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Tailored sequential drug release from bilayered calcium sulfate composites

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ABSTRACT

The current standard for treating infected bony defects, such as those caused by periodontal disease, requires multiple time-consuming steps and often multiple procedures to fight the infection and recover lost tissue. Releasing an antibiotic followed by an osteogenic agent from a synthetic bone graft substitute could allow for a streamlined treatment, reducing the need for multiple surgeries and thereby shortening recovery time. Tailorable bilayered calcium sulfate (CS) bone graft substitutes were developed with the ability to sequentially release multiple therapeutic agents. Bilayered composite samples having a shell and core geometry were fabricated with varying amounts (1 or 10 wt.%) of metronidazole-loaded poly(lactic-co-glycolic acid) (PLGA) particles embedded in the shell and simvastatin directly loaded into either the shell, core, or both. Microcomputed tomography showed the overall layered geometry as well as the uniform distribution of PLGA within the shells. Dissolution studies demonstrated that the amount of PLGA particles (i.e., 1 vs. 10 wt.%) had a small but significant effect on the erosion rate (3% vs. 3.4%/d). Mechanical testing determined that introducing a layered geometry had a significant effect on the compressive strength, with an average reduction of 35%, but properties were comparable to those of mandibular trabecular bone. Sustained release of simvastatin directly loaded into CS demonstrated that changing the shell to core volume ratio dictates the duration of drug release from each layer. When loaded together in the shell or in separate layers, sequential release of metronidazole and simvastatin was achieved. By introducing a tunable, layered geometry capable of releasing multiple drugs, CS-based bone graft substitutes could be tailored in order to help streamline the multiple steps needed to regenerate tissue in infected defects.

1. Introduction

Healing of infected bony defects, such as those resulting from periodontal disease, has become a major focus in the field of bone tissue engineering [1]. Onset of periodontitis, a bacterial infection affecting the gingiva, alveolar bone, periodontal ligament, and root cementum, leads to the initiation and propagation of chronic inflammation, eventually causing the destruction of surrounding connective tissue and bone [2–7]. Periodontal infections are first treated with extensive debridement and scaling of plaque [4–6,8]. Some pathogens may not be susceptible to mechanical removal, however, because they often 'hide' deep in gingival pockets around compromised bony tissue [5,9]. These bacteria can frequently trigger reoccurrence of the initial infection, which greatly affects restoration or preservation of lost bone [8]. Antibiotics systemically delivered orally or locally administered using topical gels, creams, or films are then used to eliminate the pathogens prior to implantation of grafting material [1,5,8].

An infected periodontal pocket contains an abundance of microflora, such as *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* [5,10]. These pathogens are often protected within a biofilm, which allows them to flourish and requires a substantially higher dose of an antibiotic to fully eradicate the bacteria [5,10]. Due to clearance in the blood during systemic administration, the resulting low local concentrations of antimicrobial agents at the infected site often do not meet the levels required to kill the microbes [3,6,7,11]. As a result, long-term therapy with high systemic doses of antimicrobial agents may be needed to fully eliminate the infection. However, this treatment could potentially cause adverse effects, such as liver and kidney damage, or lead to drug resistance [12]. Consequently, local administration of drugs directly to the site of infection may prove more effective by providing a higher concentration while using a smaller dose [5,6]. Of the different antibiotics used for dental applications, metronidazole significantly reduces periodontal infection when compared to others [11,13]. In addition, using a biodegradable material capable of controlling the amount of metronidazole released may allow for higher sustained and more effective concentrations to be obtained at the site.

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Following treatment of periodontal infections, regeneration of bony tissue can begin. Bone regeneration using autografts is considered the 'gold standard'. Intra-oral donor sites are limited, however, and harvesting can lead to undesirable donor site morbidity and chronic discomfort [14–28]. Another option is the use of allografts harvested from cadaveric bone tissue. Although frozen, freeze-dried, and/or demineralized, patients receiving the grafts are still at risk of immunologic rejection or disease transmission [28–30]. Allografts have been combined with osteoinductive growth factors to make them more effective [15]. Because of the limitations, and even risks, involved with autogenous and allogeneic materials, much attention has turned to the development of innovative bone graft substitutes. Calcium sulfate (CS) represents a promising alternative [15]. CS becomes osteogenic in the presence of bone, and through dissolution, the material is completely absorbed without inducing a significant inflammatory response [24,31–34]. Like many other synthetic grafting alternatives, however, the efficacy of CS can be further enhanced with the aid of bioactive agents.

A beneficial addition would be the incorporation of statin drugs. Statins are widely known as inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase that help control cholesterol levels, but these pleiotropic drugs also have osteogenic activity [29,35–40]. In vitro and in vivo studies have demonstrated the positive effects of simvastatin through stimulation of osteoblastic activity and inhibition of osteoclastic activity [29,35–39,41]. Simvastatin was shown to be effective when released from a CS matrix in vivo [15].

A bone grafting device capable of sequentially releasing an antibiotic followed by an osteogenic agent may be able to treat infection while still being able to regenerate bone. Little research has investigated the release kinetics of antimicrobial and osteogenic drugs from the same grafting device [1]. In the present study, dual drug-loaded, bilayered CS composites comprising a shell and core geometry with embedded poly(lactic-co-glycolic acid) (PLGA) particles were developed. After evaluating the compressive strength and modulus, dissolution, and morphology of bilayered composites, the tunable sequential release kinetics of metronidazole and simvastatin from the composites were explored.

2. Materials and methods

2.1. Metronidazole-loaded PLGA particles

Poly(lactic-co-glycolic acid) (Durect Corp., Birmingham, AL; 50:50; inherent viscosity: 0.55–0.75 dL/g; carboxylate end group) particles loaded with metronidazole (Sigma-Aldrich, St. Louis, MO) were created by film-casting and hand-grinding. Initially, 25 mg of metronidazole were combined with 200 mg of PLGA and dissolved in 1 mL of dimethyl sulfoxide (DMSO; an FDA Q3C Class 3 solvent). The solution was poured into a circular Teflon mold, frozen quickly at −80 °C, and lyophilized to remove the DMSO. The dried film was hand ground to obtain particle sizes between 150 and 250 μm. A small amount of CS was used to prevent the polymer from sticking during grinding. The particles were washed with ethanol to remove residual CS powder on the surface of the polymer. Ethanol was chosen for washing to prevent drug loss because of the low solubility of metronidazole in this solvent. Washed particles were vacuum-filtered, rapidly air-dried, and stored at −20 °C until used.

A short-term study of metronidazole-loaded microparticles was conducted to determine how much drug may be released during the setting phase of composite formation. For this purpose, 10 mg of washed PLGA particles were incubated at 37 °C in 1 mL of phosphate-buffered saline (PBS), pH 7.4. Supernatant was collected and replaced with fresh PBS every 15 min for the first hour, every 30 min for the 2nd hour, every hour for the 3rd and 4th hour, and finally increased to every 2 h for the 6th and 8th hour time points. Supernatants were filtered (0.45 μm) and the absorbance measured at 318 nm.

2.2. Bilayered calcium sulfate composites

Fabrication of the bilayered composites is illustrated in Fig. 1. The composites consisted of calcium sulfate hemihydrate (Sigma-Aldrich) as the structural matrix. First, blank CS samples without layers were produced by combining 1 g of CS with 800 μL of deionized (DI) water. The slurry was injected into a mold having a diameter of 6.3 mm and a height of 12.6 mm. The loaded mold was placed in a 43 °C oven for 24 h to allow for the CS to completely set.

To begin formation of bilayered composites, cylindrical cores were produced in Teflon molds having a diameter of 4.7 mm and a height of 10 mm. A small, 8 mm long metal peg with a 0.63 mm diameter was fitted precisely in the center of the mold, with about 2.5 mm of the peg embedded into the core. The pegs suspended and centered the cores for shell production later. To make blank CS cores, 800 μL of DI water was added to 1 g of CS powder and mixed thoroughly in 3 mL non-sterile syringes fitted with a 16 gauge blunt needle. The slurry was loaded into the custom-fabricated Teflon mold and placed in a 43 °C oven for 24 h to set the CS. For cores loaded with simvastatin, the same process was used, however 20 mg of simvastatin (Haouri Pharma-Chem, Inc., Edison, NJ) were mixed along with the CS and DI water. Pegs were removed from the cores when they were dried, and the cores were stored at room temperature with desiccant until used.

To form the shell around the cores, another Teflon mold was created with cylindrical holes having a diameter of 6.3 mm and a height of 12.6 mm. The base plate was fabricated with 3.5 mm deep holes into which metal pegs were securely inserted. This depth allowed the cores to be positioned precisely in the center of the mold, thus allowing the shell to surround the core. Blank and simvastatin-loaded shells were created using the same method described above for the cores, where 1 g of CS was mixed with 800 μL of DI water, and in the case of simvastatin-loaded shells, 20 mg of drug were directly added. For samples containing metronidazole-loaded PLGA, the particles were added to both the blank and simvastatin-loaded shells at either 1 or 10 wt.% and then mixed with 850 μL DI water in 3 mL syringes fitted with a blunt-tipped needle for easy, consistent filling of the mold. Using these formulations for the shells, bilayered composites were formed by filling the molds about half full. Next, prefabricated cores were quickly dipped in DI water to wet the surface, which allowed for smooth coverage of the shell slurry around the core, inserted into the mold, and pressed down onto the metal pegs until they stopped. The pegs positioned the cores and held them in place during setting of the shell slurry. The filled mold was placed into a 37 °C oven and allowed to dry overnight. For simplification, the different types of samples were given abbreviated names (Table 1).

The shells and cores described had a volume ratio of 50:50. Two other ratios were tested with simvastatin loaded into the shell only (SSBC), core only (BSSC), or both layers (SSSC). These samples were used to demonstrate how a change in the volume ratio would affect drug release from the composites. Table 2 lists the volume ratios used and the dimensions of the respective core and shell components. Custom molds were created to accommodate the different sizes, but the rest of the fabrication process was the same as described previously.

2.3. Composite microarchitecture

To monitor the distribution of PLGA particles within the CS shell matrix as well as the interface between shell and core, microcomputed tomography (microCT) was employed. Using a Scanco Medical μCT-40 scanner, specimens were evaluated at high resolution. Other parameters were set as follows: 156 μm increments, 0° angle, 70 kVp, 114 μA, 0.5 mm Al filter, and a voxel size of 8 μm. The raw images were qualitatively investigated for particle distribution trends, core orientation, and shell–core interaction. In addition, qualitative and quantitative assessments of the composites were conducted using a built-in 'bone trabecular morphometry' analytical tool with a lower threshold level