amount of antibiotics can be incorporated as high antibiotic concentrations increase suspension viscosity and scaffold fabrication is hampered.

In this study we present an alternative, versatile route that permits the fabrication of customizable, resorbable open porous calcium phosphate scaffolds with a high drug loading capacity as well as well-controlled, prolonged release. For a targeted release with high drug loading capacity hydroxyapatite and β-tricalcium phosphate beads obtained by ionotropic gelation were loaded with vancomycin and incorporated in an open porous hydroxyapatite matrix scaffold fabricated via freeze gelation. Hydroxyapatite and β-tricalcium phosphate were used due to similar composition to bone and due to their slower resorption and release properties than calcium sulfates [14,15,37].

Vancomycin, a relatively large glycopeptide (MW 1450 Da) and one of the most commonly used drugs for the treatment of infections induced by gram-positive bacteria such as staphylococci and streptococci [38–43] was used as model antibiotic. Vancomycin loading capacity and release were evaluated spectrometrically at 280 nm. The antibacterial activity of the eluents was tested on gram-positive bacterium *Bacillus subtilis*.

Bead loaded scaffolds are envisaged as open porous bone substitute which can be directly inserted in bone cavities or defects. We hypothesised that by embedding antibiotic loaded beads in a porous ceramic matrix antibiotic migration through tissue is prevented and a higher drug concentration at the implantation site can be achieved. The influence of bead incorporation in the scaffold on antibiotic release was assessed likewise spectrometrically.

So far, usually calcium phosphate or calcium sulfate drug carrier can be loaded with drugs either directly during sample preparation, where the drug is mixed with the ceramic [26,28,29,33,35], or the sample is impregnated with the drug afterwards [30,31]. To further enhance the drug loading capacity and to extend drug release even over long time periods, both methods were combined in this study to design multi-loaded beads/matrix composites.

2. Materials and methods

2.1. Materials

Hydroxyapatite (HAP, lot. A3420, specific surface area of 65 m^2/g) powder, β-tricalcium phosphate (TCP, lot. BCBB7609, specific surface area of 1.1 m^2/g) powder, anhydrous citric acid (lot. BCBB7128), concentrated ammonium hydroxide solution (lot. SZBA1400, ≥25%), tris(hydroxymethyl)aminomethane (Tris, lot. MKBD9221V), and agar (lot. 050M0202V, ash 2.0–4.5%) were purchased from Sigma Aldrich (Germany). Calcium chloride dihydrate (lot. BCBK7809V) and hydrochloric acid solution (lot. SZBB2900V, 1 M) were obtained from Fluka (Germany) and tri-sodium citrate dihydrate (lot. 3Z003926) and Mueller-Hinton broth (lot. 2W000933) from AppliChem (Germany). Sodium alginate (lot. 9O008361, viscosity of 350–550 mPas (1%; 20 °C), pH of 5.5–8.0 (1%; H_2O), BioChemica, Germany), ammonia stabilised silica sol with a SiO_2 content of 30% (lot. 0590b, particle size of 5–8 nm, surface area of 230–360 m^2/g, BINDZIL® 30NH3/220, Eka Chemicals, Germany), vancomycin (lot. 172186178, ≥900 I.U./mg, Carl Roth, Germany), ethanol abs. (lot. 15B130503, ≥99.8%,VWR, France) and the gram-positive bacterium *Bacillus subtilis* (*B. subtilis*, DMS cat. no. 1088, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, DSZM, Germany) were purchased from different suppliers as indicated. Double deionised water (ddH_2O) with a conductivity of 0.05 μS/cm obtained from an ultra-pure water system (Synergy System, Millipore, Germany) was used for all studies. All chemicals were utilised as received without further purification.

2.2. Bead preparation

HAP or TCP beads were prepared via droplet extrusion coupled with ionotropic gelation in the presence of calcium ions as described by Klein et al. [44]. Briefly, a ceramic/alginate suspension was prepared by adding the HAP or TCP (15.4 wt.%) stepwise to a water-based suspension containing 0.7 wt.% sodium alginate, 0.2 wt.% sodium citrate and 30.3 wt.% silica sol (Fig. 1A). The suspension was evenly stirred (Dispermat LC-2, VMA-Getzmann, Germany; rotating speed: 1000 r/min) and homogenized for 15 min with an ultrasound horn (Sonifier 450, Branson, Germany; power: 150 W, pulse rate: 0.5 s) to remove possible agglomerates. Subsequently, the ceramic/alginate suspension was dropped with a syringe (5 ml Injekt® Luer Solo; needle diameter: 0.55 mm) in a cross-linking solution consisting of ddH_2O, ethanol (ddH_2O/ethanol ratio: 80/20 v/v) and 0.1 mol/l calcium chloride. Beads were left in the cross-linking solution for 18 h. Afterwards they were washed three times with ddH_2O to remove calcium ions in excess. The beads were shortly frozen for 15 min at −150 °C (Ultra-Low Temperature Freezer MDF-1155, Sanyo Electric Biomedical, Japan) and subsequently freeze dried at −20 °C (P8K-E-80-4 −80 °C, Piatkowski, Germany). Part of the beads was rapidly sintered in a tube furnace (VTF1, Vecstar, United Kingdom) for 5 min at two different temperatures, namely 800 °C and 1200 °C. Beads sintered at 800 °C or at 1200 °C are labelled with the suffix: −800 (−1200), respectively (Table 1). Part of the beads was not sintered (suffix: -ns).

2.3. Drug loading

Concentrated vancomycin solutions with three different initial concentrations (1 mg/ml, 10 mg/ml and 100 mg/ml) were used to load −ns or −800 beads. Due to their small surface size, beads sintered at 1200 °C were not further considered. 1 ml vancomycin solution was mixed with 0.11 g beads and incubated at 37 °C under continuous shaking at 100 r/min (Unimax 1010 with Inkubator 1000, Heidolph Instruments, Germany) to guarantee a homogenous drug load. After 15 min the supernatants were removed. Preliminary kinetic drug loading measurements indicated that the largest vancomycin percentage was loaded in the first 5 to 10 min (see supporting data: Fig. B.1 in the online version at