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Gentamicin-loaded bioresorbable films for prevention of bacterial infections associated with orthopedic implants

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Abstract: Adhesion of bacteria to biomaterials and the ability of many microorganisms to form biofilms on foreign bodies are well-established as major contributors to the pathogenesis of implant-associated infections. Treatment of bone infection remains problematic, due to the difficulty of systemically administered antibiotics to locally penetrate bone. The current research addresses this issue by focusing on the development and study of novel gentamicin-loaded bioresorbable films designed to serve as "coatings" for fracture fixation devices and prevent implant-associated infections. Poly(L-lactic acid) and poly(D,L-lactic-*co*-glycolic acid) films containing gentamicin were developed through solution processing. The effects of polymer type, drug content, and processing conditions on the drug release profile were studied with respect to film morphology. The examined films generally exhibited a burst effect followed by a moderate approximately constant rate of release. The drug contents in the surrounding medium exceeded the required minimal effective concentration. Various gentamicin concentrations that were released from the films with time exhibited efficacy against bacterial species known to be involved in orthopedic infections. The developed systems can be applied on the surface of any metallic or polymeric fracture fixation device, and may therefore comprise a significant contribution to the field of orthopedic implants. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res 83A: 10–19, 2007

Key words: bioresorbable films; poly(lactic acid); poly(D,L-lactic-*co*-glycolic acid); gentamicin; controlled drug delivery

INTRODUCTION

Implant-associated infections are grave complications that currently restrict the use of biomaterials in humans. These infections require long periods of antibiotic therapy and repeated surgical procedures. Bacterial adhesion to biomaterials and the ability of many microorganisms to form biofilms on foreign bodies are well-established as major contributors to the pathogenesis of implant-associated infections. Bacteria in a biofilm are protected from the host's immune defenses and exhibit a marked increase in antibiotic resistance.[1] Treatment of bone infection therefore remains problematic, due to the difficulty of systemically administered antibiotics to locally penetrate bone. Furthermore, bacteria adhere to the bone matrix and to orthopedic implants and elude both the host's defenses and antibiotics by developing a slimy film or acquiring a very slow metabolic rate.[2] In the worst cases the implant can therefore become the source of infection and the only currently available treatment is to remove the implant and perform another implantation, which is a horrible ordeal for the patient as well as for the doctor. Conventional therapy with systemic antibiotics is expensive, prone to complications and often unsuccessful.[3] Major problems in treating osteomyelitis include poor antimicrobial distribution at the site of infection because of limited blood circulation to infected skeletal tissue, and inability to directly address the biofilm pathogen scenario. A high systemic dosage of antibiotics to facilitate sufficient tissue and biofilm penetration is undesirable due to possible grave toxic side effects. Controlled antimicrobial release systems inside orthopedic devices thus represent alternatives to conventional systemic treatments.[4] These include PMMA bone cements loaded with antibiotics, drug-containing polymeric coatings, and

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drug-loaded bioresorbable implants in addition to the fracture fixation device.

One commonly used infection management method with orthopedic implants utilizes antibiotics that are loaded into clinical bone cements based on polymethylmethacrylate (PMMA). These nonbiodegradable polymer cements have been employed clinically in various forms for nearly four decades to prevent or treat osteomyelitis.[5–8] Several such commercial antibiotic-impregnated bone cements have been approved, including Simplex™ P with erythromycin, colistin tobramycin (Stryker, UK) which has been sold in Europe for over 20 years and is now also sold in the USA and gentamicin-containing Palacos™ PMMA cement (refobacin palacos r-Knochenzement®, Merck, Austria). PMMA is not biodegradable. Therefore, when clinical failure occurs, secondary surgery is necessary to remove the PMMA before new bone can regenerate. Furthermore, PMMA polymerization exhibits a well-known, prominent exothermic effect.[9] This heat as well as residual MMA monomers can kill healthy surrounding bone cells.[7] Additional disadvantages are the low bonding strength of PMMA to the implant surface and known soft tissue encapsulation of PMMA.

Researchers have coated metal implants such as plates and wires with a polymer/gentamicin layer, using the dipping technique.[10–12] The obtained release profile demonstrated that most of the drug was released within several hours after exposure to an aqueous environment. Even when achieving better control over release kinetics, the system was only able to provide the desired drug release for less than 12 days. Melt processing techniques, such as compression molding and extrusion, were used to develop bioresorbable implants loaded with gentamicin to serve as an additional part of the fracture fixation device. Cylinders, films, and disks were developed and studied.[13–17] Other delivery systems combined bioresorbable polymers with osteoconductive materials such as calcium compounds. Several studies have focused on the inclusion of ceramic materials such as tricalcium phosphate and hydroxyapatite in antibiotic-loaded systems for treating bone infection, since, their chemical composition is very similar to the bone mineral phase.[18,19]

The aim of the present study was to develop and examine gentamicin-loaded bioresorbable films that are "bound" to orthopedic implants (by slightly dissolving their surface before attaching it to the implant's surface) and prevent bacterial infections by a gentamicin-controlled release phase for at least one month. These systems will provide desired drug delivery profiles and will not require an additional implant. This study focused on the effects of film components and processing conditions on the drug release profile and bacterial inhibition.

EXPERIMENTAL

Materials

Bioresorbable polymers

Two types of poly(L-lactic acid) were used:

Poly(L-lactic acid), Resomer L210 (Inherent viscosity (I.V.) = 3.9 deciliter/gram (dL/g) in $CHCl_3$ at 30°C, Molecular weight (M.W.) = ~450,000 Daltons (Da)), Boehringer Ingelheim, Germany. This relatively high molecular weight polymer will herewith be designated **PLLA1**.

Poly(L-lactic acid), Medisorb 100L (I.V= 1.6 dL/g in $CHCl_3$ at 30°C, M.W. = ~200,000 Da), Alkermes, USA. This relatively low molecular weight polymer will herewith be designated **PLLA2**.

Two types of 75/25 poly(D,L-lactide-*co*-glycolide) were used:

75/25 poly(D,L-lactide-*co*-glycolide), A123-12 (I.V. = 0.65 dL/g in $CHCl_3$ at 30°C, M.W. = ~97,100 Da), Absorbable Polymer Technologies, USA. This relatively high molecular weight polymer will herewith be designated **PDLGA1**.

75/25 poly(D,L-lactide-*co*-glycolide), A123-03 (I.V. = 0.24 dL/g in $CHCl_3$ at 30°C, M.W. = ~23,800 Da), Absorbable Polymer Technologies, USA. This relatively low molecular weight polymer will herewith be designated **PDLGA2**.

Drug: gentamicin sulfate (cell-culture tested), 590 μg gentamicin base/mg, Sigma G-1264.

Reagents

Isopropyl alcohol (propanol) was purchased from Frutarom, Israel.

O-phthaldialdehyde (P0657), sodium borate 0.04*M* (B0127) and 2-hydroxyethylmercaptan (63690) were purchased from Sigma.

Microorganisms

Staphylococcus aureus, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* were used in this study. All 3 strains were clinical isolates from orthopedic cases (the strains were kindly provided by the Department of Microbiology, Rambam Medical Center, Haifa, Israel).

Film preparation

Polymer films (0.12–0.15 mm thick) consisting of PLLA or PDLGA and gentamicin were prepared by a three-step solution processing method:

a. Components were mixed in chloroform at room temperature until polymer (1 gr) is totally dissolved in the chloroform, giving a clear solution, and gentamicin was added to the polymer solution. Two constant drug loadings were used: 100 mg gentamicin (10% w/w) and 300 mg gentamicin (30% w/w). Both a diluted and a concentrated solution were prepared for each polymer/gentamicin system, as follows:

Dilute solutions

Dilute solutions were prepared by using relatively large volumes of solvent (50 mL chloroform which creates polymer concentration of 0.02 g/mL, gentamicin concentration: 0.2 or 0.6 mg/mL). The polymer was totally dissolved, but only small part of the drug molecules was dissolved. The rest remained in particles (aggregates) and mixed with the polymer molecules in the chloroform solution.

Concentrated solutions

Concentrated solutions were prepared by using relatively small volumes of solvent (20 mL chloroform which creates a polymer concentration of 0.05 g/mL, gentamicin concentration: 0.5 or 1.5 mg/mL). In these solutions the polymer was totally dissolved, whereas the gentamicin powder was only broken into small particles (aggregates), which yielded an opaque polymer solution.

(b) Solution casting into a Petri dish and solvent drying under atmospheric pressure at room temperature. Two solvent evaporation rates were used. The Petri dish of the dilute solutions was partially covered, so as to enable a relatively slow evaporation rate of 2–5 mL/h. The films cast from dilute solutions using this slow evaporation rate are termed "A-type films". The petri dish of the concentrated solutions was not covered, so as to enable a relatively fast evaporation rate of 10–20 mL/h. The films cast from concentrated solutions using this fast evaporation rate are termed "B-type films".

(c) Isothermal heat treatment at a temperature higher than the glass transition temperature of the polymers (75°C for PLLA, 40°C for PDLGA) for 2 h in a vacuum oven. This heat treatment enabled disposal of residual solvent.

All film types used in this study are presented in Table I.

TABLE I
The Films Used in this Study

Number	Host Polymer	Gentamicin Content (% w/w)	Film Type
1	PLLA1	10	A
2	PLLA1	10	B
3	PLLA1	30	A
4	PLLA1	30	B
5	PLLA2	10	A
6	PLLA2	10	B
7	PLLA2	30	A
8	PLLA2	30	B
9	PDLGA1	10	A
10	PDLGA1	10	B
11	PDLGA1	30	A
12	PDLGA1	30	B
13	PDLGA2	10	A
14	PDLGA2	10	B
15	PDLGA2	30	A
16	PDLGA2	30	B

A-Type film was prepared using a dilute solution and slow solvent evaporation rate.

B-Type film was prepared using a concentrated solution and fast solvent evaporation rate.

Morphological characterization

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

Scanning electron microscopy (SEM) of cryogenically fractured surfaces was performed using a Jeol JSM 6300 at an accelerating voltage of 5 kV. The SEM samples were sputter-coated with AU prior to observation.

In vitro weight loss studies

The polymer films (1 × 1 cm^2) were weighed and then immersed in PBS (3 mL, in a Petri dish) at 37°C for 28 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:

$$\text{Weight loss } (\%) = 100 \times \frac{w_0 - w_f}{w_0}, \qquad (1)$$

where w_0 and w_f are the weights of the dried films before and after exposure to water, respectively. Four samples were tested for each point. The mean values and standard deviations are presented in the graphs.

In vitro gentamicin release study

Polymer/gentamicin films were immersed in 40 mL PBS at 37°C for 12 weeks (only PLLA1 was immersed for 24 weeks) in semi-static conditions, to determine the kinetics of gentamicin release from the films. Sodium azide (0.05% w/v) was added to prevent contamination by various microorganisms. 1.5 mL samples were collected at the following times: 0, 6 h, 24 h, 2 d, 3 d, 1, 2, 3, 4, 6, 8, 10, 12 weeks. The amount removed was replaced with fresh PBS and 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 gentamicin content in each sample was determined with a UV/vis scanning spectrophotometer (Anthos, Zenyth 200rt). Sampath and Robinson's procedure[20] for analyzing gentamicin sulphate was followed with a slight modification. *O*-phthaldialdehyde reagent was prepared by adding 2.5 g *o*-phthaldialdehyde, 62.5 mL methanol and 3 mL 2-hydroxyethylmercaptan to 560 mL 0.04*M* sodium borate in distilled water. 200 µl gentamicin solution, 200 µl isopropanol (to prevent sedimentation), and 200 µl *o*-phthaldialdehyde reagent were reacted for 45 min at room temperature. The absorbance, which corresponds to the gentamicin concentration, was then measured at 333 nm. The gentamicin working range was 20–140 µg/mL. The samples were therefore diluted. A calibration curve was prepared for each set of measurements. The calibration lines were the same within the experimental error for at least 1000 h (correlation coefficient >0.99, slope: 0.0129), which indicated that the formulated *o*-phthaldialdehyde reagent was stable.

TABLE II
MIC Value

Microorganism	MIC (μg/ml)
P. aeruginosa	6
S. epidermidis	10
S. aureus	10

Three samples were examined for each film type. The mean values and standard deviations are presented in the graphs.

Preparation of bacteria

All strains were grown overnight on Müller-Hinton (Difco) agar plates[21] at 37°C. The bacterial cells were collected and resuspended in saline, and adjusted to 1×10^8/mL by visual comparison with a 0.5 Mcfarland standard.

Evaluation of residual bacteria

Samples of 10 or 100 μl were collected, at the appropriate time, and spread on agar plates containing Müller-Hinton. CFU/mL (colony forming units) were counted after 24 h incubation at 37°C.

MIC (minimal inhibitory concentrations)

The MIC's of gentamicin against all tested microorganisms were determined using the twofold tube dilution method in Müller-Hinton broth, and are presented in Table II.

Microbiological experiment design

The microbiological study, that is the kinetics of residual bacteria, was studied in two different variations as follows:

Variation (a): Bacteria were added at the beginning of the release study (time 0)

In this variation the bacterial stains were added at the beginning of the release study at a concentration of 1×10^8/mL, and the effect of the gentamicin released from the films was tested. The concentration of gentamicin in each tube was estimated by the "*o*-ophthaldehyde" method. The samples were collected after 0, 2 h, 6 h, 12 h, and 24 h, and viable counts were performed. Microorganisms in the presence of PBS only served as the control. Bacterial growth was counted on Müller-Hinton agar plates.

The final calculation established the residual number of bacteria in the presence of gentamicin.

Variation (b): Bacteria were added after preparation of the films

The effect of the gentamicin released from the films (in PBS) on bacteria added after preparation of the films was studied. The film samples were collected after 0, 6 h, 24 h, 3 d, 1, 2, 3, and 4 weeks. All test tubes contained the gentamicin released according to the time of collection. The gentamicin concentration in each tube was determined by the "*o*-ophthaldehyde" method. The organisms were then added to a final concentration of 1×10^8/mL. The samples were collected after 0, 2 h, 6 h, 12 h, and 24 h for viable counting and were expressed as CFU (colony forming units)/mL. Bacteria grown in PBS served as control. Müller-Hinton agar plates were counted for bacterial growth. The calculation established the residual number of bacteria in the presence of gentamicin.

RESULTS AND DISCUSSION

Sixteen films (all in triplicates) were prepared for this study, using the method described in details in the experimental section. In general, four host polymers were used (two PLLAs and two PDLGAs), and films of each of them were prepared with two drug loadings [10% (w/w) and 30% (w/w)]. Films of each one of these eight compositions were prepared using two types of processing conditions: a dilute solution followed by slow solvent evaporation rate, and a concentrated solution followed by fast evaporation rate. All 16 films are described in Table I.

Microstructure and weight loss profile of the polymer/gentamicin films

Bioresorbable polymeric films containing gentamicin were prepared using solution processing, accompanied by a post-preparation isothermal heat treatment. In this process, the solvent evaporation rate determines the kinetics of drug and polymer solidification and thus, the drug dispersion/location in the film. In general, two types of polymer/gentamicin film structures were created and studied for all matrix polymer types, as presented in Figure 1 for the PLLA1 films:

a. A polymer film with drug particles located on its surface, as presented in Figure 1(a). This structure, derived from a dilute solution, was obtained using the slow solvent evaporation rate, which enables prior drug nucleation and growth on the polymer solution surface. This skin formation is accompanied by a later polymer core formation/solidification. This structure was named the "A-type".

b. A polymer film with most of drug particles distributed within the bulk, as presented in Figure 1(b). This structure, derived from a concentrated solution,

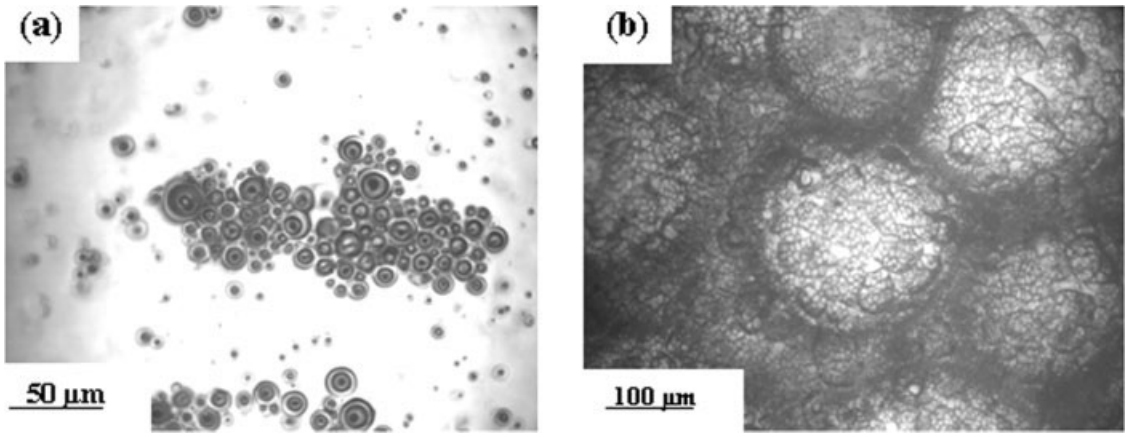

Figure 1. Light micrographs of PLLA1/gentamicin 30% (w/w) films: (a) A-type film, (b) B-type film.

was obtained using the fast solvent evaporation rate, and resulted from drug nucleation and segregation within a dense polymer solution. Solidification of drug and polymer occurred concomitantly. This structure was named the "B-type". Since gentamicin is a water-soluble drug, which practically does not dissolve in chloroform, some of its particles diffused out towards the surface during solvent evaporation. The drug concentration near the surface is therefore probably higher than in the center. Furthermore, the relatively fast evaporation rate affects the quality of the polymeric film and voids were actually created. The drug molecules are located above, below and around these voids.

We have previously developed equivalent structures for bioresorbable films loaded with steroids such as dexamethasone.[22] The morphology and formation process of these structures have been studied extensively and a detailed model describing the structuring of these films is described elsewhere.[22]

The microstructure of the degrading PLLA films is presented in Figure 2. The SEM fractographs indicate

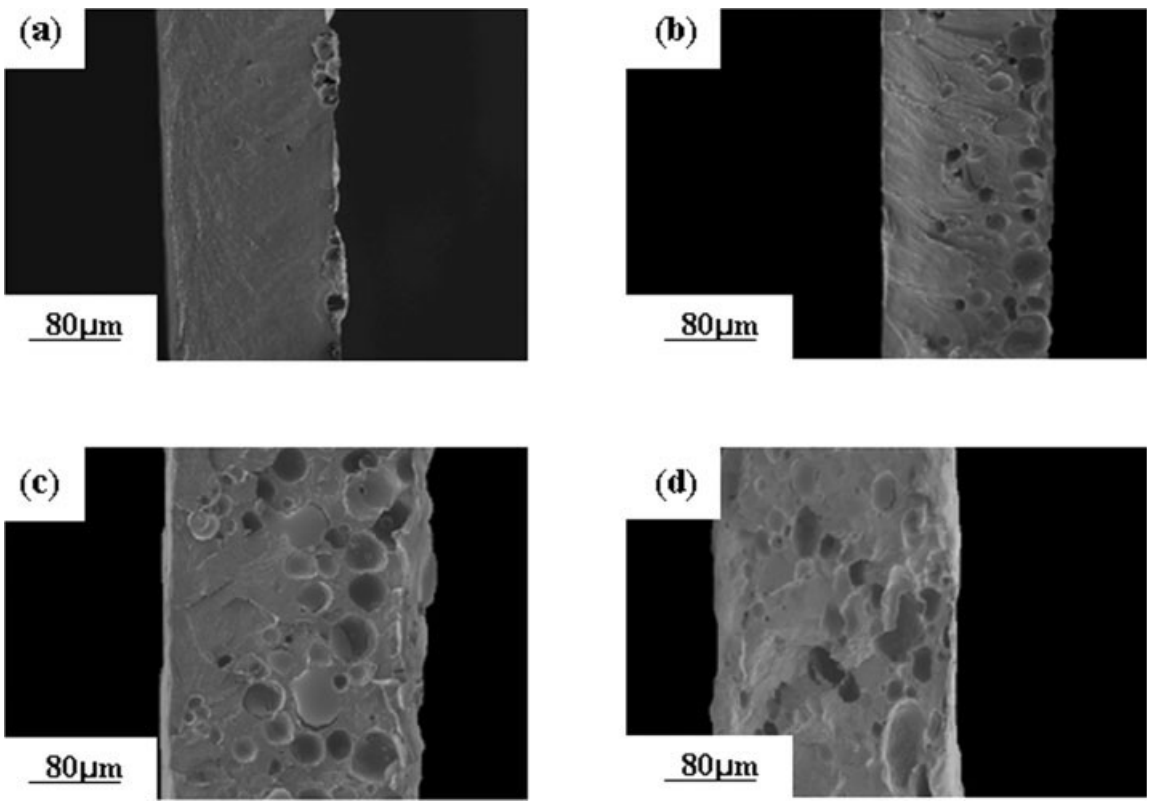

Figure 2. SEM micrographs of A-type PLLA1/gentamicin 30% (w/w) film after the following degradation times: (a) start, (b) one week, (c) four weeks, (d) five months.

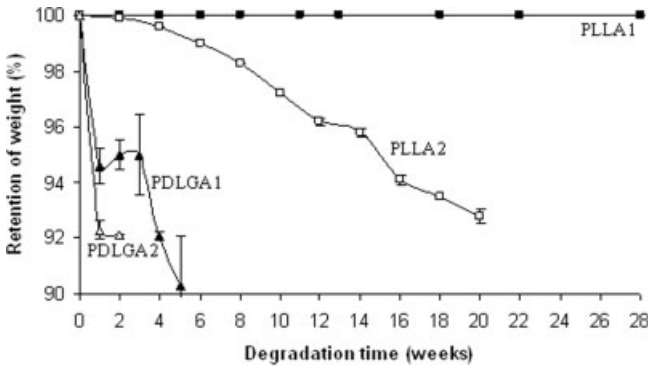

Figure 3. Weight retention of various polymer films as a function of degradation time. The host polymer is indicated.

that the drug particles are indeed located on the surface of the A-type film [Fig. 2(a)]. Since they are relatively large and adhere poorly to the polymer, they probably left the film during its fracturing in liquid nitrogen and the holes are observed. Fractured surfaces of the degrading films indicate that degradation and weight loss begin near the surface, where water molecules are first absorbed, and proceed in the bulk towards the bottom of the film. After one week of degradation, relatively large holes were created near the surface and smaller ones appear in deeper locations towards the center of the film [Fig. 1(b)]. After four weeks of degradation, holes appeared in most of the film volume [Fig. 2(c)]. After 5 months of degradation, relatively large holes were observed throughout the entire film [Fig. 2(d)]. Although PLLA is a hydrophobic polymer, it absorbs water and the thickness of the film therefore increased with time from 160 to 230 μm after 5 months in PBS.

The neat films were exposed to PBS at 37°C and their weight loss was measured at weekly intervals for PDLGAs and 2-week intervals for PLLAs. The weight loss profiles are presented in Figure 3. PLLA1 did not undergo any weight loss, whereas PLLA2 lost ~7% of its weight after 20 weeks of degradation. This difference results from differences in the initial molecular weight. The initial molecular weight of PLLA2 (200,000 Da) was lower than that of PLLA1 (450,000 Da) and therefore low molecular weight fragments that could diffuse out from the film were created in the former but not in the latter. PDLGA2 exhibited a weight loss of 8% after one week of degradation, while PDLGA2 exhibited the same weight loss after 5 weeks of degradation. These differences also result from differences in the initial molecular weight. The relatively low weight loss rate of the PLLAs compared to that of PDLGAs results from the more hydrophobic nature of the former and also from its semicrystalline structure (compared to the amorphous PDLGA).

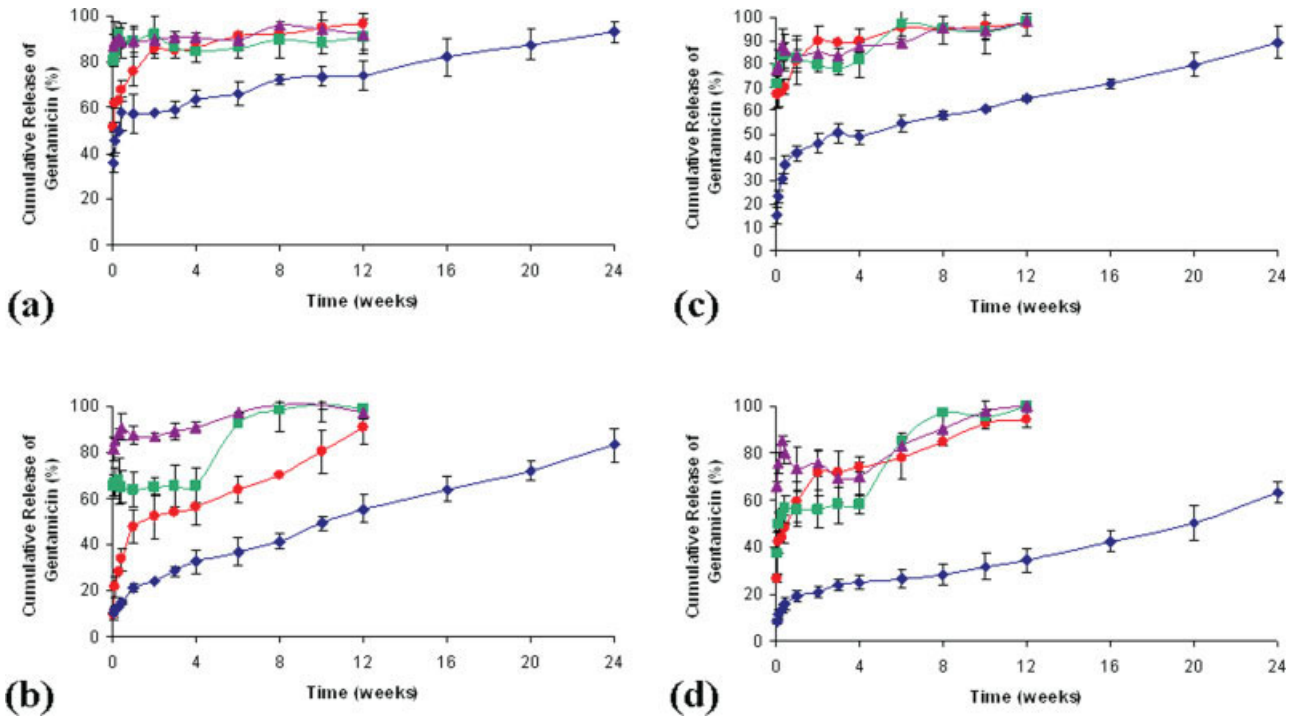

Figure 4. *In vitro* cumulative gentamicin release from polymer/gentamicin films: (a) A-type films containing 10% (w/w) gentamicin, (b) B-type films containing 10% (w/w) gentamicin, (c) A-type films containing 30% (w/w) gentamicin, (d) B-type films containing 30% (w/w) gentamicin: ◆- PLLA1; ●- PLLA2; ■- PDLGA1; ▲- PDLGA2. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

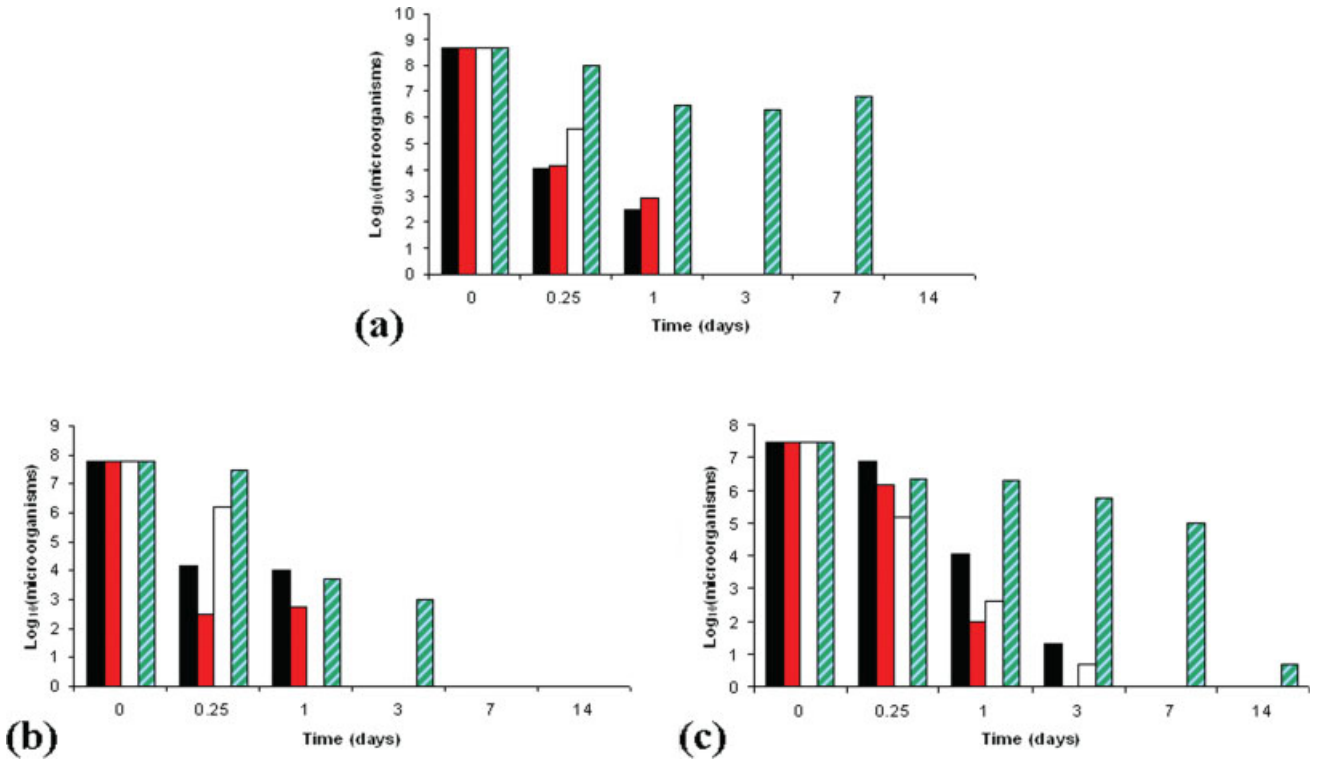

Figure 5. Number of colony forming units (CFU) versus time for variation A experiment: (a) *Pseudomonas aeruginosa*, (b) *Staphylococcus epidermidis*, (c) *Staphylococcus aureus*. The releasing films are: ■- A-type PLLA film containing 30% (w/w) gentamicin; ■- B-type PLLA film containing 30% (w/w) gentamicin; □- B-Type PDLGA film containing 10% (w/w) gentamicin; ▨- control - A-type PLLA film without gentamicin. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Gentamicin release from bioresorbable films

The effects of polymer type, initial molecular weight, film morphology (drug location/dispersion) and drug loading on the gentamicin release profile were examined. The cumulative gentamicin release profiles from films of various polymer/gentamicin systems are presented in Figure 4. All release profiles exhibited a burst release followed by a relatively slow release phase. Such profiles are desirable for applications such as fracture fixation, where a burst release is needed to prevent infection and kill the microorganisms found in the implant area before they settle and create a biofilm which antibiotics cannot easily penetrate. A second phase of slow drug release is necessary to prevent microbial infections at the implant site during healing. The burst effect is obtained due to diffusion of drug molecules located on the surface and in polymer layers close to the surface, while the continuing release is obtained due to diffusion of drug molecules from the bulk and is affected by the host polymer's degradation rate.

Gentamicin's therapeutic level in serum is 4–8 μg/mL and its toxic level is 12 μg/mL.[23] The MIC values for the bacteria used in the current study are presented in Table II. All studied films released gentamicin at levels higher than the MIC. B-type PLLA1/gentamicin (10%) exhibited the lowest burst release (35.6 mg). The burst release of gentamicin from several films was slightly higher than its toxic level. In such cases, when burst effect is suspected of exceeding the toxic level, films with a smaller total area can be used.

Effect of polymer type and initial molecular weight

The PLLA films exhibited lower burst effects followed by slower release rates, compared to PDLGA films. The latter are more hydrophilic and therefore absorb water more easily. They therefore exhibit a higher burst release. This is true mainly for B-type films, whereas A-type films release most of the water-soluble gentamicin located on their surface within 6 h. The weight loss rate of the PLLA films is lower than that of PDLGA films (Figure 3). The constant release rate from the PLLA films is therefore lower than that from PDLGA films.

As expected, lower molecular weight polymers exhibited higher burst effects and higher release rates, due to a higher quantity of hydroxylic and carboxylic edge groups, which make it more hy-

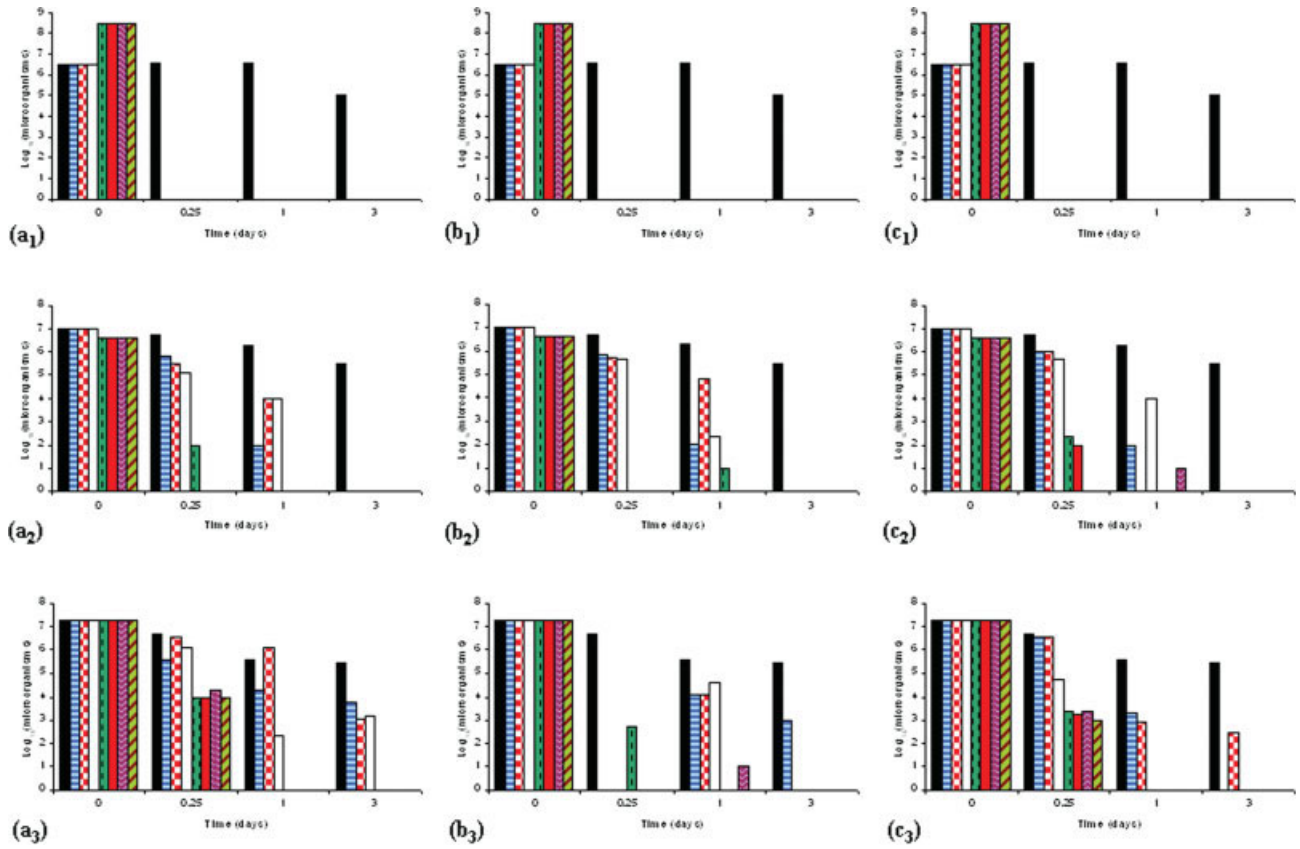

Figure 6. Number of CFU of: (1) *Pseudomonas aeruginosa,* (2) *Staphylococcus epidermidis,* (3) *Staphylococcus aureus,* versus time, according to variation B. The releasing films are: (a) A-type PLLA film containing 30% (w/w) gentamicin; (b) B-type PLLA film containing 30% (w/w) gentamicin; (c) B-type PDLGA film containing 10% (w/w) gentamicin: ■- Control - initial time; ■ - 6 hours ■- 1 day □- 3 days ■- 7 days ■- 14 days ■- 21 days ■- 28 days. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

drophilic. Furthermore, a lower molecular weight results in a lower glass transition temperature, which facilitates faster drug release from the polymer.

Effect of drug location/dispersion in the film

Processing conditions strongly affected the release profile through morphology for both PLLAs and PDLGA1. Thus, dilute solutions and slow evaporation rates resulted in A-type films with the drug located on the surface [Fig. 1(a)], which exhibited a relatively high burst effect followed by a slow release rate. In contradistinction, concentrated solutions and fast evaporation rates resulted in B-type films, in which most of the drug is located in the polymeric film and some is located on the surface [Fig. 1(b)], which exhibited a relatively low burst effect followed by a lower release rate. Only the PDLGA2 films exhibited similar release profiles for A and B-type films. This behavior is obtained due to the polymer's relatively high degradation rate (Fig. 3) and gentamicin's extremely hydrophilic nature.

Effect of drug content

When gentamicin release from films containing 10% gentamicin was compared to its release from films containing 30% gentamicin by putting them on the same graph, it was found that the release profiles are similar in most cases, except for A-type PLLA1 films.

In conclusion, the studied systems demonstrated two-phase gentamicin release profiles, that is a burst release followed by a slow continuing release, which are optimal for preventing bacterial infection associated with orthopedic implants. In all systems the released gentamicin quantities exceeded the minimal effective concentration and in most systems the released quantities were lower than the toxic level. The gentamicin release profiles from the various systems is determined by the host polymer, its initial molecular weight and the processing conditions, which affect the drug location/dispersion in the film. Drug loading has a minor effect on the release profile.

Microbiological evaluation of the effect of gentamicin 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 with the initial number of bacteria. Bacteria present in PBS only served as the control.

Variation a

In this variation the bacteria were added at the beginning of the films' release, in order to simulate contamination at the time of implantation. The results are presented in Figure 5. No bacteria were left after 1 d – 3 d, compared to the control where all bacteria survived even after 7 d, in the presence of a very high concentration of the starter (1×10^8/mL CFU). All films exhibited marked gentamicin release, which was responsible for the dramatic decrease in bacterial survival (10^3/mL CFU after 1 d). Moreover, the film preparation did not affect gentamicin's activity as an antimicrobial agent.

Variation b

In this variation the bacteria were added to tubes containing priorly-released drug. The samples were collected after 6 h, 1 d, 3 d, 1 w, 2 w, 3 w, and 4 w. The bacteria were added to these tubes and their number was monitored. This was performed in order to evaluate the integrity of gentamicin in PBS after long release times and to simulate systemic bacterial infection of the implant site after a prolonged surgical procedure. The results are presented in Figure 6. The released amount of gentamicin decreased the number of *P. aeruginosa* bacteria (Fig. 6 a1, b1, c1) after 6 h and the number of *Staph. epidermidis* (Fig. 6 a2, b2, c2) and *Staph. aureus* (Fig. 6 a3, b3, c3) after 3 days. Gentamicin was effective in all samples tested. The films' collection and preparation method did not affect gentamicin's antibiotic potency. The differences in survival times are probably due to the bacteria's different MIC values.

SUMMARY AND CONCLUSIONS

PLLA and PDLGA films containing gentamicin were prepared by solution processing. These films are designed to serve as "coatings" for fracture fixation devices and prevent implant-associated infection. Investigation of the films focused on cumulative gentamicin release as affected by film processing conditions, drug content, polymer type and initial molecular weight. Controlled gentamicin release profiles from selected film prototypes in the presence of various bacteria were studied.

The examined films generally exhibited a burst effect followed by a moderate release at an approximately constant rate. The drug contents in the surrounding medium exceeded the required minimal effective concentration. The released gentamicin also resulted in a significant decrease in bacterial viability, even at very high bacterial concentrations (1×10^8/mL CFU). Practically no bacteria survived after 1–3 days. The film preparation did not affect gentamicin's potency as an antibacterial agent.

A dilute solution and slow evaporation rate resulted in films with most of the drug located on the surface - A-type film, whereas a concentrated solution and fast evaporation rate resulted in films with most of the drug located in the bulk - B-type film. In most cases both the initial burst release and the later constant release are higher for the A-type film than for the B-type. However, the release profile from A-type films is similar to the release profile from B-type films only for systems based on host polymers with fast degradation rates.

This study demonstrates that the release profiles of gentamicin from the various systems is determined by the host polymer type, its initial molecular weight and the film processing conditions, which affect the drug location/dispersion within the film. Drug loading has a minor effect on the release profile.

This study enables in-depth understanding of gentamicin-loaded films and as a result, the production of systems with desired gentamicin controlled release profiles, that is with the desired burst effect and continuing release rate (within the therapeutic window) for several weeks. The developed systems can be applied on the surface of any metallic or polymeric fracture fixation device, and can therefore make a significant contribution to the field of orthopedic implants.

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