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In vitro studies on controlled-release cellulose acetate films for local delivery of chlorhexidine, indomethacin, and meloxicam

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Abstract

Background: Delivery of medications into periodontal pockets to suppress or eradicate the pathogenic microbiota or modulate the inflammatory response, thereby limiting periodontal tissue destruction, has attracted significant interest with the purpose of effective periodontal treatment. However, no study has previously attempted to develop a controlled-release formulation of anti-inflammatory agents to be used in the field of periodontology. The aim of the present study was to examine the in vitro release profile of chlorhexidine gluconate, indomethacin, and meloxicam from cellulose acetate films.

Methods: Cellulose acetate films containing chlorhexidine gluconate, indomethacin, and meloxicam were prepared and cut in a form to fit to the periodontal pocket anatomy. The release of active agents was studied in 10 ml artificial saliva at 37°C. Apparatus Vibrax was used at 150 r.p.m. Determinations were carried out spectrophotometrically and the release profiles were plotted as a function of time.

Results: The formulations showed two different release patterns for a total observation period of approximately 120 h. When the formulations of the three active agents were compared, the release patterns of meloxicam and chlorhexidine gluconate were found to be similar, while the indomethacin-containing formulation exhibited the fastest release rate.

Conclusions: As a conclusion, cellulose acetate may be a suitable inert material for obtaining a prolonged local release of various anti-inflammatory agents like meloxicam. Further in vitro and in vivo studies are required before starting clinical applications of these controlled-release formulations of anti-inflammatory agents.

Key words: cellulose acetate films; chlorhexidine gluconate; controlled drug delivery; indomethacin; meloxicam; periodontal disease; rate measurements

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Periodontal disease is a general term encompassing several pathological conditions such as chronic periodontitis, aggressive periodontitis, systemic disease-associated periodontitis, and necrotizing periodontitis (Armitage 1999). The clinical signs of periodontitis include changes in the morphology of gingival tissues such as oedema, redness, reduction in consistence, and bleeding upon probing as well as loss of attachment and periodontal pocket formation. Periodontal pocket provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria (Marsh 1989). The microflora present in periodontal pockets is complex in nature and is mainly composed of Gram-negative anaerobic bacteria (Haffajee & Socransky 1994). The destruction of the periodontium is a result of interactions between a complex subgingival bacterial population and specific host defence mechanisms. Suspected periodontal pathogens have been shown to produce a large number of biologically active molecules that may act directly on host tissues. Subsequent production of various inflammatory and immune mediators by the host may cause further tissue destruction.

The main therapeutic approaches for periodontal diseases include mechanical scaling and root planing, thereby removing the bacterial deposits from the tooth surface and shifting the pathogenic

microbiota to one compatible with periodontal health. However, the pocket anatomy is a significant limiting factor, because mechanical access may not always be possible. Because of the complex anatomy of the roots and the contours of the lesion, the mechanical conventional periodontal treatment alone may not be effective, and sufficient reduction of the bacterial load may not be provided to make the tooth surface biologically acceptable. Moreover, the success of mechanical periodontal treatment is closely related to the patient's performance of daily plaque control. Recurrent periodontal tissue destruction is almost inevitable in patients who fail to achieve an acceptable plaque control during the active treatment or maintenance phase of periodontal therapy.

Adjunctive administration of systemic antimicrobials has been useful in treating recurrent periodontal pockets (Slots 1979, Lundström et al. 1984, Gusberti et al. 1988). However, the doses necessary to achieve sufficient local concentrations of antimicrobials in the periodontal environment might be associated with undesirable side-effects. Local administration, therefore, may be considered as an alternative to overcome these problems. The site specificity of periodontal disease also suggests that local predisposing factors must come into play.

Chlorhexidine is an antifungal and antibacterial agent, usage of which in dentistry is well documented. Local administration of chlorhexidine has been demonstrated to be effective in periodontal treatment (Soskolne 1997, Goffin 1998, Heasman et al. 2001). Its mechanism of action relates to reduction in pellicle formation, alteration of bacterial adherence to teeth, and an alteration of bacterial cell walls, causing lysis (Fiorellini & Paquette 1992), where its long-term efficacy is dependent on the duration of exposure.

On the other hand, non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin have been reported to induce promising results in periodontal treatment by reducing the inflammatory destruction (Jeffcoat et al. 1988, Williams et al. 1991, Reddy et al. 1993). However, the systemic usage of classical NSAIDs has limitations because of their adverse effects arising from the inhibition of cyclooxygenase-1 (COX-1), while selective COX-2 inhibitors have very little side-effects if any, because of their preferential inhibition of COX-2 rather than COX-1. Meloxicam is a new NSAID of the acidic enolic class preferentially inhibiting COX-2 enzyme and is not only a potent inhibitor of acute exudation but also an intense inhibitor of bone and cartilage destruction (Engelhardt 1996). Recently, meloxicam was reported to be effective in preventing alveolar bone loss in experimental periodontitis in rats with a better risk/benefit ratio than non-selective COX inhibitors (Bezerra et al. 2000).

The important factor in the success of local antimicrobial/anti-inflammatory drug administration is the ability to control and to prolong the release of the therapeutic agent from the delivery device. The controlled-release system is expected to maintain effective concentrations of drugs at the site of action for longer periods. Various techniques are being developed for local drug delivery into the periodontal pocket because the periodontal pocket is relatively inaccessible.

To our knowledge, there is no published study on the development of a controlled-release formulation consisting of anti-inflammatory agents. Therefore, the present study was planned to develop a controlled-release formulation using cellulose acetate polymer as a drug carrier material and to evaluate the release rates of different active agents: chlorhexidine gluconate, indomethacin, and meloxicam.

Material and Methods

Preparation of drug-loaded films

The cellulose acetate films containing chlorhexidine gluconate, indomethacin, or meloxicam were prepared by the solvent evaporation technique. Three hundred and fifty milligrams of cellulose acetate was dissolved in 5 ml acetone and 350 mg of either of the active agents: chlorhexidine gluconate, indomethacin, or meloxicam, was dispersed in the cellulose acetate (Sigma Chemical Co., St Louis, MO, USA) solutions. These mixtures were poured on Teflon (Horoz Polyester Co., Izmir, Turkey) plates (5.5 × 17 cm) and evaporated at room temperature overnight. The films were then cut in a desired form with the aid of a cutting machine so that they would fit to the periodontal pocket anatomy. The schematic representation of the preparation technique is shown in Fig. 1. The mean drug content

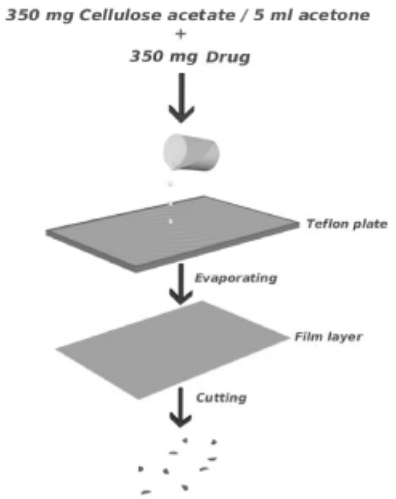

Fig. 1. Scheme of preparation of drug-loaded cellulose acetate films.

of the films was 1 mg for chlorhexidine gluconate, 2.05 mg for indomethacin, and 2.25 mg for meloxicam. Chlorhexidine gluconate, indomethacin, and meloxicam were kindly provided as gifts by Nobel Drug Industry (İstanbul, Turkey), Deva Drug Industry (İstanbul, Turkey), and Drogsan Drug Industry (Ankara, Turkey), respectively. All other reagents used in the present study were of analytical grade.

The release studies

The release studies were carried out in 10 ml artificial saliva prepared at 37°C at 150 r.p.m. according to the formula by Kawai et al. (1997). For this purpose, apparatus Vibrax (IKA-VXR, Staufen, Germany) was used. Samples were taken at pre-determined time intervals t (hour) for chlorhexidine: 0, 1, 2, 3, 4, 5, 7, 8, 24, 48, 55, 119; t (hour) for indomethacin: 0, 1, 2, 3, 4, 5, 7, 8, 24, 25, 27, 29, 31, 32, 47, 48, 49, 51, 53, 54, 118; t (hour) for meloxicam: 0, 1, 3, 4, 5, 6, 7, 8, 24, 27, 29, 31, 32, 47, 48, 49, 51, 53, 54, 118. Determinations were carried out spectrophotometrically (Shimadzu UV-1208, Kyoto, Japan). The released amounts of chlorhexidine gluconate, indomethacin, and meloxicam were determined at 362, 320, and 254 nm, respectively, and the release profiles were plotted as a function of time. The results were expressed as the mean values of three experiments for formulations containing chlorhexidine, indomethacin, and meloxicam, respectively.

Results

The cellulose acetate films containing chlorhexidine gluconate, indomethacin, or meloxicam were all stable at room temperature. The results of in vitro release studies of the drug-containing films in artificial saliva are shown in Figs 2 – 4. The formulations exhibited two different release patterns for a total observation period of 119 h for chlorhexidine, 118 h for indomethacin, and meloxicam. The release results were evaluated in two parts (0 – 8 and 24 – 119 h). The formulations had bimodal release patterns. The first release pattern was faster than the second one. The release results were applied to the zero-order kinetic model and two different release rates were calculated. The chlorhexidine formulation exhibited a release rate of 0.025 mg/h during the first 8 h (Fig. 2), and 0.0017 mg/h during the period between 24 and 119 h. The release results showed that 14.5% of chlorhexidine was released in 6 h from the present cellulose acetate formulation. Approximately 40% of chlorhexidine was released at the end of the 119 h observation period.

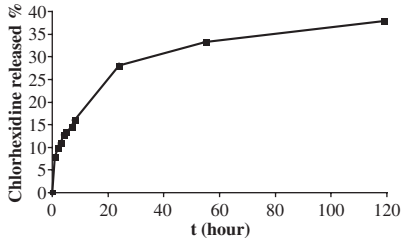

Fig. 2. Release of chlorhexidine from cellulose acetate films ($n = 3$).

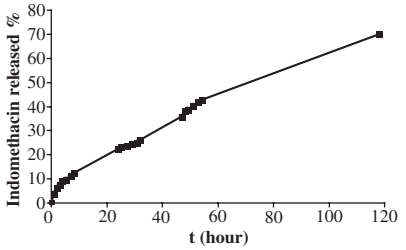

Fig. 3. Release of indomethacin from cellulose acetate film as a function of time ($n = 3$).

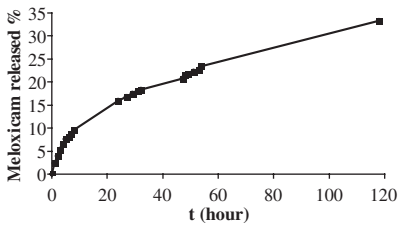

Fig. 4. Release of meloxicam from cellulose acetate film as a function of time ($n = 3$).

The fastest release rates were observed with indomethacin-containing formulations (Fig. 3). The release rates were found to be 0.0222 mg/h during the first 8 h and 0.0105 mg/h during the period between 24 and 118 h. Approximately 70% of the active agent was released from the films.

The release of meloxicam was more rapid during the first 8 h (Fig. 4). The release rate was calculated to be 0.02 mg/h. Then, the drug release slowed down and was found to be 0.0037 mg/h during the period between 24and 118 h. It was found that approximately 30% of meloxicam was released at the end of this release study.

Discussion

A local delivery device in the form of either non-resorbable or bioabsorbable matrices consists of a drug reservoir and a limiting element that controls the rate of medicament release. The present study was planned to develop a controlled-release formulation of some selected antimicrobial/anti-inflammatory agents to be used in the periodontal pocket as an adjunct to the conventional periodontal treatment. As a first step, the cellulose acetate films containing chlorhexidine gluconate, indomethacin, and meloxicam were prepared using cellulose acetate polymer by the solvent evaporation technique. The in vitro release profiles of these three formulations were then observed for a period of 120 h. The use of polymeric drug delivery devices in dentistry is a relatively new area of research, although cellulose acetate is a widely used matrix-forming material for controlled-release formulations (Papadokostaki & Petropoulus 1998, Picker & Bikane 1998).

Most of the intrapocket controlled-release devices like the one we used in the present in vitro study are composed of non-degradable matrixes (Goodson et al. 1983). The main benefits of a controlled delivery device include: improved patient compliance, improved pharmacokinetics, improved drug access to the site of disease, and lower total drug dosage (Goodson 1989). Furthermore, the physical characteristics and working properties of the delivery system may influence the degree of acceptance by the professional community as well as the patient population. The present study indicates that multiple therapeutic agents show a sustained rate of drug release from cellulose acetate films over extended periods of time.

There is substantial evidence that the main inflammatory mediators derived from host cells acting in periodontal tissue destruction are also synthesized locally from the periodontal tissues (Garito et al. 1995). Therefore, it is important to obtain sufficient gingival crevicular fluid concentrations of anti-inflammatory agents to be able to control the excess production of inflammatory mediators. Most of the previous in vitro studies on release of antimicrobials from local devices have investigated the materials in water (Addy et al. 1982, Goodson et al. 1983). To date, there is no communication on a formulation for artificial gingival crevicular fluid, and considering the biochemical similarities as well as direct contacts between gingival crevicular fluid and saliva, we chose artificial saliva as the drug release medium.

In controlled-release systems, it is generally difficult to obtain a constant release rate. In the present study, the release rate was rapid in the beginning period and maintained the loading dose. Later on, slower release rates were observed, but again maintained sustaining dose of the active agent. The difference between release rates obtained from the three drug formulations developed in the present study may be important in periodontal treatment. When the three formulations used in the present study were compared, the releases of chlorhexidine and meloxicam were similar, while the fastest release was observed with the indomethacin-containing formulation. Therefore, the indomethacin formulation does not seem to be promising much success, because most of the active agent was released rapidly.

Chlorhexidine is a well-documented and widely used antimicrobial agent in periodontal treatment and it has wide-spectrum antibacterial activity encompassing Gram-positive and Gram-negative bacteria, yeasts, dermatophytes, and some lipophilic viruses. The antibacterial mode of action is explained by the fact that the cationic chlorhexidine molecule is rapidly attracted by the negatively charged bacterial cell surface. After adsorption, the integrity of the bacterial cell membrane is altered,

which results in a reversible leakage of bacterial low-molecular-weight components at low dosage or more severe membrane damage at higher doses. Moreover, chlorhexidine has the advantage of prolonged supragingival substantivity because it can bind to the intraoral soft and hard tissues.

A biodegradable chlorhexidine chip has been introduced enabling slow subgingival release of chlorhexidine and maintaining an average concentration of more than 125 μg/ml in the crevicular fluid over 7–10 days, which would inhibit 99% of the gingival bacteria in in vitro conditions (Quirynen et al. 2002). In a previous study, we have evaluated the release of biodegradable Periochip® (PerioChip, Peno Products Ltd, Jerusalem, Israel) in artificial saliva (Buduneli et al. 2001a). When the present release results were compared with the release profile of commercially available chlorhexidine formulation: Periochip®; 96% of chlorhexidine was released from Periochip® in 6 h, while this rate was 14.5% for the present cellulose acetate formulation. Therefore, it was seen that the new formulation released chlorhexidine more slowly than Periochip® and these results indicated that this new periodontal delivery device may be used as an adjunct in the treatment of periodontal diseases.

Two isoforms of COX catalyse the synthesis of prostaglandins from arachidonic acid: COX-1 and COX-2. Prostaglandin E_2 (PGE_2) not only induces vasodilatation and capillary permeability but also stimulates osteoclastic bone resorption (Rifkin et al. 1980). Elevated levels of PGE_2 have been detected in gingivae and gingival crevicular fluid of patients with chronic periodontitis (Offenbacher et al. 1986). Selective COX-2 inhibitors are likely to exhibit a better clinical risk/benefit ratio than the classical NSAIDs (Engelhardt 1996). Selective COX-2 inhibitors have attracted great interest in the field of periodontal treatment because of their good effectiveness as well as good tolerance. Adjunctive use of either systemic or local NSAIDs provides additional reductions in both alveolar bone resorption and gingival inflammation (Jeffcoat et al. 1988, Reddy et al. 1993). However, the side effects of the non-selective NSAIDs prevent their routine and long-term application in periodontal treatment. Many of the side-effects limit the usage of non-selective NSAIDs. COX-1 plays a role in platelet aggregation, haemostasis, and the protection of gastric mucosa. COX-2 is a crucial mediator of pain, inflammation, and fever. Non-selective NSAIDs inhibit PGs, broadly inhibiting both COX-2 and COX-1 isoenzymes, but coxibs inhibit only COX-2 (Sinatra et al. 2002). NSAIDs' toxic effects are mostly caused by COX-1 inhibition in the stomach, kidney, and platelets. COX-2 inhibitors were developed for the treatment of inflammation while avoiding adverse reactions caused by COX-1 inhibition, especially in the gastrointestinal system (Osiri & Moreland 1999). These agents offer potentially significant advantages because of their relative lack of gastrointestinal tract irritation. Because of this, these medications are likely to be frequently used in the management of dental and medical conditions (May et al. 2001). Recently, we have used systemic meloxicam as an adjunct to scaling and root planing in the treatment of chronic periodontitis patients and observed promising inhibitory effects of meloxicam on the gingival crevicular fluid levels of interleukin-6 (Buduneli et al. 2001b) as well as matrix metalloproteinase-8 (Buduneli et al. 2002).

COX-2 can be considered as a rate-limiting enzyme in the PG biosynthetic pathway. The continued expression of COX-2 over time could contribute to a subclinical inflammatory state leading to progressive attachment loss (Zhang et al. 2003). Oral rinse formulations of NSAIDs have been developed to be used in periodontal treatment in an attempt to avoid the gastrointestinal side-effects associated with systemic use of non-selective NSAIDs (Kelm et al. 1996). However, oral rinse formulations are far from providing prolonged effects and only a controlled-release local delivery formulation can sustain the active anti-inflammatory agent in the periodontal pocket, while overcoming the systemic side-effects. On the other hand, it is reasonable to think that different species of periodontal pathogens will stimulate COX-2 expression to varying degrees. Therefore, a controlled release local delivery system of an anti-inflammatory agent either administrated alone or in combination with an antimicrobial agent could play a valuable role in periodontal therapy.

The initial peak concentration of chlorhexidine in gingival crevicular fluid was reported to be 2000 μg/ml, when used in situ (Soskolne et al. 1998). The concentration of the drug remains above the minimum inhibitory concentration for more than 99% of periodontal pocket flora for up to 9 days (Stanley et al. 1989). At present, we do not know the optimal gingival crevicular fluid concentrations of meloxicam. The next step, therefore, should be determination of the optimal gingival crevicular fluid concentrations of meloxicam to be effective as a controlled-release formulation in the periodontal pocket.

To our knowledge, this is the first study aimed at the development of controlled-release formulations of indomethacin and meloxicam suitable for use in the periodontal pocket. If and when successful controlled-release formulations of anti-inflammatory agents are developed, the inflammatory cytokines and enzymes derived from the host cells can be effectively controlled, eventually controlling periodontal tissue destruction.

In conclusion, it was observed that cellulose acetate is a convenient inert material for obtaining a prolonged drug release. Cellulose acetate films containing chlorhexidine may be a new alternative for the commercially available Periochip® only after obtaining results from advanced clinical studies. Meloxicam, on the other hand, is a selective COX-2 inhibitor and as yet, there exists no local delivery product of meloxicam in the market. COX-2 represents a valid pharmacologic target for the treatment and prevention of periodontitis and therefore, designing a new controlled-release formulation that contains meloxicam deserves significant attention. Further in vitro and in vivo studies will be performed before starting clinical applications of these formulations.

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