Metronidazole-Loaded Bioabsorbable Films as Local Antibacterial Treatment of Infected Periodontal Pockets

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Background: Periodontal disease is infectious in nature and leads to an inflammatory response. It arises from the accumulation of subgingival bacterial plaque and leads to the loss of attachment, increased probing depth, and bone loss. It is one of the world’s most prevalent chronic diseases. In this study we developed and studied metronidazole-loaded 50/50 poly(DL-lactide-co-glycolide) (PDLGA), 75/25 PDLGA, and poly(DL-lactic acid) (PDLLA) films. These films are designed to be inserted into the periodontal pocket and treat infections with controlled-release metronidazole for ≥1 month.

Methods: The structured films were prepared using the solution-casting technique. Concentrated solutions and high solvent-evaporation rates were used to get most of the drug located in the bulk, i.e., in whole film’s volume. The effects of copolymer composition and drug content on the release profile, cell growth, and bacterial inhibition were investigated.

Results: The PDLLA and 75/25 PDLGA films generally exhibited a low- or medium-burst release followed by a moderate release at an approximately constant rate, whereas the 50/50 PDLGA films exhibited a biphasic release profile. The drug released from films loaded with 10% weight/weight metronidazole resulted in a significant decrease in bacterial viability within several days. When exposed to human gingival fibroblasts in cell culture conditions, these films maintained their normal fibroblastic features.

Conclusions: This study enabled the understanding of metronidazole-release kinetics from bioabsorbable polymeric films. The developed systems demonstrated good biocompatibility and the ability to inhibit Bacteroides fragilis growth; therefore, they may be useful in the treatment of periodontal diseases. J Periodontol 2009;80:330-337.

KEY WORDS
Biocompatibility; drug delivery; metronidazole.

Periodontal disease, a localized inflammatory response due to infection of a periodontal pocket arising from the accumulation of subgingival plaque, is one of the world’s most prevalent chronic diseases. With 36.8% of American adults estimated to have the disease, the prevalence of periodontal disease is greater than cancer, heart disease, arthritis, obesity, acquired immunodeficiency syndrome, and many other diseases. The topical administration of antibacterial agents in the form of mouthwashes is ineffective in controlling disease progression because a limited amount of the drugs actually reach the periodontal pocket. Moreover, the drugs are constantly flushed because of a very high fluid-clearance rate (an estimated 40 replacements of the fluid per hour within a 5-mm pocket).

There are numerous benefits for local drug delivery to the pocket in the form of subgingivally placed systems. Fortunately, periodontal diseases are localized in the immediate environment of the pocket, which is easily accessible for the insertion of a delivery device by syringe or tweezers, depending on the physical form of the delivery system. The critical period of exposure of the pocket to the antibacterial drug is between 7 and 10 days. Intrapocket delivery systems can be divided into bioabsorbable and non-bioabsorbable systems. Non-bioabsorbable systems must be removed or discharged from the pocket after their
drug-release function has been accomplished. Examples of non-bioabsorbable systems include cellulose acetate fibers loaded with tetracycline, chlorhexidine, or metronidazole and film- or slab-based devices made of poly(methyl methacrylate) and ethylcellulose loaded with the three aforementioned drugs. The most studied systems showed promising clinical results in the maintenance of periodontal pockets over a 2-year period.

Bioabsorbable systems are usually polymeric or protein in nature and undergo natural degradation in response to gingival fluid components. The first bioabsorbable systems to be developed were based on hydroxypropylcellulose loaded with various agents: tetracycline, chlorhexidine, and ofloxacin. The researchers reported on the fast drug release from the film within 2 hours with tetracycline remaining within the pocket for 24 hours after insertion. Several modifications have been made to overcome the rapid degradation and short duration of drug release, e.g., incorporating methacrylic acid copolymer particles into the film to get ofloxacin release for 7 days.

A bioabsorbable device based on hydrolyzed gelatin cross-linked by formaldehyde, as reported by Steinberg et al., has evolved to the commercial chlorhexidine in a gelatin matrix. This United States Food and Drug Administration–approved device releases chlorhexidine. A different system is based on a water-free mixture of melted glycerol mono-oleate and metronidazole to which sesame oil was added to improve its flow properties in the syringe. The gel flows deeply into the periodontal pockets and readily adapts to root morphology. It sets in a liquid crystalline state when it comes in contact with water. The matrix is degraded as a result of neutrophil and bacterial activity within the pocket. Effective doses of metronidazole within the pocket are maintained for 24 to 36 hours.

Metronidazole-loaded poly(vinyl alcohol) films demonstrated a biphasic release profile, but the drug was released within several hours because of the high hydrophilic nature of the host polymer. Metronidazole and amoxicillin were loaded in poly(DL-lactide-co-glycolide) (PDLGA) and poly(DL-lactic acid) (PDLLA) films. The drug-release study showed that during the first 16 days, the released quantities of drugs were higher than the minimum inhibitory concentration (MIC) needed against various microbes causing periodontal diseases.

Metronidazole is a partially hydrophilic drug, which effectively inhibits anaerobic microorganisms and protozoan infections. This drug is used for the treatment of many infections, including periodontal and vaginal infections. The drug was incorporated in drug-delivery systems for various applications, such as tablets for the treatment of peptic ulcers, microspheres for the treatment of diseases associated with the colon and the gastric mucosa, alginate gel beads for gastric applications, and various systems for the treatment of periodontal diseases, as described above.

Solution casting of polymers is a well-known method for preparing polymer films. To incorporate a drug using this method, the polymer is dissolved in a solvent and mixed with the drug prior to casting. Then the solvent is evaporated, and the polymer/drug film is created. We reported a method for controlling drug location/dispersion within the film. In this process, the solvent evaporation rate determines the kinetics of drug and polymer solidification and, thus, the drug dispersion/location within the film. Solubility effects in the starting solution also contribute to the postcasting diffusion processes and occur concomitantly with the drying step. In general, two types of polymer/drug film structures were created and studied: a polymer film with large drug crystals located on the film surface (A type) and a polymer film with small drug particles and crystals distributed within the bulk (B type). This structure enables control of the drug-release profile and results in desired release behavior. The two types of films, A and B, were developed and studied for two types of drugs: highly hydrophobic drugs, such as dexamethasone, and highly hydrophilic drugs, such as gentamicin. Metronidazole has moderate hydrophilicity (10 mg/ml). Therefore, it is of interest to study the controlled-release characteristics of polymer/metronidazole-loaded films.

The aim of the present study was to develop and evaluate metronidazole-loaded bioabsorbable films, designed to be inserted into the periodontal pocket and treat infections during metronidazole controlled-release phase. This study focused on the effects of drug content and the type of host polymer on the drug-release profile. Selected films were studied for cell growth and bacterial inhibition. We believe that a relatively long release time (4 weeks) would be beneficial for the healing process.

**MATERIALS AND METHODS**

All experiments and analyses were conducted in the Biomaterials Laboratory at Tel-Aviv University.

**Materials**

Bioabsorbable polymers. The following three bioabsorbable polymers were used: PDLLA with an inherent viscosity (IV) of 0.62 dl/g in CHCl₃ at 30°C, which corresponds to a molecular weight (MW) of 90,177 Da; 75/25 PDLGA with an IV of 0.65 dl/g in CHCl₃ at 30°C, which corresponds to a MW of 100,050 Da;...
50/50 PDLGA with an IV of 0.57 dl/g in ChCl 3 at 30°C, which corresponds to a MW of 71,500 Da.

The drug used in the study was metronidazole.**

** Microorganisms. A clinical isolate of *Bacteroides fragilis* was used in this study. Centers for Disease Control and Prevention (CDC) anaerobic blood agar†† was used to grow the microorganisms. This medium is highly recommended for the anaerobic growth of bacteria.

**Film Preparation**

Polymer films (0.12 to 0.15 mm thick) consisting of PDLLA, 75/25 PDLGA, or 50/50 PDLGA and metronidazole were prepared by a three-step solution-processing method. The polymer (1 g) was mixed with a relatively small volume of methylene chloride (20 ml) at room temperature until it was totally dissolved, giving a clear solution; metronidazole was added to the polymer solution to give a relatively concentrated solution. Two constant drug loadings were used: 20 mg metronidazole (2% weight/weight [w/w]) and 100 mg metronidazole (10% w/w). The drug was fully dissolved in both solutions. The following steps were solution casting into a petri dish and solvent drying under atmospheric pressure at room temperature. The petri dish was not covered, so as to enable a relatively fast evaporation rate of 10 to 20 ml/hour. Afterwards, the solution was cast into a petri dish, and the solvent was dried under atmospheric pressure at room temperature. The petri dish was not covered, so as to enable a relatively fast evaporation rate of 10 to 20 ml/hour. Afterwards, the solution was cast into a petri dish, and the solvent was dried under atmospheric pressure at room temperature. Following this step, the film underwent isothermal heat treatment at 33°C for 23 hours in a vacuum oven. This heat treatment enabled disposal of residual solvent.

**Morphologic Characterization**

Polarized light microscopy was performed using a microscope†† (transmission mode).

**In Vitro Weight-Loss Studies**

The polymer films (1 x 1 cm) were weighed and then immersed in phosphate buffered saline (PBS; 50 ml in a petri dish) at 37°C for 20 weeks to determine their weight-loss profiles. Samples (also those broken into small pieces) were removed every week, dried in a vacuum oven, and weighed. The weight loss was calculated as follows:

\[
\text{weight loss (\%) = 100} \times \frac{w_0 - w_f}{w_0},
\]

where \(w_0\) and \(w_f\) are the weights of the dried films before and after exposure to water, respectively. Four samples were tested at each point. The means and SD values are presented in the relevant figures.

**In Vitro Metronidazole Release Study**

Polymer/metronidazole films with a diameter of 10 cm were immersed in 40 ml PBS at 37°C for 7 weeks (50/50 PDLGA films were immersed for 5 weeks) in semi-static conditions to determine the kinetics of metronidazole release from the films. The films were weighed before the experiment to permit determination of 100% release. Sodium azide (0.05% weight/volume) was added to prevent contamination by various microorganisms. At the following times, 1.5-ml samples were collected: 1, 6, and 24 hours; 2, 3, and 8 days; and 2, 3, 4, 5, and 7 weeks. The maximum metronidazole concentration in the release medium was ≥10 times less than its water solubility so as not to affect the release kinetics. The amount removed was replaced with fresh PBS, and the correction factor was applied as follows:

\[
\text{correction factor} = \left(\frac{40}{40 - 1.5}\right)^{n-1},
\]

where \(n\) is the sequential sample number. The metronidazole content in each sample was determined with an ultraviolet/visible scanning spectrophotometer§§ at 320 nm. The metronidazole working range was 10 to 140 μg/ml; therefore, the samples were diluted. A calibration curve was prepared for each set of measurements, with a correlation coefficient >0.99. Three samples were examined for each film type. The means and SD values are presented in the relevant figures.

**Preparation of Bacteria**

*B. fragilis* was grown overnight on CDC anaerobic blood agar at 37°C under anaerobic conditions. The bacterial cells were collected and resuspended in saline and adjusted to 1 x 10^8/ml by visual comparison to a 0.5 McFarland standard. Ten-fold dilutions were performed in Eppendorf tubes. Aliquots of 100 μl were spread on CDC agar, and three repetitions were conducted.

**Evaluation of Residual Bacteria**

Samples of 10 or 100 μl were collected or diluted to a final concentration, at the appropriate time, and spread on CDC anaerobic agar plates. Colony forming units (CFU)/ml were counted after 24 hours of incubation at 37°C.

**MIC**

The MIC of metronidazole against *B. fragilis* was determined using the E-test method, and it was found to be 1 μg/ml. Special strips¶¶ for detecting MIC values (using the E-test method) were used.

**Microbiologic Experiment Design**

The microbiologic study, i.e., the kinetics of residual bacteria, was performed as follows. The effect of metronidazole released from the films (in PBS) on bacterial inhibition was studied. The release medium samples

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**Notes:**

1. Absorbable Polymers International.
2. Sigma, St. Louis, MO.
3. Difco Microbiology, Detroit, MI.
4. Leica, Wetzlar, Germany.
5. Zenyth 200rt, Anthos, Eugendorf, Australia.
were collected after 0, 5, and 48 hours and 1, 2, and 3 weeks. The organisms were added to a final concentration of $1 \times 10^7$ CFU/ml. The samples were collected (after 0 and 24 hours and 2 and 7 days in the case of 50/50 PDLGA films and after 0 and 24 hours and 3 and 8 days in the case of PDLLA films) for viable counting and were expressed as CFU/ml. Bacteria in PBS served as the control. CDC anaerobic blood agar plates were used for counting the residual bacterial. The calculation established the residual number of bacteria in the presence of metronidazole.

Because *B. fragilis* is an obligatory anaerobe, all incubations were performed in anaerobic jars in the presence of anaerobic bags.

**Biocompatibility Experiment**

Films of $5 \times 5$ mm, which were loaded with 2% w/w and 10% w/w of metronidazole, were placed in 35-mm cell culture dishes and covered with 70% alcohol solution for 30 minutes to achieve sterilization of the films. The alcohol was decanted and replaced by three washes of sterile PBS and left to dry in the laminar flow hood, blowing sterile air. Human gingival fibroblasts (HGFs; three to four subcultures of human gingival explants) were seeded at $1 \times 10^5$ cells/dish, with 2 ml growth medium covering the films. The cells were cultured in minimum essential medium supplemented with 10% newborn bovine serum. The medium was replaced every 4 days. After 4 to 6 days, subconfluent cultures were observed under phase-contrast microscopy. The medium was decanted, and the cells were washed with PBS and fixed with 5% formalin in PBS. Two hours later, the formalin was removed, and the cells were washed in PBS and stained with Coomassie blue to expose the cytoskeleton F-actin. Cells on the films and the culture dish exhibited normal fibroblastic morphologic features.

**RESULTS**

**Microstructure of Polymer/Metronidazole Films**

Bioabsorbable polymeric films containing metronidazole were prepared using solution processing, accompanied by a postpreparation isothermal heat treatment. Our structuring technique enabled us to control the drug location/dispersion in the film. Using this technique, the films were prepared from concentrated solutions using a fast evaporation rate to get films with the most drug molecules located in the bulk, i.e., in whole film’s volume, rather than on the surface. The microstructure of the metronidazole-loaded 50/50 PDLGA, 75/25 PDLGA, and PDLLA films is presented in Figure 1. In the 50/50 and 75/25 PDLGA-based films, drug crystals, particles, and aggregates (2 to 100 μm) are dispersed in the bulk. Only a small amount of the drug is located on the surface of the film.

Conversely, in the PDLLA-based films, more drug crystals and particles are located on the surface of the film. The effect of this difference in microstructure on the drug-release profile is described in the next section.

¶¶ Oxoid, Cambridge, U.K.
Metronidazole Release From Bioabsorbable Films and Their Weight-Loss Profile

The effects of polymer type and drug loading on the metronidazole-release profile were examined. The cumulative metronidazole-release profiles from various polymer/metronidazole films loaded with 2% w/w metronidazole and 10% w/w metronidazole are presented in Figure 2. A small or moderate burst release (during the first 48 hour) was observed for all six studied films. The films loaded with 2% w/w metronidazole released 10% to 20% of their drug within the first 48 hours (Fig. 2A), whereas the films loaded with 10% w/w metronidazole released 20% to 38% of their drug (Fig. 2B).

The PDLLA/metronidazole and 75/25 PDLGA/metronidazole films exhibited a constant rate of release during the first 2 to 3 weeks; thereafter, only a small amount of drug was released through week 7. Conversely, the 50/50 PDLGA/metronidazole film exhibited a two-phase release: the first release occurred during the first 3 days for the 2% w/w loaded films and during the first 7 days for the 10% w/w loaded films, and the second phase started after 14 days for the 2% w/w loaded films and after 20 days for the 10% w/w loaded films. All encapsulated drug was released by 35 days.

The weight-loss (erosion) profile of PDLLA and 50/50 PDLGA is presented in Figure 3. Although the PDLLA films lost only 2% of their initial weight during the first 7 weeks of degradation, the 50/50 PDLGA films lost 18% of their initial weight.

Microbiologic Evaluation of the Effect of Metronidazole Release on Bacterial Viability

The purpose of these experiments was to monitor the effectiveness of various concentrations of the antibiotic released from the films in terms of the residual bacteria compared to the initial number of bacteria. Bacteria present in PBS only served as the control. We chose the following two types of films with different release profiles: 50/50 PDLGA films loaded with 2% w/w metronidazole, which demonstrated a biphasic release profile with a relatively high–burst release of 20% (compared to the other samples containing 2% w/w drug), and PDLLA films loaded with 10% w/w metronidazole, which demonstrated the highest burst release of ~38% and the highest release rate during the first 2 weeks of release.

Figure 4 presents the two profiles for comparison. The profiles are expressed as the percentage of the total drug encapsulated (Fig. 4A) and as the drug amount (milligrams; Fig. 4B). The samples were collected after 5 and 48 hours and after 7 and 21 days for 50/50 PDLGA film containing 2% w/w metronidazole and after 5 and 48 hours and 7 and 14 days for PDLLA loaded with 10% w/w metronidazole. B. fragilis was added to the tubes containing previously released drug, and the number of bacterial cells was monitored. The initial bacterial concentrations used in our experiment were $1 \times 10^7$ CFU/ml, which is relatively high. Most infections involve lower concentrations (usually not more than $1 \times 10^5$ CFU/ml). Therefore, we assume that if our metronidazole-eluting films were effective against such concentrations, they will be effective against serious infections. The results (bacterial inhibition kinetics) for 50/50 PDLGA film containing 2%
w/w metronidazole are presented in Figure 5A, and the results for PDLLA loaded with 10% w/w metronidazole are presented in Figure 5B.

Our results showed that, when *B. fragilis* was exposed to 50/50 PDLGA film loaded with 2% w/w metronidazole, the quantity of drug released during the first 7 days (<30%) eradicated all bacteria within less than a week (Fig. 5A), whereas the drug quantity released during the first 5 and 48 hours only reduced the bacterial concentration to <1·10³ CFU/ml within a week. Hence, relatively small amounts of drug (such as 2% w/w) start to become effective only after ≥1 week. When *B. fragilis* was exposed to the PDLLA film, which was loaded with 10% w/w metronidazole, the drug quantity released for at least 48 hours eradicated all bacteria within 3 days (Fig. 5B).

**Biocompatibility**

No differences in cell shape or number were observed between the treated (Fig. 6A) and non-treated films (Fig. 6C). Also, the gingival fibroblasts exhibited an elongated shape on the films (Figs. 6A and 6C) or on the bottom of the culture dishes (Figs. 6B and 6D). These results indicated that the films are biocompatible with HGFs.

**DISCUSSION**

Our results showed that although most metronidazole particles were dispersed in the bulk of the 50/50 and 75/25 PDLGA films, many drug particles and crystals were located on the surface of the PDLGA films (Fig. 1). These differences in drug location/dispersion in the film can be explained by the fact that lactic acid is more hydrophobic than glycolic acid. PDLLA contains only lactic acid monomers; therefore, it is more hydrophobic than 50/50 PDLGA and 75/25 PDLGA, which contain lactic and glycolic acid monomers in the polymer chains. Although we used concentrated (viscous) solutions and a relatively fast evaporation rate during the film-preparation step, it seemed that the hydrophilic metronidazole tended to diffuse to the surface of the hydrophobic PDLLA solution; therefore, part of the drug was located on the surface of the film and was not entrapped in it.

The metronidazole release pattern from the 50/50 PDLGA films was different than from the 75/25
PDLGA and PDLLA films (Fig. 2). The former films exhibited a constant rate of release during the first 2 to 3 weeks, and then only a very small amount of drug was released until week 7. This first phase of release is governed mainly by diffusion. We expected a second phase of release to occur after 7 weeks because of a combination of diffusion and erosion of the host polymer. In the case of 50/50 PDLGA films, we observed the two phases of release because that film exhibited the fastest degradation rate, and massive erosion occurred during the first 4 weeks of exposure to aqueous medium (Fig. 3). The release profiles of the films loaded with 10% w/w drug showed that larger drug quantities were released from the PDLLA films than from the 75/25 PDLGA films (Fig. 2B). This probably resulted from differences in the drug’s location in the film, i.e., in the former, larger drug quantities were located near the film’s surface (Fig. 1). Our results also showed that the drug loading affected the burst release. The higher burst release from films loaded with 10% w/w drug resulted from higher driving force for diffusion of drug molecules from polymer domains close to the surface.

Metronidazole is a relatively hydrophilic drug (10 mg/ml); therefore, we expected higher burst release and higher rates of release. The slow-moderate burst-release values and moderate drug-release rate observed in the current study resulted from the film structuring, i.e., most drug molecules were located in the film rather than on its surface. Also, specific interactions, such as hydrogen bonding, may exist between the metronidazole molecules and the bioabsorbable polyester chains. These may decrease the release rate. Our metronidazole-release profiles showed longer periods of release than those obtained for other metronidazole-eluting bioabsorbable systems (several hours to several days).14,17,18

Our microbiologic experiments showed that metronidazole was effective in the tested samples. The films’ collection and preparation method did not affect metronidazole’s antibiotic potency. Also, our results indicated that moderate drug contents (such as 10% w/w) were very effective and could eradicate the bacterial growth within several days, even when a large inoculum (1 x 10^7 CFU/ml) was created. The release profile of metronidazole from PDLLA films loaded with 10% drug was more suitable than that obtained from 50/50 PDLGA films loaded with 2% drug. In addition to their effectiveness against the relevant bacterial strain, our new structured bioabsorbable polymer/metronidazole films were biocompatible with HGFs and, therefore, may be used to treat periodontal diseases.

CONCLUSIONS

Bioabsorbable films containing metronidazole were prepared by solution processing. These films are designed to be inserted into the periodontal pockets and treat infections with controlled-release metronidazole for ≥1 month. PDLLA and 75/25 PDLGA films generally exhibited a low or medium burst release followed by a moderate release at an approximately constant rate, whereas the 50/50 PDLGA films exhibited a biphasic release profile due to the relatively high degradation rate of the host polymer. This study demonstrated that the release profile of metronidazole was determined mainly by the host polymer type. Drug loading had a minor effect on the release profile.

The drug contents in the surrounding medium exceeded the required minimum effective concentration. When relatively high drug loading was used (10% w/w), the released metronidazole resulted in a significant decrease in bacterial viability within several days,
even with relatively high bacterial concentrations (1 × 10^7/ml CFU). When relatively low drug loading was used (2% w/w), the kinetics of bacterial inhibition was relatively slow, and ≥1 week was required to eradicate bacterial growth. The film preparation did not affect metronidazole’s potency as an antibacterial agent. Our results indicated that the drug-loaded films exhibited biocompatible properties with regard to HGFs. Hence, our new metronidazole-eluting films may be useful in the treatment of periodontal diseases.

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