A New Bioerodible Polymer Insert for the Controlled Release of Metronidazole

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This study evaluates a new class of bioerodible polymers as periodontal inserts for the controlled release of metronidazole. The system is based on association polymers formed from compatible blends of cellulose acetate phthalate (CAP) and a hydrophobic block copolymer of polyoxyethylene and polyoxypropylene, Pluronic L101. In addition to characterizing these polymers by thermal analysis, their erosion and metronidazole release characteristics were determined both *in vitro*, and *in vivo* using a rat model. The results show that increasing the concentration of Pluronic L101 in the blend to 50% and above leads to a sharp reduction in the rates of polymer erosion and metronidazole release. The characteristics of these slowly eroding films are potentially suitable for use as periodontal drug inserts with an effective duration of up to several days. Depending on the blend composition, the mechanism of metronidazole release was found to range from a surface erosion-controlled process to an erosion-modulated diffusion process. In all *in vivo* experiments, no signs of adverse tissue reactions were detected. Based on these results, prototype delivery inserts were designed and subsequently evaluated in volunteer patients. Preliminary results from this pilot study show that the metronidazole concentration in the gingival crevicular fluid was significant throughout the sampling period of up to 3 hr and remained well above the minimum inhibitory concentration for most periodontal pathogens. In addition, no discomfort or irritation was reported by the test subjects.

KEY WORDS: bioerodible association polymer; metronidazole; cellulose acetate phthalate (CAP); Pluronic L101; periodontal pocket insert; controlled drug delivery.

INTRODUCTION

Pathogenic bacterial flora associated with deep periodontal pockets are believed to be the principal etiological factor in destructive periodontitis. Treatment strategies are generally based on their abilities to induce a shift in microflora comparable to that observed around healthy teeth. Periodontal disease has traditionally been treated either by physical methods or by the administration of systemic or topical antimicrobials. Conventional physical methods include mechanical debridement of the tooth surface, root planing and root scaling. These treatments show fairly high rates of disease recurrence and problems in motivating the patient to maintain good oral hygiene. For patients who fail to respond adequately to conventional mechanical procedures, adjunctive use of systemic antibiotics are usually employed. Systemic antibiotics show efficacy in controlling subgingival flora, however, they produce peak plasma antibiotic concentrations which may be associated with various side effects such as diarrhea, dizziness, headaches, nausea, tooth staining as well as drug interactions with alcohol (1). In addition, the plasma antibiotic concentration often declines rapidly to subtherapeutic drug levels requiring frequent daily administration. In contrast, local drug delivery can provide an effective concentration of antibiotics for the desired duration of treatment with minimal systemic drug levels.

Existing approaches for local drug delivery in the periodontal pocket are often unsatisfactory due to their rapid drug release and/or poor biodegradability of the polymeric carrier (2). Therefore, the need to remove the depleted devices after treatment presents a major drawback. In addition, the presence of nondegradable or slowly degradable systems for prolonged periods of time, potentially may cause irritation in the periodontal pocket. To be useful for periodontal therapy, it is desirable to have a bioerodible drug delivery system that can maintain an effective drug release rate in the periodontal pocket while simultaneously eroding throughout the duration of treatment up to several days.

Recently, a new bioerodible association polymer system with erosion periods of hours to days has been reported (3-5). These polymers are based on molecular association through intermolecular hydrogen bonding between a proton-donating carboxylic acid polymer and a proton-accepting ethoxylated non-ionic surfactant. They can range from totally insoluble at low pH, where drug release is diffusion-controlled, to erodible at physiological pH, where drug release is primarily erosion-controlled. The present study was designed to investigate the *in vitro* and *in vivo* characteristics of polymer erosion and metronidazole release from a hydrophobic association polymer system based on blends of cellulose acetate phthalate (CAP), a carboxylic acid polymer, and Pluronic L101, a hydrophobic block copolymer of polyoxyethylene and polyoxypropylene. The feasibility of applying these results to the design of a periodontal insert for the local delivery of metronidazole was subsequently investigated in a pilot clinical study, which was designed to evaluate the safety and short term release behavior of such inserts in patients.

MATERIALS AND METHODS

Materials

Cellulose acetate phthalate (CAP) was kindly donated by Eastman Chemicals (Kingsport, TN). The CAP was further purified by precipitation from acetone and washed several times with distilled, deionized water to remove any low molecular weight extractables. After drying, phthalyl and free acid content was determined according to USP methods. Purified CAP used in these studies had an average phthalyl content of 34% (w/w) and contained less than 0.5%

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(w/w) free acid. These values are well within the USP specifications. Pluronic L101 (average MW = 3800 with 10% repeating ethylene oxide units), was obtained as a gift from BASF (Parsippany, NJ) and used without any further purification. Metronidazole (free base) was purchased from Sigma (St. Louis, MO). All reagents and organic solvents used in the HPLC assay of metronidazole were of HPLC grade and were obtained from Fisher Scientific (Fair Lawn, NJ). All other reagents were analytical grade.

Sample Preparation

CAP and Pluronic L101 were dissolved in acetone containing a trace amount of water to form 50/50 or 30/70 blends. Metronidazole was also incorporated to give a 10% drug loading in the resulting dry sample. Film samples were produced using a multiple casting technique on a glass plate. The films were dried in a vertical flow fume hood for 24 hr and then vacuum dried for an additional 24 hr to remove any residual solvent. Subsequently, the samples were carefully separated from the glass and stored at room temperature in a dessicator prior to use. The film thickness ranged from 0.29 - 0.34 mm.

Differential Scanning Calorimetry (DSC)

The association polymer films were analyzed for their glass transition temperatures (Tg) using a Perkin-Elmer Model DSC-2 differential scanning calorimeter (Norwalk, CT) equipped with a dual-stage cooler (Intercooler II) and a thermal analyses data station (TADS). DSC thermalgrams were obtained for 30/70 and 50/50 CAP/Pluronic L101 blends as well as for pure CAP, using a scanning rate of 10 K/min over a temperature range of 210 - 500 K.

In Vitro Dissolution Analysis

The polymer erosion and drug release rates were determined using a rotating-disk apparatus as depicted in Fig. 1. The rotating-disk apparatus consists of a modified Hannson dissolution apparatus (Northridge, CA) with the paddles replaced by rotating sample holders. The UV absorbance of CAP and metronidazole were monitored continuously at 240 and 320 nm, respectively, using a quantition software package on a HP8452 Diode Array Spectrophotometer (Fort Collins, CO). Drug loaded polymer discs 1.74 cm in diameter and approximately 0.3 mm thick (mean dry weight = 77.9) were die cut from the polymer films and mounted in the sample chamber. During a typical experiment, the disc was rotated at 60 rpm in 400 ml of pH 7 Sørenson isotonic sodium phosphate buffer. In all cases triplicate runs were carried out and the results averaged.

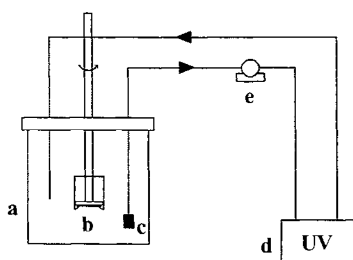

Fig. 1. Schematic representation of the rotating-disk apparatus for studying polymer erosion and drug release. a, water bath; b, sample holder in cross-sectional view; c, filter; d, flow-through cell and UV-vis spectrophotometer assembly; e, peristaltic pump.

In Vivo Polymer Erosion and Drug Release Analysis

Polymer implants containing 10% metronidazole were prepared from CAP/Pluronic L101 films with 50/50 and 30/70 polymer blend ratios. The implants of 1 mm wide, 10 mm long, 0.3 mm thick with a mean dry weight of 3.46 mg were prepared and injected subcutaneously into the back of 8 anesthetized (2.86 mg/100 g i.p. of sodium pentobarbital) Sprague Dawley rats (average weight = 350 g) using an 14 gauge cannula disinfected with a 70% ethanol/water solution. This site was chosen because it allows the implants to be exposed to an *in vivo* environment supplied with a limited amount of interstitial fluid similar to that found in diseased human GCF (both resemble a physiological plasma exudate) (6). A total of 6 implants were placed in each rat. Prior to implant injection, the rat's dorsal torso was shaved and the dermis disinfected with a 70% ethanol solution. At predetermined time periods the rats were sacrificed with sodium pentobarbital and the dorsal skin layers were cut away to expose the polymer implants. The implants were retrieved and wiped free of blood, dried first at room temperature for 24 hr and then in a vacuum oven for an additional 24 hr. Their weights were then determined and the percentage of implant remaining was calculated on a gravimetric basis. To determine the percentage of metronidazole remaining in the implants, the dried samples were extracted with 3 ml of a 1:1 methanol:water solution for 24 hr on a Fisher Scientific hematology mixer (Ivyland, PA). The metronidazole concentration in the extracting solvent was subsequently determined at 320 nm using the UV-visible spectrophotometer and the amount of drug remaining was determined.

Pilot Clinical Study

The pilot clinical study was conducted at the Graduate Clinic of the Faculty of Dentistry, University of Toronto and was based on a protocol approved by the Human Subjects Review Committee at the University. For the determination of clinical parameters relevant to the performance of the present bioerodible association polymer, prototype periodontal inserts were produced from 30/70 CAP/Pluronic L101 polymer films containing 10% (w/w) metronidazole. These film samples were prepared in a chemically disinfected laminar flow environment and were allowed to air dry for 24 hr before being dried for an additional 6 hrs in a slab gel dryer (Hoeffer Scientific Instruments Model SE 1160; San Francisco, CA). Periodontal inserts were die cut from the films using a sterilized, stainless steel "dumbbell" shaped punch and stored in a vacuum dessicator until clinical insertion. The drug containing inserts (mean dry weight = 7.6 ± 0.20 mg) were placed in at least six sites of 8 volunteer patients exhibiting periodontal pocket depths of ≥ 6 mm. Two inserts were retrieved from each patient after 60, 120, and 180 min respectively, during which time the patients abstained from eating and drinking (7). The crevicular fluid flow in the periodontal pocket was determined using a Interstate Periotron 6000 (Amityville, NY) both before placement

and after removal of the inserts. The retrieved inserts were dried and their erosion rates were determined gravimetrically. The amount of metronidazole remained in the inserts was determined by the methanol extraction technique described in the previous section. Crevicular fluid samples were obtained for the above mentioned time periods and the metronidazole concentration was determined using a modified HPLC assay (8). The chromatographic analysis was performed using a Perkin-Elmer Series 2 liquid chromatography, a Shimadzu variable wavelength detector set at 312 nm, a 023 linear recorder and a 5 micron C18 reverse phase Ultrasphere ODS column (4.6 mm ID × 150 mm) (Beckmann Instruments, Toronto, ON). The mobile phase consisted of acetonitrile, methanol and 10 mM potassium dihydrogen phosphate (pH = 3.0) in a ratio of 3%, 4% and 93% respectively and was delivered at a rate of 1.5 ml/min at room temperature. An external standard technique was utilized to determine the unknown metronidazole concentrations.

RESULTS

Association polymer samples prepared in the present study were transparent without drug loading, however, the 10% metronidazole loaded films were opaque due to the presence of dispersed drug in the polymer matrix. All film samples appeared to be mechanically strong and resilient. In particular, the 30/70 CAP/Pluronic L101 blends were very well plasticized and flexible.

Thermal Properties

Results from the DSC analysis and CAP/Pluronic L101 blends are summarized in Table I, where the Tg of the 50/50 blend is seen to be reduced from that of pure CAP (382 K) by about 12 K. However, at a low CAP content of 30% (or high Pluronic L101 content of 70%), the Tg is reduced significantly from that of CAP by about 112 K, and an exothermic peak similar to that of a crystallization peak is observed at 394 K.

Erosion and Drug Release Properties

In vitro and *in vivo* studies have been conducted to elucidate drug release and polymer erosion processes in the present association polymer blends. Figs 2 & 3 show typical *in vitro* characteristics of polymer (CAP) erosion and metronidazole release from 50/50 and 30/70 CAP/Pluronic L101 films loaded with 10% metronidazole. From the time scales involved, it is apparent that increasing the Pluronic L101 content from 50% to 70% leads to a significant reduction of both the rate and duration of the polymer erosion and metronidazole release (changing from 1.737 to 0.003%/min and 1.866 to 0.0125%/min, respectively). Consequently, the duration of metronidazole release is increased from 1 hr to over 13 hr, and that of polymer erosion from 1 hr to about 10 days. Figs. 4 & 5 summarize the *in vivo* results of polymer erosion and metronidazole release from 50/50 and 30/70 CAP/Pluronic L101 blends after implantation in a dorsal rat back model. Similar to the *in vitro* results of Figs. 2 & 3, a significant reduction in both the rates of polymer erosion and metronidazole release occurs as the blend ratio is changed from 50/50 to 30/70 (changing from 1.51 to 0.0134%/min and 1.55 to 0.067%/min, respectively). As a result, the duration of metronidazole release is increased from about 1 hr to over 24 hr, whereas that of polymer erosion is extended from about 1 hr to over 4 days.

Table I. Thermal Properties of CAP/Pluronic L101 Blends

CAP (wt. %)	T_g (K)	Crystallization Peak (K)
30	270	394
50	370	—
100	382	—

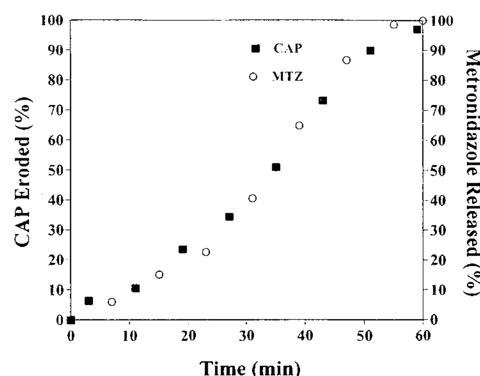

Fig. 2. Characteristics of *in vitro* CAP erosion and metronidazole (MTZ) release from 50/50 CAP/Pluronic L101 films (with 10% metronidazole loading) in isotonic phosphate buffer (pH = 7) at 37°C.

Pilot Clinical Study

Preliminary results from pilot clinical trials (shown in Figs. 6 and 7) indicate that the amount of drug in the crevicular fluid was significant during the three sampling periods (60, 120 and 180 min) and remained well above the minimum inhibitory concentrations for most periodontal pathogens (47.45 ± 6.21 mg/ml, 25.10 ± 4.9 mg/ml and 21.20 ± 4.7 mg/ml, respectively). The dry weight of the retrieved inserts over the three sampling times was 6.56 ± 0.42 mg, 4.54 ± 0.64 mg and 5.31 ± 0.71 mg respectively. Although the erosion of the periodontal inserts show an average of 30% lost during the sampling period, the corresponding metronida-

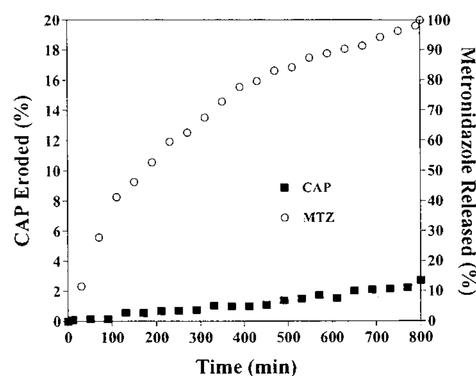

Fig. 3. Characteristics of *in vitro* CAP erosion and metronidazole (MTZ) release from 30/70 CAP/Pluronic L101 films (with 10% metronidazole loading) in isotonic phosphate buffer (pH = 7) at 37°C.

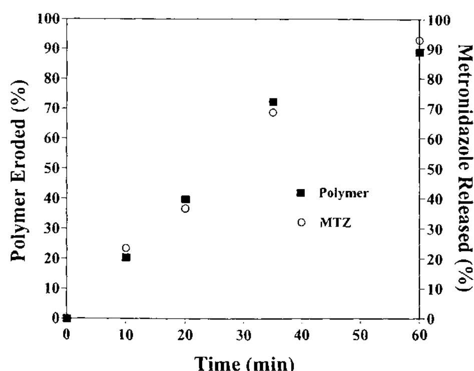

Fig. 4. Characteristics of *in vivo* polymer erosion and metronidazole (MTZ) release from 50/50 CAP/Pluronic L101 films (with 10% metronidazole loading) in a dorsal rat model.

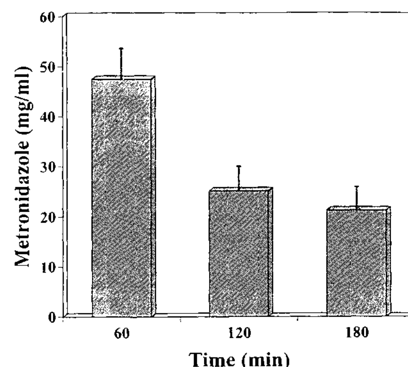

Fig. 6. Metronidazole concentration in the crevicular fluid during drug release from the present periodontal insert in test subjects.

zole release reached about 80%, over the entire sampling period. No discomfort or irritation was reported by the test subjects. Six patients detected a slight bitterness during the first 45 min after the insert placement but, overall, patients found the insert highly acceptable.

DISCUSSION

With regard to the selection of polymer components, CAP and Pluronic L101 have been widely used in the pharmaceutical, cosmetic and food industries as an enteric coating material (9) and a spreading agent (10), respectively. In medical applications, Pluronic L101 has also been employed as an immunological adjuvant (11). As to the antimicrobial agent, metronidazole was selected for this study because it has been shown to be highly effective in treating adult periodontal disease (1).

Upon mixing acetone solutions of CAP and Pluronic L101, an appreciable increase in viscosity of the solution was noted. This is attributed to the formation of an association polymer through intermolecular hydrogen bonding, between the ether oxygen in the Pluronic L101 and the carboxylic acid of the CAP. Similar observation was noted earlier between a hydrophilic Pluronic F127 and CAP, and the existence of intermolecular hydrogen bonding was confirmed by Fourier transform infrared spectroscopy (5).

The presence of a single but lower Tg in the polymer composition containing 50% Pluronic L101 (Table I) indicates that a miscible phase is formed in the blend, where the intermolecular hydrogen bonding between the ether oxygen and carboxylic acid groups is playing a key role in enhancing the compatibility of the blend components. At an even higher Pluronic L101 content of 70% (or lower CAP content of 30%), the appearance of an exothermic peak during heating suggests the formation of some ordered structure in the polymer blend, and the drastic reduction in the observed Tg indicates a transition of polymer morphology to that dominated by the major component Pluronic L101, a water-insoluble hydrophobic liquid. Such transition is believed to be directly responsible for the drastic reduction (as much as 2 orders of magnitude) observed in both the *in vitro* and *in vivo* rates of polymer erosion and metronidazole release as the Pluronic L101 content in the blends with CAP is increased from 50% to 70%. This finding is significant in that the duration of polymer erosion and metronidazole release up to several days, ideal for periodontal applications, may be attained by the present polymer system. The liquid crystalline properties associated with Pluronic L101 as cited previously (12) may also affect the polymer morphology and the characteristics of polymer erosion and drug release. Additional investigations are in progress to address this aspect.

For the 50/50 CAP/Pluronic L101 blend, both the *in vitro* and *in vivo* results show that the percentage metronidazole release and polymer erosion curves are substantially linear and almost superimposable, supporting a primarily surface

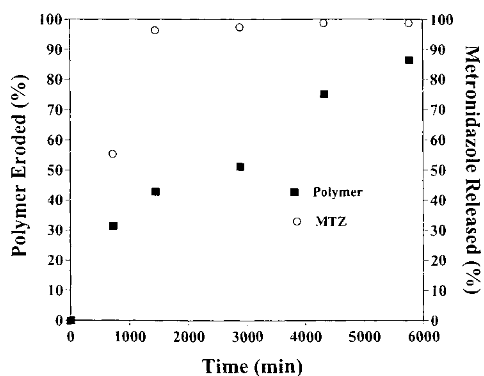

Fig. 5. Characteristics of *in vivo* polymer erosion and metronidazole release from 30/70 CAP/Pluronic L101 films (with 10% metronidazole loading) in a dorsal rat model.

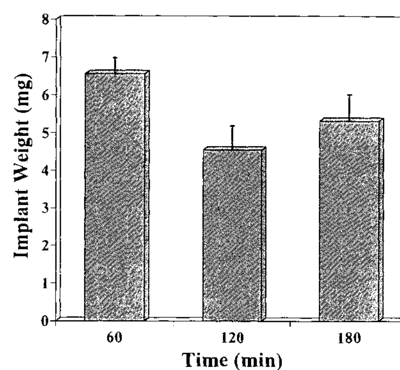

Fig. 7. Dry weight of remaining inserts retrieved from test subjects over the three sampling periods (dry weight of initial insert = 7.6 ± 0.20 mg).

erosion-controlled release mechanism (Figs. 2 & 4). On the other hand, the corresponding *in vitro* and *in vivo* results for the 30/70 CAP/Pluronic L101 blend ratio show a metronidazole release faster than that of polymer erosion, suggesting a diffusion-controlled release process with limited modulation from polymer erosion (Figs. 3 & 5). The reduction in the *in vitro* polymer erosion and drug release rates as a result of increasing Pluronic L101 content in the polymer blend is reflected in the *in vivo* rat model but in varying degrees. Therefore, in a diffusion-controlled system such as the 30/70 blend, the *in vivo* metronidazole release appears to be slower than that of the *in vitro* release due to obvious differences in their respective local hydrodynamic conditions. However, quite unexpectedly, the *in vivo* polymer erosion is observed to be faster than that of the *in vitro* erosion in both blend compositions, possibly due to differences in the local ionic compositions.

During the *in vivo* rat experiments, no signs of adverse tissue reactions were observed. The removal of implants in the rat model was occasionally complicated by the fact that upon hydration, the polymers became somewhat adhesive and eventually turned into a liquid-gel like state before complete eroded. Therefore, incomplete retrieval of implants due to fragmentation and dispersion during removal may results in some overestimation of the metronidazole release and polymer erosion in the *in vivo* results. In such cases, the effective rate for the *in vivo* drug release and polymer erosion may be smaller and the effective duration may be longer than those presented in Figs. 4 & 5.

The duration of the *in vivo* metronidazole release and polymer erosion favors the use of the 30/70 blend composition as a bioerodible insert for the delivery of metronidazole in periodontal patients. Results from the present pilot clinical study are consistent with the *in vivo* rat results in that the polymer erosion lags behind the metronidazole release. However, the extent of the erosion of the inserts (30% in 3 hr) and the corresponding metronidazole release (80% in 3 hr) are more rapid than that observed in the *in vivo* rat study. The exact reason for this discrepancy is not entirely clear at this moment. However, difficulties in the retrieval of hydrated inserts from inflamed periodontal pockets similar to those encountered in the rat model may account at least partially for the possible overestimation of results. Additionally, the higher pH of human gingival crevicular fluid at the inflamed site (pH = 8) (13) can result in a higher erosion rate of the present association polymer than that carried out at the normal physiological pH (pH = 7). With the information gained from this pilot clinical study, further investigations are being carried out to refine the insert design and to examine long term drug release and polymer erosion and its effect on clinical parameters such as probing depth and bleeding on probing as well as possible shifts in the microbial flora of the periodontal pocket.

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