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Design of a multiple drug delivery system directed at periodontitis

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ABSTRACT

Periodontal disease is highly prevalent, with 90% of the world population affected by either periodontitis or its preceding condition, gingivitis. These conditions are caused by bacterial biofilms on teeth, which stimulate a chronic inflammatory response that leads to loss of alveolar bone and, ultimately, the tooth. Current treatment methods for periodontitis address specific parts of the disease, with no individual treatment serving as a complete therapy. The present research sought to demonstrate development of a multiple drug delivery system for stepwise treatment of different stages of periodontal disease. More specifically, multilayered films were fabricated from an association polymer comprising cellulose acetate phthalate and Pluronic F-127 to achieve sequential release of drugs. The four types of drugs used were metronidazole, ketoprofen, doxycycline, and simvastatin to eliminate infection, inhibit inflammation, prevent tissue destruction, and aid bone regeneration, respectively. Different erosion times and adjustable sequential release profiles were achieved by modifying the number of layers or by inclusion of a slower-eroding polymer layer. Analysis of antibiotic and anti-inflammatory bioactivity showed that drugs released from the devices retained 100% bioactivity. The multilayered CAPP delivery system offers a versatile approach for releasing different drugs based on the pathogenesis of periodontitis and other conditions.

1. Introduction

Periodontitis is one of the most common inflammatory diseases and is a leading cause of tooth loss in adults [1,2]. It is also related to systemic disorders, such as coronary artery disease, stroke, and diabetes [3,4]. In the initial stages of periodontitis, the onset of bacterial infection is followed by the host response of active and progressive inflammation, leading to resorption and loss of tissue [5]. When periodontitis is well-established, effective therapeutic and surgical intervention is required for the removal of bacterial plaque, control of inflammation, and inhibition of progressive bone loss with subsequent complete repair and regeneration of functional periodontium [3]. One of the most common methods for treating chronic periodontitis involves mechanical debridement of periodontal pockets by scaling and root planning along with effective plaque control to eliminate bacterial infection [6]. Subsequent periodontal regenerative procedures are time-consuming and financially demanding [7], and currently there is no ideal therapeutic approach to completely cure periodontitis and achieve predictable tissue regeneration [3]. Because the progression of periodontitis involves a complex, sequential relationship between infection, inflammation, and tissue loss [8], treatment might be improved by controlled release of multiple biologically active agents in an appropriate sequence [9].

Vyas et al. reviewed controlled drug delivery systems that have been employed for treating periodontal diseases [10]. Some approaches involved localized delivery of antibiotics for elimination of bacterial infection [6,11], while others have addressed inflammation [12,13] or bone resorption [14]. Periodontal regeneration has also been attempted using local delivery of osteogenic agents [15–18]. None of these methods, however, addressed all aspects of the disease to achieve comprehensive treatment.

The present research was aimed at developing an “all-encompassing”, multiple drug delivery system capable of delivering antibacterial, anti-inflammatory, anti-resorptive, and osteogenic agents in the appropriate sequence for potential treatment of periodontitis. Fig. 1 shows a schematic representation of the order

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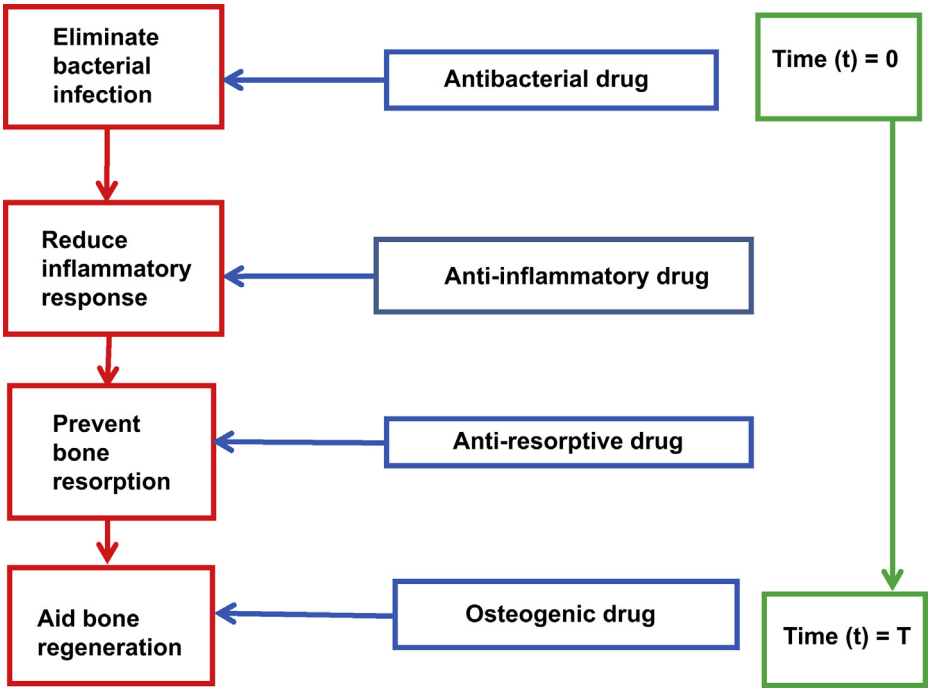

Fig. 1. Proposed sequential drug delivery based on the pathogenesis of periodontal disease.

in which the drugs will be delivered at different stages based on pathogenesis of the disease.

2. Materials and methods

2.1. Fabrication of multilayered devices

Devices were fabricated using a surface-eroding association polymer system (CAPP) comprising cellulose acetate phthalate (CAP) (Sigma–Aldrich, St. Louis, MO) and Pluronic F-127 (P) (Sigma–Aldrich) [19,20]. CAPP films, prepared by a solvent evaporation technique, were used to fabricate the multilayer devices. CAP and Pluronic F-127 were mixed together in the weight ratio of 70:30, respectively, and dissolved in acetone to obtain an 8% polymer solution. The drug of interest (5 wt%) was added to the acetone-polymer solution and mixed thoroughly until the drug was completely dissolved. The drug-polymer solution was poured in a Teflon dish and stored at 4 °C for 24 h for slow evaporation of the solvent. Blank CAPP films were prepared in the same way but without the addition of drugs. For this study, CAPP films were loaded with metronidazole (Sigma–Aldrich), ketoprofen (Sigma–Aldrich), doxycycline (Sigma–Aldrich), or simvastatin (Haorui Pharma-Chem, Inc., Edison, NJ). Samples with diameter of around 6 mm and thickness of 0.5 mm were punched out of the CAPP films. The drug-loaded discs were arranged in the desired sequence with alternating layers of blank CAPP films (Fig. 2). The stack of the CAPP films was bonded together by compressing them after acetone had been applied between the layers. The multilayered device was then coated with poly(sebacic acid) (diacetoxy-terminated; PSA; Sigma–Aldrich), which acted as a barrier to enable unidirectional erosion and drug release. Blank (drug-free) multilayered devices were used for comparison. Three different device designs were investigated for increasing the duration of erosion and release. In addition to the single CAPP blank layers, either two blank layers were used or a thin PSA layer was included between the blank layers.

2.2. Mass loss and drug release

The multilayered devices were eroded in phosphate-buffered saline (PBS), pH 7.4, during incubation at 37 °C with gentle shaking. After collecting the supernatants at regular time intervals, the samples were weighed and then fresh PBS was added. The measured mass of the samples was used to construct the mass loss profiles of the multilayered CAPP devices. Collected supernatants were used to determine the amount of metronidazole, ketoprofen, doxycycline, and simvastatin using high performance liquid chromatography (HPLC; Shimadzu Prominence). For measuring the concentration of ketoprofen, an isocratic mobile phase composed of acetonitrile (60%) and 0.1% trifluoroacetic acid (TFA) in DI (deionized) water (40%) was used with UV detection at 260 nm, and for simvastatin, the isocratic mobile phase was acetonitrile (70%) and 0.1% TFA in DI water (30%) with UV detection at 240 nm. A gradient mobile phase with acetonitrile and 0.1% TFA in DI water was developed for measuring the concentration of metronidazole and doxycycline with UV detection at 318 and 350 nm, respectively.

2.3. Mathematical modeling

Profiles of drugs released from the multilayered CAPP device were evaluated using Hopfenberg's model for controlled release from erodible slabs (Eq. (1)):

$$\frac{M_t}{M_\infty} = 1 - \left[1 - \frac{k_0\, t}{C_0\, a}\right] \tag{1}$$

where M_t is the amount of drug released (mg) at time t (hours), M_∞ the total amount of drug released from the device (mg), k_0 the erosion constant (mg/hr/mm^2), C_0 initial concentration of the drug in the device (mg/mm^3), a the half thickness of the slab, and $n = 1$ for a slab [21]. Because only one side of the CAPP layer (slab) was exposed for polymer erosion and drug release due to the presence of the PSA barrier, the term a (half the thickness of the slab) was replaced with 2a (total thickness of the slab in mm) in Eq. (1). The release profiles predicted using this mathematical model were compared with the experimentally-obtained cumulative release profiles of the four drugs.

2.4. Bioactivity

Bioactivity of released metronidazole or ketoprofen was measured to assess effects of encapsulation and release. The Kirby–Bauer assay was performed to test the antibacterial activity of metronidazole. An aliquot of *Porphyromonas gingivalis* (FDC381) (*P. gingivalis*) culture was uniformly spread on blood agar plates using polystyrene beads. Release supernatant (7 μL) containing metronidazole was added to 7 mm diameter filter paper discs and placed on the *P. gingivalis*-inoculated plates. After 24 h incubation under anaerobic conditions, the plates were imaged, and the area of inhibition (clear zone) around the filter papers was measured using ImageJ software. Results from the release supernatants were compared to the clear zones obtained using serial dilutions of fresh antibiotic to determine the percent bioactivity. A cyclooxygenase (COX) inhibitor assay kit (Cayman Chemical Company, Ann Arbor, MI) was used to determine the bioactivity of the ketoprofen released from the CAPP films. Activity against COX-1 enzyme was measured using the manufacturer's protocol. As for metronidazole, COX inhibition by the release supernatants was compared with that of freshly prepared standard dilutions of ketoprofen to determine the percent bioactivity.

2.5. Statistical analysis

Mass loss and drug release profiles, both experimental and predicted from mathematical modeling, were analyzed by linear regression using

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