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Compression Molding of Biodegradable Drug-Eluting Implants for Sustained Release of Metronidazole and Doxycycline

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ABSTRACT: The purpose of this report was to develop solvent-free biodegradable drug-eluting implants that provide sustained release of metronidazole and doxycycline. The drug-eluting implants were prepared using the compression molding technique. To fabricate the implants, polylactide-polyglycolide copolymers were premixed with metronidazole or doxycycline. The mixture was then compression molded and sintered to form implants of various sizes and geometries. An elution method and an HPLC assay were used to characterize the *in vitro* release rates of the antibiotics over a 28-day period. A bacterial inhibition test was also carried out to determine the bioactivity of released antibiotics. The concentrations of both metronidazole and doxycycline were much greater than the minimum inhibitory concentration of *Escherichia coli* for up to 3 and 4 weeks, respectively, and the bioactivities of the antibiotics remained high after the fabrication process. Furthermore, the initial burst could be minimized and the release rate could be reduced by increasing the size of the implants and by adopting low drug to polymer ratios. By using this compression molding technique and appropriate processing parameters, we will be able to fabricate biodegradable implants of various types of antibacterial drugs for long-term local deliveries. Eventually, biodegradable drug-eluting implants may be used to treat various periodontal diseases. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: compression molding; biodegradable drug-eluting implants; thermal stability; *in vitro* release

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INTRODUCTION

Periodontitis is a difficult infection to treat and eradicate. The opportunistic microflora present in the periodontal pocket provides an ideal environment for the growth and proliferation of bacteria.[1] The pathogenic microflora is complex in nature and is mainly anaerobic Gram-negative bacteria.[2] Long-term local antibiotic deliveries in the periodontal pockets are often required for effective therapy. The treatment of periodontal disease begins with the removal of subgingival calculus. This is commonly addressed by the surgical procedures known as root planing and scaling. These procedures debride calculus by mechanically scraping it from tooth surfaces. Following debridement surgery, the pocket space created by the removal of the root accretions and diseased cementum and dentin must be managed. Delivering an effective antimicrobial at sufficiently high concentrations to the area of infection in combination with surgery is a recognized treatment for periodontal infection.[3,4] Based on this concept, several research groups have been focusing on developing drug-eluting implants for a long-term delivery of antibiotics.[5–9] This release profile of a drug-eluting implant should have an initial high release rate to accommodate the infection, followed by a sustained period of a relatively constant release above the minimum inhibitory concentrations. Release kinetics was found to be influenced by the type of polymer utilized for the implants. Differences in materials degradability may influence not only the antibiotic release rate but also its release mechanism.

Biodegradable polymers such as polylactide-polyglycolide (PLGA) have received increasingly attention in the past few decades. They have been used to make biodegradable drug-eluting implants for the purpose of pharmaceutics delivery. In most previous studies, the drug-eluting implants were prepared using

Figure 1. Photo of the setup for compression molding of the antibiotic pellets and disks.

a solvent-casting method.[5–9] To remove the solvent, a wide range of protocols using different solvents, drying times, drying temperature, and molds are reported, with the most commonly reported drying time consisting of between 24 and 28 h at room temperature followed by a further 24–48 h in vacuum at 40–50°C.[10] However, Ferier et al.[11] reported that residual solvent levels would remain relatively similar after 14 days and would not completely be removed until ~700 days drying.

This article adopts a solvent-free compression molding method[12] of manufacturing biodegradable drug-eluting implants for drug delivery. Doxycycline and metronidazole were incorporated in the implants. No solvent was needed during the manufacturing process, which would provide the advantage of avoiding the problems associated with the use of solvents. Doxycycline is effective against a broad range of Gram-positive and Gram-negative bacteria and is widely used to treat many different bacterial infections including periodontitis, whereas metronidazole is a nitroimidazole antibiotic medication used particularly for anaerobic bacteria. After fabrication, the drug-eluting implants were evaluated by an *in-vitro* elution method. A high performance liquid chromatography (HPLC) analysis was utilized to evaluate the release characteristics of doxycycline and metronidazole[13] from the biodegradable implants. In addition, an antibiotic disk diffusion method was used to examine the bioactivities of eluted pharmaceuticals. The final goal of this research was to develop a biodegradable system for the delivery of antibiotics into the periodontal pockets for the treatment of periodontitis.

MATERIALS AND METHOD

Materials

The polymers used were poly (D,L)-lactide-*co*-glycolide with a ratio of 50: 50 and an intrinsic viscosity of 0.4 (Resomer RG 503, Boehringer, Germany) and with a glass transition temperature of ~47.5°C.[12] All polymers were available in powder form with particle size ranges from 100 to 200 μm. The antibiotics used included commercial grade doxycycline hyclate crystal (D9891-5G, Sigma-Aldrich) and metronidazole (M3761-5G, MW 171.51 g/mol, Sigma-Aldrich) in powder form with an approximately average particle size of 100 μm.

Thermal Stability of Antibiotics

Thermal stability[14] tests of both doxycycline and metronidazole were performed. The bioactivities of the incubated doxycycline to *Escherichia coli* (ATCC25922) were determined by the antibiotic disk diffusion method. Twenty milligrams of doxycycline was incubated in an oven for 30 min at various temperatures ranging from 40 to 100°C, with a negative control that was not incubated. Each drug sample was dissolved in 20 mL of normal saline. The concentration equaled to 1 mg/mL. Eight microliters of the buffer samples were pipetted onto 6 mm absorption disks. The disks were placed on the nutrient agar plates that were seeded with a layer of *E. coli*, and the zones of inhibition were measured with a micrometer after 16–18 h of incubation at 37°C. The bioactivity of the released antibiotic was defined as:

$$\text{Bioactivity}(\%) = (\text{diameter of sample inhibition zone} / \text{diameter of control inhibition zone}) \quad (1)$$

The thermal stability of metronidazole was also completed following the same procedure.

Fabrication of Drug-Eluting Implants

The biodegradable drug-eluting implants were fabricated using a compression molding technique. PLGA copolymers were premixed with either doxycycline or metronidazole using a laboratory scale dry mixer. The mixture was compressed under a pressure of 247.13 MPa by a mold shown photographically in Figure 1 to form implants of two different geometries: pellets with a height of 3.0 mm and diameters of 1.0 and 2.0 mm, and disks with a diameter of 3.0 mm and thickness of 0.3, 0.8, 1.3, and 1.8 mm. The compressed implants with the mold were then placed in an oven (that is equipped with a circulation fan and operates with a temperature range of 30–300°C) for sintering. The sintering temperature was set at 55°C, which was higher than the polymers' glass transition temperature (i.e., 47.5°C), but low enough to avoid destroying the antibiotics. The sintering time used was 30 min to attain an isothermal

Figure 2. Photo of the biodegradable implants for drug delivery. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

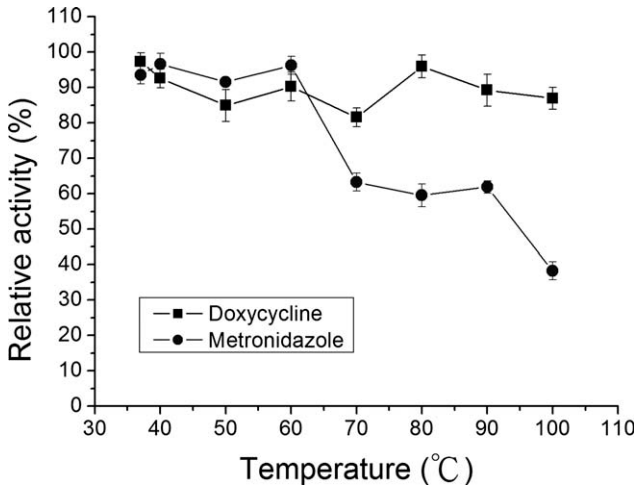

Figure 3. Thermal stability of metronidazole and doxycycline.

sintering of the materials. After sintering, drug-eluting implants of various size and geometry were then obtained as shown photographically in Figure 2.

In-Vitro Elution

An *in-vitro* elution method was utilized to determine the release characteristics of antibiotics from the implants. A phosphate buffer, 0.15 mol/L (pH 7.4), was used as the dissolution medium. The implants were placed in glass test tubes with 1 mL of phosphate buffer. All tubes were incubated at 37°C. The dissolution medium was collected for subsequent analyses at every 24-h interval. Fresh phosphate buffer (1 mL) was then added for the next 24-h period, and this procedure was repeated until the implant was fully dissolved.

HPLC Analysis

The antibiotic concentrations in buffer for the elution studies were determined by a HPLC assay standard curve for doxycycline and metronidazole. The HPLC analyses were conducted on a Hitachi L-2200 Multisolvent Delivery System. The column used for separation of metronidazole was an Agilent Zorbax ODS 5 μm, 4.6 × 150 mm^2 HPLC column. The mobile phase contained 24.7 mol methanol and distilled water (25/75, v/v). The absorbency was monitored at 254 nm and the flow rate was 1.0 mL/min. All samples were assayed in triplicate, and sample dilutions were performed to bring the unknown concentrations into the

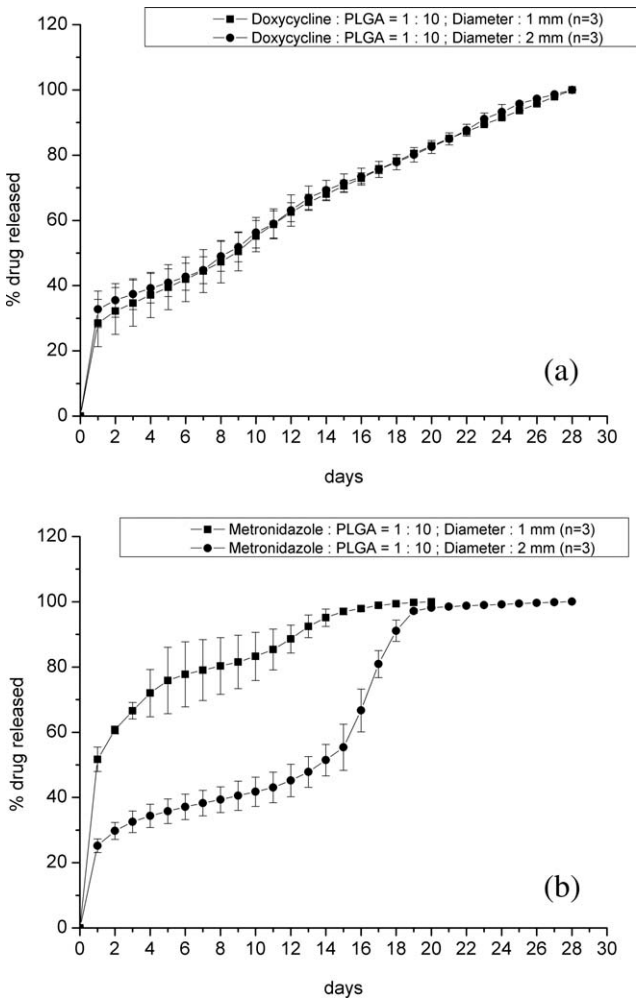

Figure 4. Release curves of (a) doxycycline and (b) metronidazole from biodegradable drug-eluting pellets of different diameters.

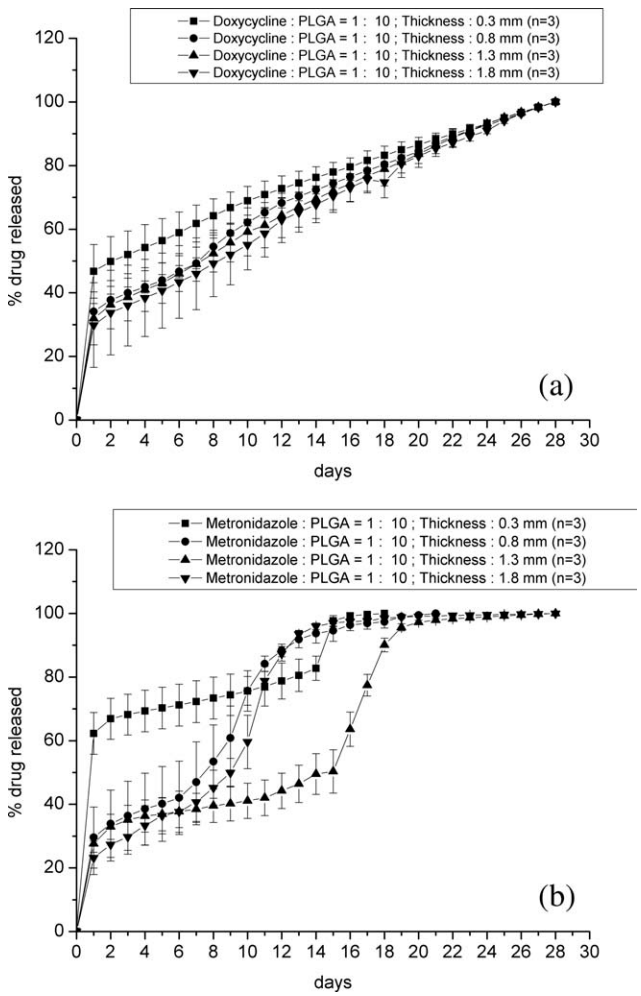

Figure 5. Release curves of (a) doxycycline and (b) metronidazole from biodegradable drug-eluting disks of various thicknesses.

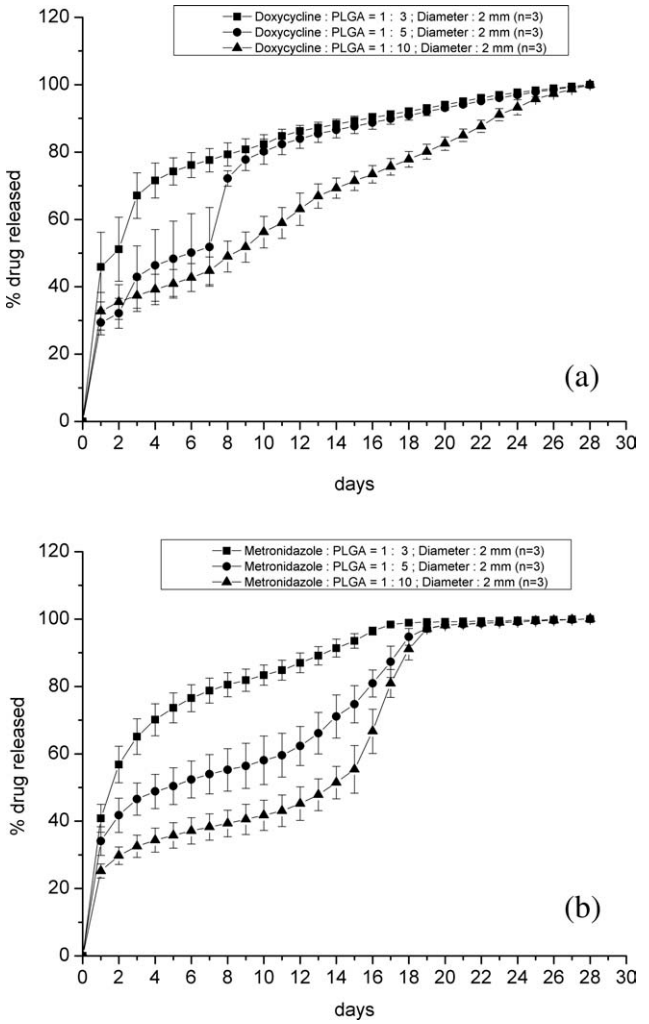

Figure 6. Release curves of (a) doxycycline and (b) metronidazole from biodegradable drug-eluting implants of different drug to polymer ratios.

range of the assay standard curve. A calibration curve was made for each set of the measurements (correlation coefficient > 0.99). The elution product can be specifically identified and quantified with high sensitivity using the HPLC system.

Bioactivities of Released Antibiotics

The bioactivity of released doxycycline and metronidazole to *E. coli* (ATCC25922) were determined using an antibiotic disk diffusion method in Nutrient Broth (beef extract, peptone, Difco Laboratories). Eight microliters of the buffer sample from each daily buffer sample was pipetted onto 6 mm absorption disks. The disks were placed on the nutrient agar plates that were seeded with a layer of *E. coli*, and the zones of inhibition were measured with a micrometer after 16–18 h of incubation at 37°C. A drug solution of 50 μg/mL was used as a reference. Each sample was first diluted or concentrated to 50 μg/mL, based on the drug concentration data from HPLC analysis. The bioactivity of the released antibiotics were calculated according to:

$$\text{Bioactivity}(\%) = (\text{diameter of sample inhibition zone} / \text{diameter of reference inhibition zone}) \quad (2)$$

The eluent of the implants was tested up to 28 days.

RESULTS AND DISCUSSION

Figure 3 shows the thermal stability of the antibiotics used in this study. Cleary, the metronidazole remained fully active for the temperature up to 60°C, whereas the doxycycline still kept 90% of its activity at 80°C. The thermal stability of metronidazole is inferior to that of doxycycline. The results also suggest that the sintering temperature used (55°C) would not deactivate the effectiveness of the drugs. The release characteristics of the antibiotics from the biodegradable implants were examined. Figure 4 shows the accumulated release of doxycycline and metronidazole from drug-eluting pellets of different diameters, whereas Figure 5 shows the accumulated release of the antibiotics from drug-eluting disks of various thicknesses. Generally, the doxycycline showed a biphase drug release characteristics, i.e., an initial burst followed by a diffusion-control release.[15] Doxycycline diffusion release from the PLGA implants proceeded relatively fast until release reached completion at around day 28. Despite increasing the thickness of the disks reduces somewhat the initial burst release [Figure 5(a)],

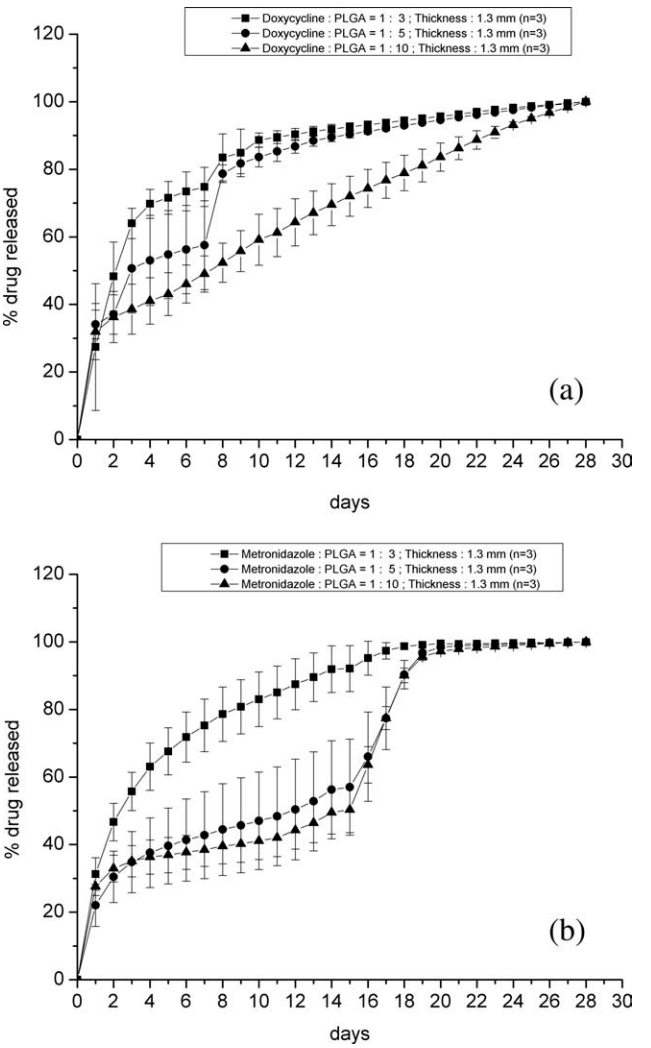

Figure 7. Release curves of (a) doxycycline and (b) metronidazole from biodegradable drug-eluting disks of different drug to polymer ratios.

Table I. Bioactivities of the Released Drugs

	Drug			
	Doxycycline		Metronidazole	
Day	Concentration (μg/mL)	Bioactivity (%)	Concentration (μg/mL)	Bioactivity (%)
1	171.47	96.0 (±3.4)	568.94	84.3 (±1.7)
2	37.52	65.7 (±7.7)	100.17	49.4 (±4.9)
3	18.01	67.5 (±8.0)	39.59	49.2 (±0.0)
4	21.89	55.6 (±6.6)	15.93	59.6 (±0.0)
5	19.10	63.7 (±7.6)	9.77	46.1 (±0.0)
6	25.26	48.2 (±5.7)	10.26	43.8 (±0.0)
7	24.45	49.8 (±5.9)	11.62	38.7 (±0.0)
8	19.14	63.6 (±7.5)	22.41	64.7 (±0.0)
9	26.89	45.2 (±5.4)	12.68	35.5 (±0.0)
10	20.49	59.3 (±7.0)	15.16	57.2 (±9.5)
11	20.65	58.9 (±6.9)	14.35	54.6 (±10.1)
12	29.89	40.7 (±4.8)	31.93	45.5 (±0.0)
13	21.00	57.9 (±6.9)	32.65	44.4 (±0.0)
14	18.48	65.8 (±7.8)	59.49	60.8 (±9.7)
15	20.10	60.5 (±7.1)	12.74	35.3 (±0.0)
16	18.24	39.3 (±7.9)	215.33	70.2 (±9.4)
17	18.81	38.1 (±7.7)	242.54	74.0 (±4.1)
18	15.58	45.9 (±9.3)	255.00	68.4 (±7.1)
19	17.31	41.4 (±8.3)	113.06	52.6 (±7.7)
20	19.66	36.4 (±7.9)	33.42	58.3 (±0.0)
21	20.50	34.9 (±7.0)	11.88	51.9 (±12.2)
22	21.88	32.7 (±6.6)	6.22	32.2 (±0.0)
23	24.98	28.6 (±5.8)	5.23	38.3 (±0.0)
24	24.32	29.5 (±5.9)	4.95	40.4 (±0.0)
25	17.29	41.5 (±0)	4.83	41.4 (±0.0)
26	13.09	22.9 (±0)	4.46	44.8 (±0.0)
27	12.61	23.8 (±0)	4.44	45.1 (±0.0)
28	12.60	23.8 (±0)	4.45	45.0 (±0.0)

The MIC of doxycycline and metronidazole against *Escherichia coli* were determined to be 0.4 μg/mL.

most of the release curves are comparable [Figures 4(a) and 5(a)]. On the other hand, metronidazole exhibited a triphase release behavior, i.e., a burst, a diffusion-controlled release, and a degradation-dominated release. Implants' size has substantial influence on the release behavior of metronidazole. The initial burst decreased significantly with the implant size [Figures 4(b) and 5(b)]. The drug particle size will make a difference in the elution process. If the particles are too large, they can not be completed encapsulated by the polymers, and their release will depend on particle dissolution rate as well as the size of resultant channels or diffusion pathways. Furthermore, because of smaller thickness of the thinner disks, the drug particles might not be completed encapsulated by the polymeric materials. Channel diffusion thus dominated, which in turn accelerated the release rates.

Figure 6 shows the release curves of doxycycline and metronidazole from drug-eluting pellets of different drug to polymer ratios, whereas Figure 7 shows the release behavior of the antibiotics from drug-eluting disks of various drug-to-polymer ratios. The initial burst release was reduced as the drug to polymer ratio decreased. The 1: 10 drug to polymer ratio implants exhibited the least initial burst, whereas the 1: 3 drug-to-polymer ration one showed the greatest burst release. Furthermore, the release rates of the antibiotics increased with the drug to polymer ratios, whereas the duration period of drug release decreased somewhat with the drug to polymer ratios. The HPLC analysis results from the *in-vitro* elution test showed that the drug-eluting implants could release effective doxycycline and metronidazole for ~4 weeks and 3 weeks, respectively.

The bioactivity test of eluted antibiotics to *E. coli* (ATCC25922) were determined by using an antibiotic disk diffusion method in the Nutrient Broth. Three samples were evaluated for each test. The measured results are listed in Table I. The activity of

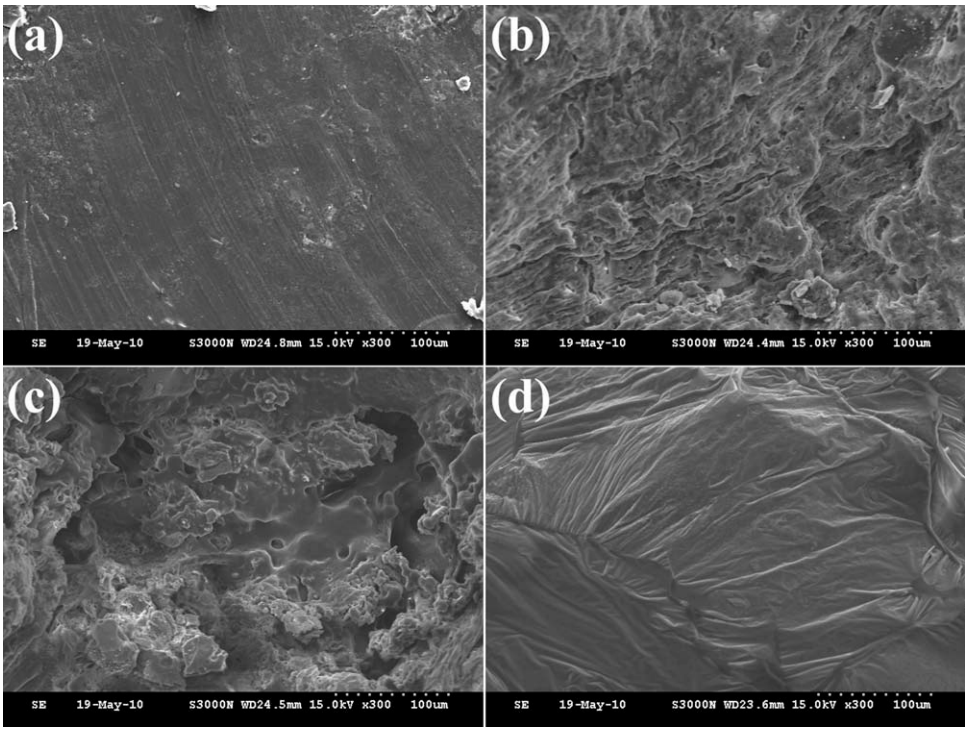

Figure 8. SEM photo of the metronidazole-eluting implant surface (a) as fabricated, and after (b) 4 days, (c) 7 days, and (d) 14 days elution.

doxycycline and metronidazole ranged from 30% to 100% and 27% to 100%, respectively. The results of bioactivity test suggested that the compression molding technique did not significantly deactivate the pharmaceuticals used in this study. A more detailed bioactivity analysis should be done in the future to further investigate the influence of various processing parameters on the activity of released antibiotics.

The release mechanism of antibiotics from the drug-eluting implants is also an important consideration. During the manufacture of drug-eluting implants, the formation of a homogeneous melt from powder particles involves two steps: First, the polymeric particles stick or fuse together at their points of contact around the antibiotic particles. This fusion zone grows until the mass becomes a three-dimensional network, with relatively little density change. Second, at some point in the fusion process, the network begins to collapse into the void spaces between the polymer and the antibiotics. These spaces are filled with molten polymer that is drawn into the region by capillary forces. The antibiotic is then encapsulated by the polymer to form a composite for the implants.[16]

The experimental results in this study suggest that doxycycline exhibited a biphase release from the drug-eluting implants: an initial burst followed by a diffusion-controlled release. After the fabrication process, although most pharmaceutics particles were dispersed in the bulk of the PLGA implants, some drug particles and crystals were located on the surface of the implants [Figure 8(a)]. This in turn led to the initial burst release of the drugs. Following the initial burst, drug release was controlled solely by diffusion. For a water-soluble antibiotic in a hydrophobic PLGA matrix, the release mechanisms are controlled by channel diffusion, osmotic pressure, and polymer degradation.[17] PLGA is a hydrophobic and glassy polymer with T_g of ~40–45°C. Only limited free volume was available for drug transport pathway. It has to rely on degradation to break long chains and polymer relaxation to create a porous network for the drugs to be diffused [Figure 8(b)]. The onset of channel diffusion was delayed by the time taken for pore formation and coalescence. Doxycycline is a hydrophilic pharmaceutics and can be easily dissolved in water and eluted by channel diffusion. The release of doxycycline from the drug-eluting implants thus exhibit diffusion-controlled release behavior. Relatively, metronidazole is a partially hydrophilic drug, which effectively inhibits anerobic microorganisms and protozoan infections.[13] The low aqueous solubility of metronidazole hindered water penetration and additional release. However, water will be taken up by a water-soluble antibiotic with a high osmotic pressure through the polymer, causing swelling of the particle. The polymer matrix may break under this swelling to form openings for antibiotic release [Figure 8(c)]. Finally, when the molecular weight of the polymer decreases sufficiently, loss of polymer begins. The antibiotic will be released along with this polymer loss [Figure 8(d)]. Furthermore, the amines degenerated from the metronidazole in acidic condition of the eluent may also accelerate degradation of the polymeric materials. The degradation-controlled release could thus be observed. This may explain why metronidazole showed a triphase release behavior from the drug-eluting implants.

A significant advantage of the drug-eluting implants is that the local antibiotic concentrations are much greater than the minimum inhibitory concentration (MIC) for most pathogens commonly isolated in the periodontal pockets.[18,19] Furthermore, the

concentrations of both doxycycline and metronidazole eluted from the drug-eluting implants were much greater than the MIC for up to 4 and 3 weeks, respectively (Table I), and the bioactivities of the antibiotics remained high after the fabrication process. Finally, more sophisticated measures, such as fabricating the biodegradable implants in a clean room, should be taken to assure that no kind of contamination occurs during the manufacturing process. In addition, the result of our recent study[20] suggested that the biodegradable composites can be sterilized by gamma irradiation of less than 25 kGy and release high concentrations of antibiotics (well above the MIC) *in vitro* for up to 28 days after sterilization. These should help ensure the safety of the biodegradable drug-eluting devices before their implantation and will be the topics of our future studies.

CONCLUSIONS

This article has adopted a solvent-free method of processing biodegradable polymers as antibiotic implants for a long-term drug release in periodontal pockets. An elution method was utilized to characterize the *in-vitro* release rates of the antibiotics from the implants over a 28-day period. The HPLC analysis and the bacterial inhibition test showed that drug-eluting implants released high concentrations and activity of doxycycline and metronidazole (well above the minimum inhibition concentration) *in vitro* for the period of time needed to treat bone infection; i.e., 3 to 4 weeks. The bioactivities of the released antibiotic remained high after the manufacturing process. The initial burst and the total release period of the drugs can be adjusted by varying the processing parameters. By adopting this novel technique, we will be able to fabricate biodegradable implants of various pharmaceuticals for a long-term drug deliveries.

Further studies being conducted in our laboratory are investigating the drug-eluting implants in animal model, such as the localized periodontitis models. Eventually, biodegradable drug-eluting implants may be used in humans for the treatment of various periodontal diseases.

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