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Efficacy of antimicrobial polymer coatings in an animal model of bacterial infection associated with foreign body implants

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Objectives: To assess support discs, comprising polyethylene terephthalate (PET), coated with different polymer/levofloxacin combinations for antimicrobial activity in an animal model of infection, in order to explore the use of specific polymer coatings incorporating levofloxacin as a means of reducing device-related infections.

Methods: Aliphatic polyester-polyurethanes containing different ratios of poly(lactic acid) diol and poly(caprolactone) diol were prepared, blended with levofloxacin and then used to coat support discs. The *in vitro* levofloxacin release profiles from these discs were measured in aqueous solution. Mice were surgically implanted with the coated discs placed subcutaneously and infection was initiated by injection of 10^6 cfu of *Staphylococcus aureus* into the subcutaneous pocket containing the implant. After 5, 10, 20 and 30 days, the discs were removed, and the number of bacteria adhering to the implant and the residual antimicrobial activity of the discs were determined.

Results: *In vitro*, the release of levofloxacin from the coated discs occurred at a constant rate and then reached a plateau at different timepoints, depending on the polymer preparation used. *In vivo*, none of the discs coated with polymer blends containing levofloxacin was colonized by *S. aureus*, whereas 94% of the discs coated with polymer alone were infected. All discs coated with levofloxacin-blended polymers displayed residual antimicrobial activity for at least 20 days post-implantation.

Conclusions: Bioerodable polyester-polyurethane polymer coatings containing levofloxacin can prevent bacterial colonization of implants in an intra-operative model of device-related infections.

Keywords: *Staphylococcus aureus*, implant-associated infections, levofloxacin

Introduction

The use of indwelling medical devices has become an essential part of modern day medicine. While these prosthetic implants have improved clinical outcomes for patients, particularly for the critically ill, their increased use has given rise to an increase in implant-associated infections.[1] The consequences of device-related infections can be substantial, both medically, in terms of morbidity and mortality, and economically, in terms of expended financial resources. While the mortality rates associated with the use of urinary catheters, for example, are low in comparison with the mortality associated with central venous lines, a comparatively higher number of cases of infection and more frequent application result in significant morbidity.[1] Conversely, although heart assist devices are implanted less frequently, there is a significant risk of infection and >25% attributable mortality.[1] The burdens these respective situations place on healthcare systems illustrate how the type of implant and location within the body can determine the risk and outcome of associated disease.

To establish infection, bacteria first adhere to and then proliferate on the surface of the implanted device. During this process they form dense microbial communities called biofilms, which exhibit a profound ability to evade host defences and resist antimicrobial therapy.[2] Bacterial biofilms are difficult to eradicate and often lead to persistent and recurrent infections, which can adversely affect the function of the indwelling device, necessitate the use of aggressive therapies and require eventual removal of the device with devastating consequences for the patient. In addition, systemic life-threatening infections can occur from bacteria detaching from biofilms and seeding throughout the body.

Current strategies aimed at preventing device-related infections focus primarily on the use of antimicrobial therapies, and include systemic antibiotic prophylaxis and local administration of antimicrobial agents through irrigation of the surgical site, placement of antibiotic carriers and coating of the device itself.[3] The systemic delivery of antibiotics and intra-operative administration through irrigation and the immersion of devices in an antimicrobial solution immediately prior to implantation are hampered by multiple variables and the lack of an established method of application.[4] Accordingly, antimicrobial coatings have emerged as a desirable method to prevent implant-related infections, because of the potential for persistent antimicrobial activity, with high local concentrations and low systemic levels of the drug being delivered.

The development of biologically inert coating materials that release antimicrobial compounds is complicated by the chemical properties of the components used in the development of these coatings. The dispersal of antibacterial agents into both biostable and biodegradable polymer matrices has proven to be a major challenge, due to the limited solubility of various antibacterial agents in the polymer matrix.[5–7]

A number of features of the antimicrobial agent and polymer matrix can influence the degree of solubility, including: (i) polarity; (ii) hydrophobicity/hydrophilicity; (iii) crystallinity; (iv) differential solubility of the polymer and antibacterial agents in different solvents; and (v) differential melting temperatures, whereby the requirement for excessive heat to disperse one agent can result in the degradation of the other. Consequently, variation in the release profile of the antimicrobial agent between samples can occur if there is inadequate dispersal of the chemical agent within the polymer matrix.

Adopting new approaches for the development of polymer conjugates used to coat implantable devices provides an opportunity to apply antimicrobial agents directly to the device surface, thus preventing bacterial colonization of the implant and inhibiting implant-associated infection. Here, we describe how a bioerodable polymer coating containing levofloxacin can prevent colonization of implants in a murine model of subcutaneous staphylococcal infection associated with foreign body implantation.

Methods

Bacterial strains

Staphylococcus aureus strains Xen29[8] (kanamycin resistant) and ATCC 29213 were routinely grown aerobically at 37°C on horse blood agar. Tryptone soya broth supplemented with 0.25% glucose (TSBG) and Luria–Bertani (LB) agar supplemented with 200 mg/L kanamycin were used to isolate bacteria from excised implants.

Synthesis of polyester-polyurethane (PE-PU) polymers

An amorphous aliphatic PE-PU polymer made from poly(lactic acid) diol (DLLA), poly(caprolactone) diol (PCLD) (ERA Polymers Pty Ltd, NSW, Australia) and 1,6-hexamethylene diisocyanate (HDI) was developed for blending with levofloxacin. DLLA was produced by the polycondensation reaction of an 88% DL-lactic acid solution (Sigma–Aldrich, St Louis, MO, USA) using 1-4-butanediol (Sigma–Aldrich) as the initiator and 2-ethylhexanoate (Sigma–Aldrich) as the catalyst. DLLA (1000 g/mol) and PCLD (1000 g/mol) were both dried under high vacuum overnight at 75°C, and HDI was distilled before use. Polyol mixtures were catalysed by the addition of dibutyltin dilaurate and after the addition of HDI were cured at 80°C overnight. The formulations for the PE-PU polymers prepared for blending with levofloxacin are shown in Table 1.

The molecular weights of the polyester polyols and PE-PU polymers were determined by gel permeation chromatography (GPC) performed in tetrahydrofuran or dimethylformamide at 1.0 mL/min at 25°C, using a Waters

Table 1. Composition of polyester-polyurethane (PE-PU) polymer blend preparations used in this study

Polymer	DLLA 1000[a]	PCLD 1000[b]	HDI 1000[c]	Mn (Mw)	LVX[d]	Polymer appearance
PU1	90	10	100	46360 (77953)	0	clear, flexible PU
PU2	80	20	100	50245 (85116)	0	clear, flexible PU
PU3	70	30	100	60642 (106541)	0	clear, flexible PU
PU4	60	40	100	69805 (123594)	0	clear, flexible PU
LPU1	90	10	100	46360 (77953)	10	opaque, evenly dispersed
LPU2	80	20	100	50245 (85116)	10	opaque, evenly dispersed
LPU3	70	30	100	60642 (106541)	10	opaque, unevenly dispersed
LPU4	60	40	100	69805 (123594)	10	opaque, unevenly dispersed
LPU5	60	40	100	69805 (123594)	5	unevenly dispersed

Mn, number average molecular weight; Mw, weight average molecular weight.
[a]DLLA, poly(lactic acid) diol (mole-%).
[b]PCLD, poly(caprolactone) diol (mole-%).
[c]HDI, 1,6-hexamethylene diisocyanate (mole-%).
[d]LVX, levofloxacin (weight-%).

GPC instrument with a Waters 2414 Refractive Index Detector (Waters Corporation, Milford, MA, USA), a PLGel column series (3×5 μm Mixed-C, 1×3 μm Mixed-E) (Varian Inc., Mulgrave, Australia) and Empower™ Pro Software (Waters Corporation). The GPC was calibrated with narrow polydispersity polystyrene standards (EasiCal™ 264000–256000 Da; Varian Inc.) and the molecular weight reported as polystyrene equivalents.

Polyester polyol and PE-PU polymer composition was characterized by proton NMR spectroscopy using a Bruker AV400 and Bruker AV200 spectrometer (400 and 200 MHz, respectively; 23°C) (Bruker BioSpin, NSW, Australia).

Coating of implants with polymer preparations

Levofloxacin was shown to disperse more readily into the PE-PU preparations than ciprofloxacin, due to the absence of free amine and acid groups. To reduce the potential for *trans*-esterification between the acid groups of levofloxacin and the polyesters within the PE-PU polymer matrix, the length of time for which the preparations were exposed to high temperatures was minimized.

Levofloxacin and the PE-PU polymer were heated to the melting temperature of the polymer, blended and then transferred onto a polyethylene terephthalate (transparency film) substrate. The substrate was then placed between glass plates with a Teflon sheet and a brass shim spacer (100 μm thick), and formed into a thin film (100 μm) by hot pressing at 145°C. Discs (6 mm in diameter) were punched out using a hole punch. Double-sided discs were produced by sticking together two single-sided discs with contact adhesive (Selleys Gel-Grip; Selleys, NSW, Australia).

In vitro assessment of drug release

Following *in vitro* release guidelines recommended by the International Organization for Standardization,[9] polymer-coated discs were suspended in wire baskets that were immersed in 15 mL of pre-heated degassed isotonic phosphate buffer (IPB), adjusted to pH 7.4 using orthophosphoric acid and containing 0.01% sodium azide as a preservative, and incubated at 37°C with continuous stirring. Aliquots (90 μL) of the receptor solution were removed for analysis at predetermined timepoints until the release from the polymer no longer increased.

The amount of levofloxacin released from four discs (6 mm in diameter) coated on one side with the polymer preparations was quantified by reverse-phase HPLC at various timepoints. The HPLC system consisted of a Waters 2690 Alliance Separation Module with a Waters 2487 Dual Absorbance Detector, and drug separation was performed on a Waters Symmetry® C18 column (5 μm particle size, 3.9×150 mm) (Waters Corporation). Levofloxacin was eluted isocratically at a flow rate of 1.2 mL/min using a degassed mobile phase of water and acetonitrile (85:15, v/v), 0.6% v/v triethylamine and adjusted to pH 3 using orthophosphoric acid. The sample injection volume was 30 μL and detection was performed at 293 nm. The retention time for levofloxacin under these conditions was ∼3 min. Data integration was performed using Empower™ Software (Waters Corporation).

In vitro assessment of antimicrobial activity

The antimicrobial activity of polymer-coated discs was assessed using the disc diffusion test, based on protocols recommended by the CLSI (formerly the NCCLS).[10] Colonies selected from an overnight culture of *S. aureus* ATCC strain 29213 were suspended in PBS at a density of 1.5×10^8 cfu/mL. The suspension was then used to inoculate the surface of a Mueller–Hinton agar plate. Discs coated on one side only were placed coated side down onto the freshly inoculated agar and the plates were incubated at 37°C in air for 18 h. Growth inhibition was inspected visually the following day. Discs were then transferred to freshly inoculated agar plates each day until no inhibition of growth occurred.

Surgical implantation and inoculation of mice

Six- to eight-week-old, specific pathogen-free, female BALB/c mice were housed in groups of three to five, and had free access to food and water. All experimental procedures were approved by the University of Melbourne Animal Experimentation and Ethics Committee, and were performed in accordance with the guidelines for animal experimentation of the Australian National Health and Medical Research Council. Mice were anaesthetized by intraperitoneal injection of a mixture containing 100 mg/mL ketamine and 20 mg/mL xylazine at 0.01 mL/g of body weight. Before implantation, both flanks of the mice were shaved, treated with a depilatory cream and disinfected with 80% (v/v) ethanol. An ∼8 mm skin incision was made and dissected to create a subcutaneous tunnel into which a disc (6 mm in diameter) coated with polymer on both sides was inserted as far from the incision site as possible. The incision was then covered with intact skin and closed with a surgical staple. This procedure was repeated on the opposite flank, with each animal receiving the same implant type in duplicate. Following implantation, discs were immediately inoculated by subcutaneous injection of a 50 μL suspension containing 1×10^6 cfu of *S. aureus* strain Xen29 in PBS into the pocket directly above the implant. Mice were given paracetamol for analgesia following surgery and then monitored daily for signs of illness.

Mice were killed 5, 10, 20 or 30 days after implantation and inoculation by inhaling carbon dioxide, and the discs were surgically removed for enumeration of bacteria. Each disc was transferred to a tube containing 1 mL of TSBG and the staphylococci adhering to the implant surface were detached by sonication for 2 min in an ice-cold ultrasonic bath (50 Hz, 40 W) (Ultrasonics, NSW, Australia), followed by 30 s of vigorous mixing using a vortex mixer. The number of viable *S. aureus* removed from the implant surface was determined by plating serial dilutions of the bacterial suspension on LB agar containing kanamycin and incubating in air at 37°C for 18 h. Assuming that all viable bacteria were dislodged from the implant surface during sonication and vigorous mixing, the limit of detection of the assay was 10 cfu/disc (equivalent to 10 cfu/mL).

Statistical analysis

Bacterial counts from excised implants were log-transformed and compared using Student's *t*-test. A value of $P<0.05$ was taken to indicate statistical significance.

Results

Release of levofloxacin into aqueous solution

The *in vitro* release studies were conducted to quantify and characterize levofloxacin release into IPB from discs (6 mm in diameter) coated on one side with polymer preparations of differing proportions of DLLA and PCLD (LPU1–5). The release profiles of the various polymer preparations (Figure 1) showed that levofloxacin release occurred at a constant rate and then reached a plateau at different timepoints, depending on the polymer preparation. After 2 days of immersion, the amount of levofloxacin released was greatest from preparations LPU4 and LPU5, which contained the lowest proportions of DLLA 1000 to PCLD 1000, with 15.98%±0.63% and 22.64%±1.50% w/w (mean±standard error, $n=4$) of the loaded dose of levofloxacin being released, respectively. Moderate levofloxacin release was demonstrated from preparations LPU2 and LPU3, with 6.20%±0.09% and 9.18%±0.18% w/w levofloxacin released, respectively. The lowest amount of levofloxacin released at this

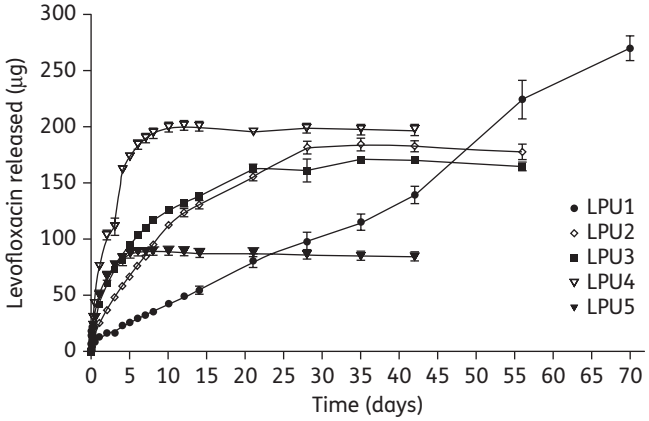

Figure 1. *In vitro* release of levofloxacin into isotonic phosphate buffer (pH 7.4; containing 0.01% w/v sodium azide) from discs (6 mm in diameter) coated on one side with polymer preparations LPU1 (filled circles), LPU2 (open diamonds), LPU3 (filled squares), LPU4 (open triangles) or LPU5 (filled triangles) (see Table 1 for a description of these compounds). Data are the mean release of levofloxacin (μg) ± standard error measured from four discs.

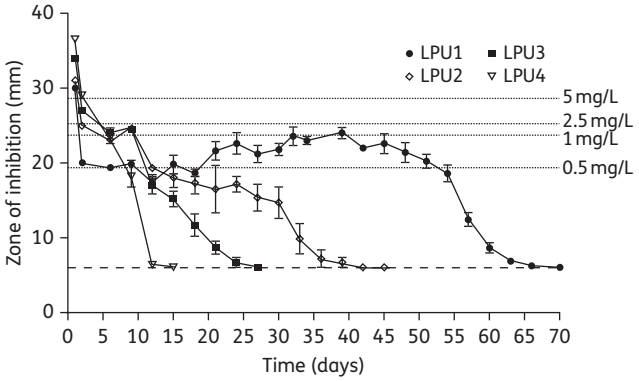

Figure 2. Antimicrobial activity of levofloxacin/polymer blends against *S. aureus* 29213. Discs (6 mm in diameter) coated on one side with polymer preparations LPU1 (filled circles), LPU2 (open diamonds), LPU3 (filled squares) or LPU4 (open triangles) were transferred daily to a freshly inoculated bacterial lawn, incubated and the zone of growth inhibition was measured. Data are the mean zone of inhibition (mm) ± standard error measured from six discs. The zones of inhibition produced by known concentrations of levofloxacin are shown as dotted lines and the diameter of the discs as a dashed line.

timepoint was from LPU1, which contained the highest proportion of DLLA 1000 to PCLD 1000, with only 2.41% ± 0.05% w/w levofloxacin released. In addition to releasing more levofloxacin over the first 2 days than preparations LPU1–3, polymer preparations LPU4 and LPU5 also exhibited the greatest initial release rate (measured on days 0 and 2) of 46.64 and 30.57 μg/day, respectively; contrasting with the respective release rates for polymer preparations LPU1, LPU2 and LPU3 of 7.25, 16.77 and 27.24 μg/day.

Although the initial release of levofloxacin from LPU4 and LPU5 was rapid, it quickly diminished to a plateau ∼10 days after commencement of the experiment (Figure 1), by which time the polymers had released 30.35% ± 0.56% and 28.21% ± 1.26% w/w of the loaded dose of levofloxacin, respectively. The similarity of the release profile between LPU4 and LPU5 (loaded with 10% w/w and 5% w/w levofloxacin, respectively) suggested that relative to the initial loading dose, it was the variations in the polymer preparation, and not the drug concentration, that determined the amount, rate and extent of levofloxacin release. Although when compared with polymers LPU4 and LPU5, preparations LPU2 and LPU3 exhibited prolonged release (56 days) and reached a plateau at a later timepoint (∼30 days), their release was comparable to that from LPU4 and LPU5, with 29.64% ± 0.99% and 25.01% ± 0.60% w/w of the loaded dose of levofloxacin, respectively. The LPU1 preparation showed both elevated and prolonged release of 39.20% ± 1.30% w/w of the loaded dose of levofloxacin by day 70. Whilst the release from the other polymer preparations (LPU2–5) diminished or ceased by day 40, the release from LPU1 continued in a linear, pseudo-zero order fashion until the experiment was stopped (day 70).

Antimicrobial activity of implants in vitro

To assess the antimicrobial activity associated with active polymer coatings—LPU1–4 (containing 10% w/w levofloxacin)—discs (6 mm in diameter) coated with these compounds on one side only were tested in a disc diffusion transfer assay against *S. aureus* strain 29213. Control polymers PU1–4 (no levofloxacin) did not inhibit growth (data not shown), indicating that the polymer preparations alone have no measurable antistaphylococcal activity. All active polymer preparations blended with levofloxacin (LPU1–4) inhibited growth of the test microorganism for at least 10 days *in vitro* (Figure 2). Preparations LPU1 and LPU2, with a high proportion of DLLA 1000 to PCLD 1000, inhibited growth for a long period of time (66 and 40 days, respectively), while preparations with a lower proportion of DLLA 1000 (LPU3 and LPU4) maintained antimicrobial activity for a much shorter period (26 and 12 days, respectively) (Figure 2). Following an initial burst effect, preparations LPU2–4 produced consistently decreasing zones of inhibition until the concentration of levofloxacin released from the polymer was insufficient to prevent the growth of *S. aureus* 29213 (Figure 2). In contrast, polymer preparation LPU1 produced a constant zone of inhibition with an average diameter of 20.5 mm for a period of 54 days before zones began to decrease, with growth inhibition no longer apparent 66 days after commencement of the experiment (Figure 2).

Bacterial colonization of implants

Due to differences in their *in vitro* release profiles, polymer preparations LPU1 and LPU3 were selected for *in vivo* analysis. In an intra-operative model of subcutaneous staphylococcal infection, 88% of PU1 (14/16) and 100% PU3 (20/20) control implants (polymer without levofloxacin) were culture positive (Figure 3). Five days after implantation and inoculation, PU1- and PU3-coated implants were colonized with a mean bacterial load of 4.1×10^2 and 1.5×10^5 cfu/disc, respectively (Figure 3). After 10 days, an average of 1.8×10^4 cfu/disc was cultured from PU1-coated implants. A similar bacterial load of

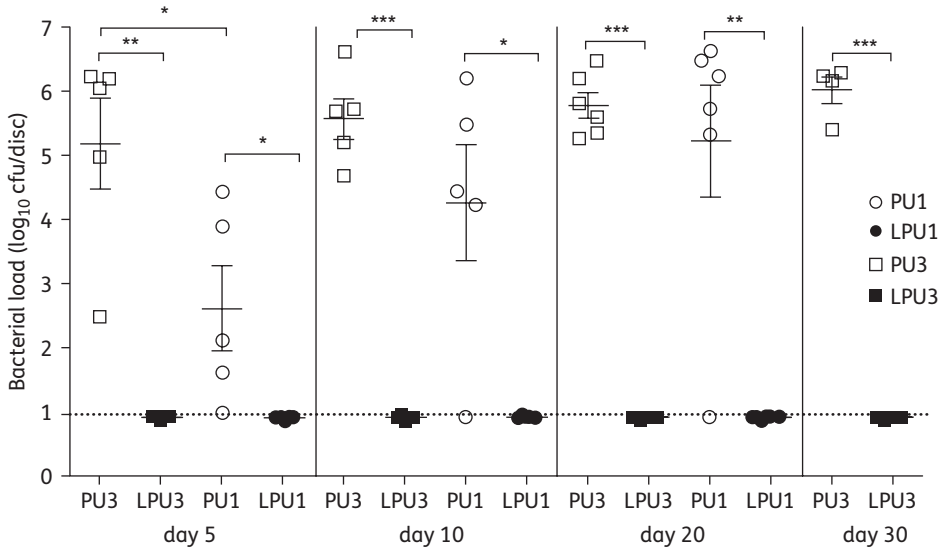

Figure 3. Bacterial load of implants after inoculation with *S. aureus* strain Xen29 in an intra-operative model of infection. Data points represent the bacterial load ($\log_{10}$ cfu/disc) of implants ($n=4–6$) recovered from individual mice between 5 and 30 days after implantation and inoculation. Implants were coated on both sides with control polymers (no levofloxacin) PU1 (open circles) or PU3 (open squares), or with active polymers [10% (w/w) levofloxacin] LPU1 (filled circles) or LPU3 (filled squares). The mean $\log_{10}$ cfu/disc (±standard error) is shown as a solid line. The limit of detection is displayed as a dotted line. Undetectable bacterial loads were arbitrarily assigned a value of 1 cfu less than this limit. *$P<0.05$; **$P<0.005$; ***$P<0.0001$ (unpaired, two-tailed Student's *t*-test).

3.7×10^5 cfu/disc was recovered from PU3-coated implants. This same pattern of colonization was observed 20 days after implantation and inoculation, where the mean bacterial load for PU1- and PU3-coated implants was 1.7×10^5 and 6.0×10^5 cfu/disc, respectively (Figure 3). At the final timepoint of 30 days, all PU3-coated implants were colonized, with a mean bacterial load of 1.1×10^6 cfu/disc (Figure 3).

In contrast, none of the implants coated with active polymers LPU1 (0/16) or LPU3 (0/22) containing levofloxacin showed evidence of colonization by *S. aureus* for up to 20 (LPU1) to 30 days (LPU3) post-implantation (Figure 3). The average number of cfu in the active-blend (containing levofloxacin) groups was significantly lower than in the groups implanted with polymer-alone controls (no levofloxacin) ($P<0.05–0.0001$ Student's *t*-test, two-tailed).

Residual antimicrobial activity of implants

Following processing for bacteriological analysis, excised implants were tested in a diffusion assay against *S. aureus* strain 29213 to determine if there was sufficient residual levofloxacin to inhibit bacterial growth. At no time did PU1- or PU3-coated implants inhibit the growth of *S. aureus* 29213 (Table 2), confirming that there was no detectable anti-staphylococcal activity associated with the polymer coatings in the absence of levofloxacin. Conversely, between 5 and 20 days after implantation, 100% of LPU1-coated (16/16) and LPU3-coated (16/16) implants containing levofloxacin caused inhibition of bacterial growth *in vitro* (Table 2). For LPU1-coated implants, the mean zones of inhibition produced by discs removed days 5, 10 and 20 after implantation were 20.8, 27.4 and 25.2 mm, respectively (Table 2). The mean zones of inhibition for LPU3-coated discs recovered at the same timepoints were 20.2, 20.2 and 18 mm, respectively (Table 2). Thirty days after implantation, none of the LPU3-coated implants recovered from the mice produced a zone of inhibition against *S. aureus* 29213 (Table 2). These results correspond with the *in vitro* release study, which showed that LPU3-coated discs had no inhibitory affect against *S. aureus* 29213 after 26 days passage in a disc diffusion transfer assay (Figure 2).

Table 2. Antimicrobial activity of implants taken from an intra-operative model of device-related subcutaneous staphylococcal infection

	Zone of inhibition (mm) on day:[a]			
Compound	5	10	20	30
PU1	0	0	0	ND
LPU1	20.8±0.84	27.4±2.30	25.2±2.71	ND
PU3	0	0	0	0
LPU3	20.2±1.30	20.2±1.79	18.0±3.95	0.17±0.41

ND, not determined.
[a]Data are the mean zone of growth inhibition (±standard deviation) from four to six discs (6 mm in diameter) excised from mice at the times indicated. Control discs were coated on both sides with polymers PU1 or PU3; active discs were coated on both sides with LPU1 or LPU3 polymers blended with 10% levofloxacin by weight.

Discussion

Over the past few decades, the use of surgically implanted devices has increased markedly as a result of their beneficial

effect on patients' life quality and expectancy. However, the expanding application of medical implants has increased the need to prevent infectious complications associated with these devices. Increasingly, medical devices are responsible for a large proportion of nosocomial infections,[1] where bacterial colonization of implant surfaces is a prelude to persistent and recurrent infection. Repeated episodes and the inability to clear an infection can cause serious complications for patients and malfunction of the device. The consequences of device-related infections also have major economic implications, making infections associated with indwelling medical devices a significant problem in healthcare.

A number of strategies have been developed in a bid to avoid infection of implantable devices,[3] including the application of antimicrobial coatings, which have emerged as a potentially effective method of preventing device-related infections. The potential benefit of these applications has been demonstrated in trials involving ventricular[11] and vascular catheters,[12–15] as well as models of infection of catheters,[16] heart valve prostheses[17] and orthopaedic devices.[18] While these methods have proven effective, application of the antimicrobial agent by spraying it onto the surface of the device or impregnating the material of the device directly means that consistently accurate dosing and release may be difficult to attain.

In this study, we investigated the efficacy of levofloxacin-blended bioerodable polymer coatings in preventing bacterial colonization of foreign body implants in a murine model of subcutaneous infection with *S. aureus*. We showed that the elution rate of levofloxacin from polymer preparations into aqueous solution varies substantially with the nature of the polymer. The similarities in the shape of the profiles for release of levofloxacin from LPU4 and LPU5, which differed only in their loading of levofloxacin, are also consistent with polymer-controlled release.

The release characteristics of all the preparations may be influenced by the proportions of DLLA, an amorphous substance with a glass transition temperature of 57°C that exists in a glassy state at 37°C,[19,20] and PCLD, a relatively hydrophobic, semi-crystalline (45%–60% crystallinity) substance with a low transition temperature (−65°C).[19–21] This presents a potential to tailor the polymer coatings to modulate drug release for different end-usage requirements.

The mechanical and degradation properties of amorphous DLLA polymers are highly dependent on the glass transition temperature.[20] The transition temperature generally increases with increasing molecular weight and proportion of DLLA in the polymer preparations.[21,22] Pitt *et al.*[19] showed that although polymers of DLLA itself are amorphous in nature, drug release from DLLA is 104 times slower than from PCLD and copolymers of DLLA and PCLD, and suggested this was a reflection of the decrease in the free volume and high glass transition temperature (57°C) of DLLA. The finding that levofloxacin release from preparations LPU2–5 (containing up to 80% w/w DLLA) was much more rapid than from LPU1 (containing 90% w/w DLLA) is consistent with the findings of Pitt *et al.*[19]

The rate of release of levofloxacin from the polymer was a function of the relative proportions of DLLA and PCLD, with polymers containing higher proportions of PCLD having higher release rates. The same trend was seen when levofloxacin release was measured chemically and microbiologically. The polymer preparation with the highest loading of DLLA polyester polyol (LPU1) released levofloxacin at a rate approaching pseudo-zero order kinetics, while the softer, slower-degrading polymer preparations containing a higher loading of the PCLD polyester polyol (e.g. LPU4) demonstrated a more rapid release of levofloxacin. It is not clear, however, why the release from polymer preparations LPU2–5 reached a plateau after ∼30% of the levofloxacin was released; but it is noteworthy that the same proportion of levofloxacin was released from LPU4 and LPU5, despite differences in overall drug loading.

In vivo, we showed that active levofloxacin/polymer blends could prevent infection of implants in an intra-operative contamination model for at least 20 to 30 days post-implantation. Colonization of almost all of the control implants coated with the polymer preparation alone indicated that all antimicrobial activity observed for the active implants was associated with the levofloxacin component of the polymer-blend coating. Assessment of the antimicrobial activity of excised implants demonstrated that discs coated with active polymer blends released levofloxacin at concentrations sufficient to inhibit bacterial growth for at least 20 days *in vivo*. Interestingly, after 30 days, LPU3 polymer coatings no longer released inhibitory concentrations of levofloxacin. Despite this, there was no growth of *S. aureus* on the excised implant surface, suggesting that the release of antibiotic from LPU3 polymer coatings was sufficient to inhibit bacterial colonization of the implant surface and prevent bacterial persistence in the surrounding tissue, despite the large number of *S. aureus* that was inoculated at the site of the implant.

Compared with intra-operative soaking and impregnation of biocompatible materials, the application of antimicrobial polymer coatings offers a number of advantages. First, antibiotic-blended polymers have the potential to be applied to the surface of any medical device, providing a broad application range. In addition, the implantation of a pre-coated device is more practical and time saving. Second, the biodegradable properties of the polymers themselves can provide a number of potential benefits. For example, by altering the chemical and physical properties of the polymer, it is possible to change the rate of degradation, allowing some control over the rate of antimicrobial release. Moreover, as seen in this study the nature of the polymer can influence the kinetics of release, whereby certain release profiles may be more desirable depending on the intended application. Third, the antibiotic dose can be altered by varying the thickness of the polymer coating. This allows for flexibility in application of a standard polymer/antimicrobial blend preparation, even when differing doses of drug are required.

In summary, we have shown that coating foreign bodies with an antimicrobial-blended polymer can prevent bacterial colonization in an intra-operative contamination model of subcutaneous infection of implanted devices with *S. aureus*. Analysis over a prolonged period demonstrated the sustained release of levofloxacin from the implant surface and confirmed the ability of blended polymer coats to prevent colonization of the device surface over a period of at least 20 to 30 days. The potential to apply this technology to a wide range of medical devices and to manipulate this system for customized applications offers exciting opportunities for the future development of coatings that prevent bacterial colonization of medical implants and, thus, decrease the incidence of device-related infections.

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Transparency declarations

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