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Tailoring antibiotic release for the treatment of periodontal infrabony defects using bioactive gelatin-alginate/apatite nanocomposite films

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ABSTRACT

Local antibiotic delivery is preferred for treating periodontitis due to the benefits of high local drug concentration and minimal side effects. However, commercial polymeric drug delivery systems lack bioactivity and need additional bone grafting to treat the periodontal bone loss. In the present work, calcium deficient hydroxyapatite (CDHA) nanoparticles incorporated gelatin-alginate (GA) films were developed as antibiotic releasing bone substitutes for treating infrabony periodontal defects. CDHA nanoparticles (<50 nm) were incorporated into GA polymer blend films prepared by solvent casting method. The degradation rate and ease of handling were controlled by optimising the concentration of polymer solution and CDHA. The effect of loading methodology on the drug release was studied by adding the antibiotic tetracycline to a) polymer blend, b) CDHA, c) polymer CDHA solution and d) both CDHA and polymer blend. Tetracycline loading on polymer matrix resulted in nearly 100% burst release while an incomplete release (40%) was observed from tetracycline loaded CDHA. Sustained drug release for 10 days suitable for treating severe periodontal infections was obtained on equal drug distribution between the polymer blend and CDHA. The bioactive CDHA incorporated GA composite films can provide sustained tetracycline release for the treatment of periodontal infections.

1. Introduction

Periodontal infections are the major cause of tooth loss in adults. An untreated infection can lead to the destruction of connective tissue attachment and resorption of the alveolar bone around the infected teeth [1]. Infrabony defects are one of the complications of chronic periodontal infection. Treatment for infrabony defects involves elimination of pathogenic microorganisms and regeneration of lost periodontium [2]. Oral administration of antibiotics is regularly employed towards infection control, while bone grafting and guided tissue regeneration are used for periodontal repair/regeneration [1]. Local drug delivery has been viewed as a promising route for delivering antibiotics to periodontium due to various complications of orally administered antibiotics such as poor bioavailability at the site of periodontal infection, high systemic dosage, toxicity and adverse effects [3]. High bioavailability, longer duration of antibiotic activity at the site of infection and low systemic toxicity are few benefits of local drug delivery systems (DDS) [3]. Many local DDS aimed towards sustained antibiotic release have been developed by impregnating drugs in carrier systems [1]. Commercial products like Actisite®, Atridox®, and Periochip® are some of the local DDS currently available for treating periodontal infections [4]. The major drawback of these commercial products is their lack of bioactivity, which necessitates additional placement of bone graft materials. The most viable option towards managing infrabony defects is to develop degradable DDS capable of delivering antibiotics as well as promoting bone repair and regeneration [3,5].

Among various degradable polymers, gelatin and alginate have been widely used for medical applications as films, scaffolds, spheres etc., due to their easy availability and low cost [6–8]. Gelatin is a partially hydrolyzed protein derived from collagen. Gelatin promotes both cell attachment and cell spreading as it contains Arg-Gly-Asp (RGD) sequence which is also present in the extracellular matrix [9]. Sodium alginate is a natural anionic polysaccharide polymer comprising (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomeric blocks [10]. Alginate is also known to improve the mechanical strength when blended with gelatin films [11]. Nevertheless, as with most polymers, alginate and gelatin are non-bioactive.

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An ideal local DDS is expected to provide therapeutically effective drug dosage with a provision for prolonged drug release. In case of periodontitis, a controlled antibiotic release for a minimum of 7 days is necessary to effect any change in the bacterial population [12]. Commercial periodontal DDS are designed to release drugs for the duration of 7 days (Periochip®, Atridox®) to 10 days (Actisite®) [13–16]. It is usually preferred for the carriers to be biodegradable in order to avoid repeat/second surgery for their removal. In addition, periodontal DDS are required to facilitate easy placement and longer retention within the periodontal cavity. Among various formulations such as gels, films, fibers or chips, drug loaded films are highly suitable as local DDS as they are flexible, easily packable and provide controlled drug release [1,17–19]. The anatomy of periodontal pocket permits easy insertion of films which can be trimmed to appropriate sizing to suit the pocket morphology [20]. Film formulations have particularly proven to be ideal DDS for periodontitis as evidenced by various clinical trials [21–23]. A recent clinical study on multi layered films composed of thiolated alginate and carboxymethyl cellulose sodium polymers for intrapocket metformin delivery have shown their effectiveness in the management of moderate periodontitis [21]. Similarly, biodegradable chitosan films for the periodontal delivery of metronidazole and levofloxacin have proved their efficacy by significantly improving the clinical markers of periodontitis such as gingival index, plaque index and pocket depth [23].

Bone defects occur commonly with moderate to severe periodontitis and are treated with bone grafts or synthetic bone substitutes. Among the many bone substitutes, calcium phosphate (CaP) nanoparticles are widely used for bone repair, regeneration and drug delivery due to the structural and compositional similarities to bone mineral [24,25]. CaPs are bioactive bone substitutes capable of not only osteoconduction but also osteoinduction by upregulating the expression of osteoblast marker genes [26]. Among them, calcium deficient hydroxyapatite (CDHA) nanoparticles [$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$; Ca/P = 1.33–1.67] have shown to be ideal DDS for antibiotics like tetracycline and doxycycline [24,27]. The CDHA has high drug loading capability due to its high specific surface area and the drug release can be varied by tailoring its Ca/P ratio [28,29]. However, drug release from CDHA nanoparticles has been observed for 5 days approximately, which is inadequate in case of severe periodontal infections [24,27]. As a possibility of achieving sustained drug release, bioactivity and ease of placement, it would be of interest to develop CDHA polymer composite films as periodontal DDS.

Thus, in this work, we have developed degradable, bioactive gelatin-alginate/CDHA nanocomposite blend films for the management of periodontal infrabony defects. Many process parameters such as chemical structure, porosity, composition, polymer ceramic ratio and degradation rate can affect the drug release from polymer ceramic composites [30–32]. For example, a study on CaP-polycaprolactone composite beads for local drug delivery application has reported the effects of CaP particle size, CaP-polymer distribution and amount of drug loading on the drug release rate [33]. However, to the best of available literature, the effect of different loading techniques on the drug release profile of polymeric composite films has not been studied. Tetracycline, a well-known antibiotic was used to study the drug release properties of the composite films. In order to obtain controlled release profiles, the drug was added to any one or both components (polymeric matrix and/or CDHA) or finally to the prepared nanocomposite solution. The release profile from these composite films was studied under physiologic conditions *in vitro* to observe the relationship between the drug loading method, drug distribution and drug release profiles.

2. Materials and methods

2.1. Materials

Analytical grade calcium hydroxide $Ca(OH)_2$, calcium chloride ($CaCl_2$), diammonium hydrogen phosphate $(NH_4)_2HPO_4$, ammonia (30% GR) and gelatin (CAS No.9000-70-8) were purchased from MERCK, India. Sodium alginate (CAS No. 9005-38-3) was purchased from ACROS Organics, USA. Tetracycline was purchased from Sigma Aldrich.

2.2. Synthesis of gelatin-alginate (GA) polymer blend film

The solvent casting method was used to prepare the polymer films at room temperature as it provides freedom to choose heat labile antibiotics. 0.5, 2 and 4 wt % gelatin solutions were prepared by dissolving 0.5, 2 and 4 g of gelatin respectively in 100 ml of deionized water. 100 ml of 4 wt % alginate solution was prepared by dissolving 4 g of sodium alginate in deionized water. The gelatin and alginate solutions were mixed at a ratio of 70:30. The resulting solution was poured into a petri dish and a polymer blend film was formed after solvent evaporation. The films were cross-linked by adding 10 ml of 5% $CaCl_2$ solution. The cross-linked films were stored in a freezer after drying at room temperature. Table 1 lists the coding and composition of the prepared polymer blend films.

2.3. Synthesis of GA-CDHA (GAC) composite films

CDHA nanoparticles were prepared by microwave accelerated wet chemical synthesis as described in our previous report [28]. Briefly, $Ca(OH)_2$ and $(NH_4)_2HPO_4$ solutions were taken as the precursors to synthesize CDHA nanocarriers of Ca/P ratios of 1.61. The $(NH_4)_2HPO_4$ solution was added dropwise with regular stirring into the $Ca(OH)_2$ solution while the pH was maintained at 10.5 using ammonia. After complete mixing, the solution was subjected to microwave irradiation in a microwave oven (BPL, India) of 800 W for 30 min at 60% power. The precipitate was washed thrice with distilled water to remove the impurities, dried in the oven at 100 °C and ground to a fine powder with a mortar and pestle. CDHA was added to the chosen GA polymer blend film at different weight percentages (1–20 wt/vol. %). CDHA nanoparticles (20, 10, 7, 5, 4, 3, 2 and 1 g) were added to 100 ml of the GA solution followed by 2 h of stirring. The resulting solution with well dispersed CDHA nanoparticles was poured in a petri dish and dried at room temperature to cast a polymer ceramic film. The films were then cross-linked, dried and stored similar to GA films. The coding and composition of GAC films are listed in Table 2.

2.4. Swelling ratio and degradation studies of GA and GAC films

The GA polymer blend films were cut to strips of similar weight and immersed in 15 ml simulated body fluid (SBF) at a pH of 7.4. At regular time intervals, the films were removed and their wet weight was determined. The films were then oven dried at 45 °C for 15 min and the dry weight was measured. The experiment was continued until no further swelling of films was observed. The

Table 1
Compositions of various GA polymer blend films.

Film Code	Gelatin	Alginate	G:A ratio
GA 1	0.5%	4%	70:30
GA 2	2%	4%	70:30
GA 3	4%	4%	70:30