Poly-D, L-Lactide and Levofloxacin-Blended Beads: A Sustained Local Releasing System to Treat Osteomyelitis

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ABSTRACT: Osteomyelitis is the inflammation of bone which is treated by a high dose of antibiotics given intravenously for 4–6 weeks. However, at present locally administered antibiotic such as gentamicin poly (methyl methacrylate) (PMMA) bead is nonbiodegradable and a secondary surgery is often inevitable. This study described the biodegradable material poly-D, L-lactide (PLA) with 80 kDa molecular weight that could be used as a potential antibiotic carrier for local drug release. PLA was first dissolved in tetrahydrofuran followed by blending with levofloxacin (LFX) in a physical way. The blend was then molded into beads. The optimized weight ratio between PLA and LFX was designated as 45 : 15. Glass transition temperature and surface ultramicrostructure of the beads were measured. *In vitro* tests of drug release and bacteriostasis demonstrated that the PLA–LFX beads released high concentrations of antibiotic for the period of time (i.e., 6 weeks), which is needed to treat bone infection. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 3678–3684, 2012

Key words: poly-D; L-lactide; levofloxacin; osteomyelitis; sustained delivery system

INTRODUCTION

Osteomyelitis is the inflammation of bone usually due to infection. It has been reported that the incidence of osteomyelitis is 2–16% after open fractures, depending on the grade of trauma and the administered treatment.[1] The inferior penetration of antibiotics into bone often brings about a long-term (i.e., 4–6 weeks) and high-dose intravenous administration to treat osteomyelitis,[2] which often leads to bacterial resistance and deep-seated mycoses.[3]

Local antibiotic administration is therefore an alternative option for clinical treatment. Gentamicin poly (methyl methacrylate) (PMMA) beads have been applied clinically to prevent or treat osteomyelitis.[4] However, since PMMA is a nonbiodegradable material, secondary surgery is often required to remove the beads after gentamicin has been released. To solve this problem, a series of biodegradable materials have been investigated as potential antibiotic carriers for local drug delivery.[5] Poly-D, L-lactide (PLA) is an amorphous, biodegradable, and aliphatic polyester polymer. Direct compression tablets based on PLA and a drug have delivered the drug according to profiles suitable for implantation.[6,7] In addition to direct compression tablets, therapeutic carriers in the nanometer scale have increasingly been used as drug delivery vehicles due to the multitude of advantages they offer by virtue of their small size and versatility. However, unlike liposomes, polymeric nanoparticles poorly encapsulate water-soluble drugs due to the rapid leakage of the drug from the nanoparticles during the high-energy emulsification step commonly employed in the nanoparticle preparation.[8–10] Levofloxacin (LFX) is a widely used fluoroquinolone antibiotic for osteomyelitis. The minimal inhibitory concentration (MIC) of LFX is as low as 0.18 μg/mL for *Staphylococcus aureus,* the pathogen that causes osteomyelitis.[11] Based on these, we developed a sustained local releasing system by taking PLA as the carrier and LFX as the releasing antibiotic in an effort to investigate the drug release property of the system and to explore its potential value for treating or preventing osteomyelitis.

EXPERIMENTAL

Materials

PLA (molecular weight = 80 kDa) was obtained from Shandong Institute of Medical Devices (Shandong, China). LFX was provided by Zhejiang Xinhua Pharmaceutical Co. (H20094174). *S. aureus* (ATCC 25923),

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Escherichia coli (*ATCC 25922), and Pseudomonas aeruginosa* (ATCC 27853) were provided by the Clinical Laboratory of Zhongnan Hospital, Wuhan University.

Preparation of PLA–LFX-blended beads

In this study, the total weight of PLA–LFX-blended beads was set to 60 mg, with PLA:LFX at 54 : 6, 51 : 9, 48 : 12, 45 : 15, and 42 : 18 (w/w). The method to prepare the beads was followed.[12] Briefly, PLA was initially weighed and dissolved in tetrahydrofuran. LFX powders with corresponding weights were then added into the solution and were ground thoroughly and evenly. After complete volatilization of the tetrahydrofuran, 60 mg blend was weighed, put into a cylindrical metal mold (6 mm in diameter), and preheated at 90°C for 10 min. Then, the blend was compressed and molded at 104 MPa for 2 min and cool to room temperature. After mold stripping, all of the beads were sealed within plastic bags and sterilized with ^{60}Co at a delivered dose of 25 kGy.

In vitro release assay

Release of LFX from the bend was studied in triplicate under sink conditions.[13–16] The PLA–LFX blend bead was placed in a vial with 10 mL of isotonic phosphate buffer solution (PBS pH 7.3) with 0.02% w/v sodium azide, which was agitated at 60 rpm in a horizontally shaking water bath maintained at 37°C. Considering a 4–6 weeks' intravenous antibiotics treatment cycle for of osteomyelitis in clinic,[2] we sampled the release medium at various time intervals for 46 days: 1, 2, 4, and 12 h after the beginning of the test; every 24 h from days 1 to 15; every 2 days from days 16 to 31; and every 3 days thereafter. At each sampling time, the release medium in the vials was replaced with fresh buffer to maintain the sink conditions. After the completion of the test, the LFX concentration in the buffer samples was analyzed by Nanodrop2000c (Thermo Fisher Scientific). In the dissolution tests, UV–vis spectra of both LFX alone in PBS and PBS samples containing PLA–LFX will be detected first, then LFX concentration was determined spectrophotometrically. From plots of cumulative LFX released versus time, LFX cumulative release (%) and daily release amount (μg/mL) were calculated.

Differential scanning calorimetry

The glass transition temperatures (T_g) of PLA and PLA–LFX blend bead were determined with a power-compensation differential scanning calorimeter (PerkinElmer). About 8 mg of sample in simply sealed aluminum pan was under a continuous nitrogen flow (20 mL/min). The measuring temperature ranged from –30 to + 100°C, it was started with heating from +25 to +100°C to destroy the thermal history and then cooled to –30°C by liquid nitrogen and reheated to +100°C. The heating rate was 10°C/min and cooling rate was 30°C/min (holding time was 1 min).

Scanning electron microscopy

The surface structure of PLA–LFX blend beads was measured by scanning electron microscopy (SEM). The micrographs were taken with a JSM-5610LV (Carl JEOL, Japan) scanning electron microscope. Before scanning, the samples were coated with platinum using a vacuum evaporator. SEM images were obtained at an accelerated voltage of 25 kV.

In vitro bacteriostasis experiment

Based on the results of the *in vitro* drug release, the beads with the best ratio were chosen for bacteriostasis experiment *in vitro*. The experiment conducted three times independently. Each PLA–LFX-blended bead was placed in the middle of an agarose culture medium that was inoculated with *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853), respectively. After being incubated at 37°C for 24 h, the culture media were examined, and the diameters of the inhibition zones of bacteria around the beads were measured. Subsequently, the beads were transferred onto new culture media, and the media were replaced every 3 days until 46 days. The PLA beads without LFX were placed onto the culture media inoculated with the aforementioned bacteria as controls.

RESULTS

Preparation and appearance

Under the compression conditions, the PLA and LFX of different weight ratios adhered tightly to each other in the mold. The beads were yellowish in appearance, cylindrical with 6 mm in diameter, and 2.36 mm in thickness.

In vitro release assay

During the dissolution tests, UV–vis spectra of LFX alone in PBS show the peak point is 287 nm, while the PBS samples contained PLA–LFX also show the same peak point (Fig. 1). The beads with different PLA:LFX weight ratios showed initial burst of drug release to differing extent during the first day. They were 17.23 ± 1.39%, 22.29 ± 1.61%, 9.83 ± 1.96%, 30.11 ± 1.19%, and 49.69 ± 1.23% for the beads with 54 : 6, 51 : 9, 48 : 12, 45 : 15, and 42 : 18 weight ratios. The beads with 42 : 18 weight ratio had the highest percentage of drug release at the first day, but after 22nd day, they had lower percentage of

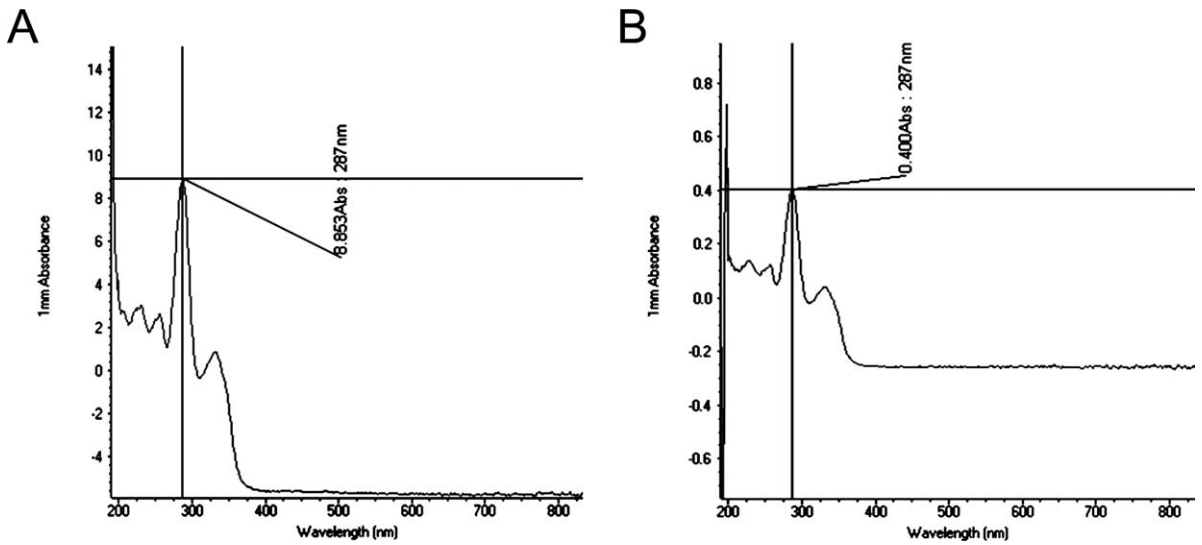

Figure 1 UV–vis spectrum of LFX alone in PBS (A) and UV–vis spectrum of the PBS samples containing PLA–LFX (B), both peak points were showed at 287 nm.

drug release than the beads with 45 : 15 weight ratio (Fig. 2). Finally, on the 46th day, it was observed that the beads volume increased, no erosion occurred (visual inspection), and the PLA–LFX beads were not disintegrated. The beads with 45 : 15 weight ratio had the highest percentage of cumulative drug release (73.87 ± 1.79%). As the dissolution profiles indicated (Fig. 3), its daily release amount of LFX was higher than 2 μg/mL per day.

Based on the above results and the normal duration of effective drug release, the PLA–LFX blend beads with 45 : 15 weight ratio were selected for the following experiments.

Differential scanning calorimetry

For the purpose of characterizing the physical state of the PLA and PLA–LFX-blended beads, differential scanning calorimetry analysis was performed. There was one predominant endothermic as showed in the heat curve (Fig. 4), T_g of PLA was detected at 51°C and PLA–LFX-blended bead was 47°C.

Scanning electron microscopy

The surface morphology of the PLA–LFX bead is shown in Figure 5. LFX was crystalline particles, while PLA was a semicrystalline polymer. Scanning

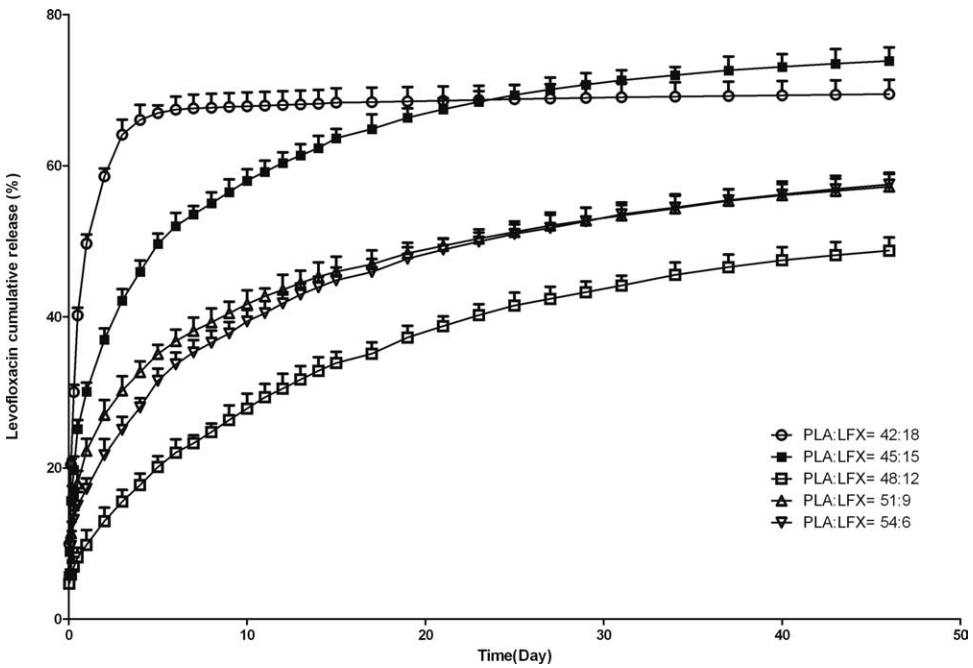

Figure 2 Levofloxacin cumulative release (%) of different PLA–LFX weight ratios, $n = 3$.

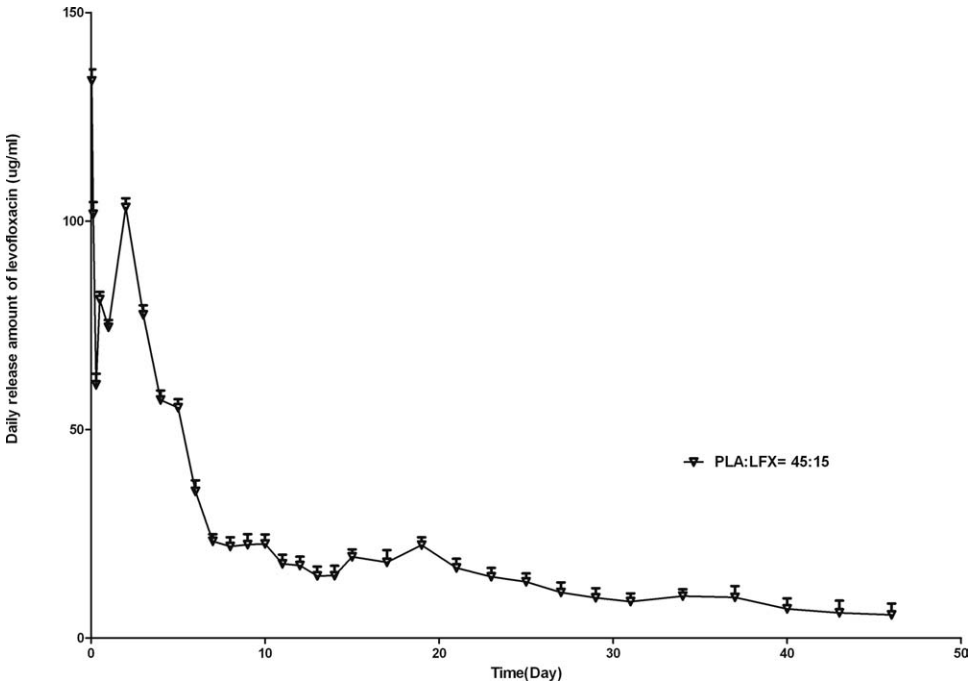

Figure 3 Daily release of levofloxacin (μg/mL) for beads with 45 : 15 weight ratio, $n = 3$.

electron micrographs showed that the beads had a homogeneous structure. LFX particles were evenly distributed throughout the bead, and some micropores were found evident in the solidified polymer matrix, which might have formed during the evaporation of the tetrahydrofuran.

In vitro bacteriostasis experiment

The beads with 45 : 15 weight ratio between PLA and FLX produced inhibition zones in the culture mediums that were respectively, inoculated with *S. aureus*, *E. coli,* and *P. aeruginosa* within the 46 days of the experiment. No inhibition zone was observed in culture medium containing the PLA beads without LFX. The overall average diameters of the inhibition zones of bacteria around the beads were 30.2 ± 2.4, 36.3 ± 1.7, and 27.1 ± 2.1 mm for *S. aureus*, *E. coli,* and *P. aeruginosa,* respectively (Fig. 6).

DISCUSSION

Drug delivery systems based on biodegradable materials have received widespread interests.[17–19]

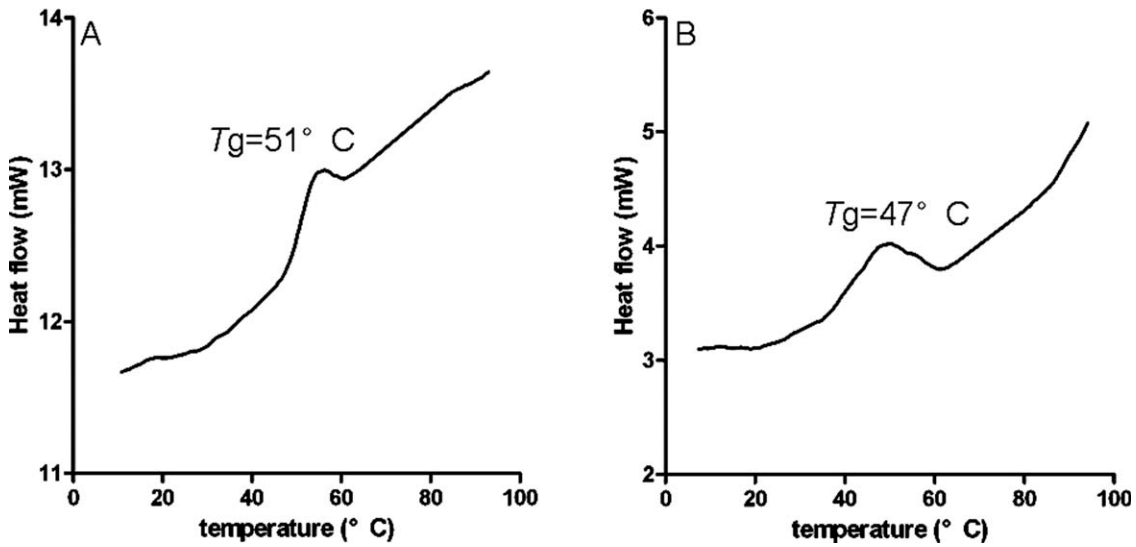

Figure 4 Heat flow of the PLA (A) and PLA–LFX bead (B) during the heating process in nitrogen measured by differential scanning calorimetry. T_g of PLA was detected at 51°C and PLA–LFX-blended bead was 47°C.

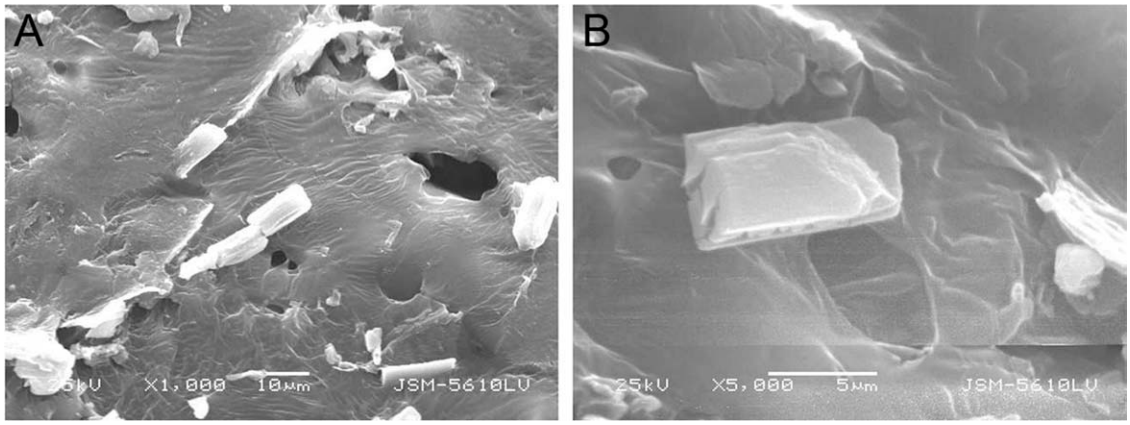

Figure 5 The surface morphology of the PLA–LFX bead; LFX particles were evenly distributed throughout the surface of the bead and some micropores were found.

Among them, antibiotic delivery system for the treatment or prevention of osteomyelitis also attracts attention of the researchers because of the clinical demand. Having been studied for decades, PLA is considered as one of the most promising biodegradable biomaterials, which is nontoxic, minimally inflammatory, and bioabsorbable without any accumulation in the vital organs.[20] Molecular weight of PLA can greatly affect its chemical and physical–mechanical properties such as degradation, diffusivity, and glass transition temperature. Previous studies have demonstrated that drug release from tablets containing low molecular weight PLA (e.g., 2 kDa) was highly pH dependent[21] and it has been concluded that low molecular weight PLA might not be suitable to be the carrier. Thus, higher molecular weight (e.g., 85 kDa) PLA is often used in the subsequent researches to avoid narrow pH range.[22] In this study, PLA with 80 kDa molecular weight was chosen, and *in vitro* drug release under the condition imitating the situation in the body showed that the PLA–LFX-blended bead was not eroded and disintegrated after 46 days while good drug release profile was obtained.

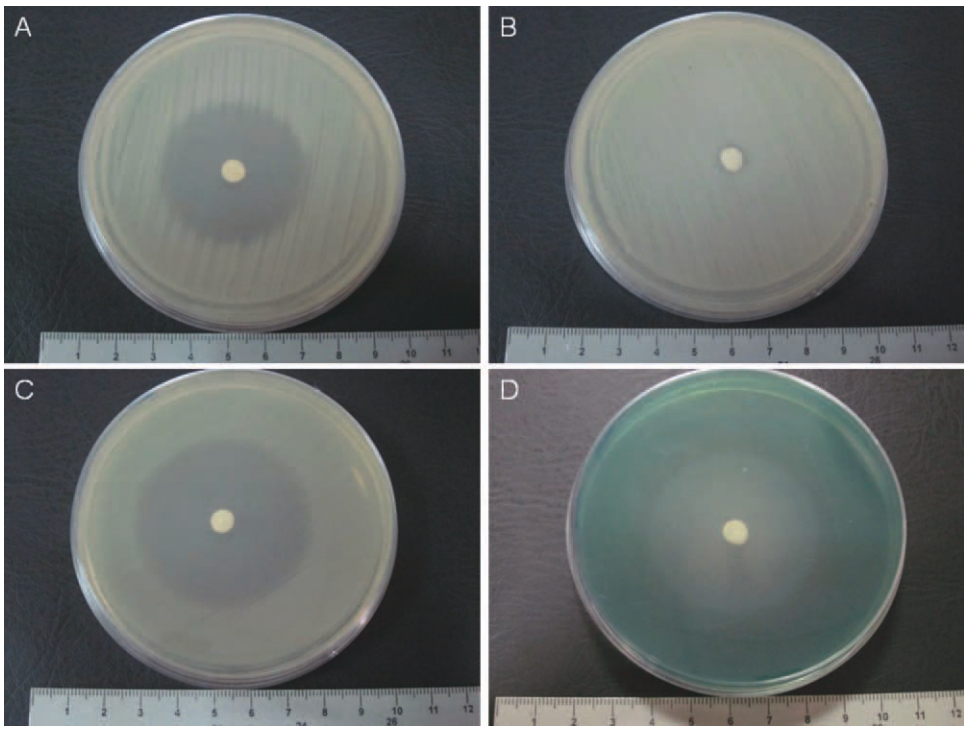

Figure 6 The PLA–LFX bead produced an inhibition zone to *Staphylococcus aureus* (A), while no inhibition zone for *Staphylococcus aureus* was observed for the PLA bead without LFX (B). The inhibition zones were also produced to *Escherichia coli* (C) and *Pseudomonas aeruginosa* (D).

During the processing of polymer beads, the formation of a homogeneous melt from powder particles involves two steps: sintering and densification.[23] The polymeric particles stick or fuse together at their points of contacting around the antibiotic particles. This fusion zone grows until the mass becomes a three-dimensional network, with relatively little density change; 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 bead. It has been suggested that sintering and densification occur because the voids in the powder interior, that are formed upon densification, are pushed ahead of the melt from the free surface.[24] In this research, tetrahydrofuran was used to dissolve PLA, and LFX powder was dispersed in the solution by the physical way while tetrahydrofuran volatilized.[12] The small standard deviation for the amount of drug release at each detection time point *in vitro* drug release demonstrated that LFX was evenly mixed into PLA by this method. The uniformly distributed crystalline particles of LFX on the surface of the bead showed by SEM also proved this from a different angle. All these indicated that the used technique is a feasible approach for the preparation of PLA–antibiotic blend beads. Differential scanning calorimetry analysis on the beads with 45 : 15 weight ratio showed that the glass transition temperature was 47°C, that was far beyond normal body temperature and higher than that of a high fever. This suggests that the beads are suitable for possible *in vivo* use without altering its physical and mechanical character.

Most of the drug release systems keep to the following rule, i.e., an evident burst release on the first day, followed by a slow, continuous release over the next days.[25,26] The initial burst must be due to the rapid diffusion of the drug close to the surface of the implant, while the slower release phase occurs through dissolution and diffusion of the remaining drug within the implant.[26] In this research, the *in vitro* release test showed the similar process. This may explain why the beads with weight ratios 42 : 18 have the highest percent of drug release at first, but has lower percent of drug release compare to the beads with weight ratios 45 : 15 after 22nd day. In clinic, treating osteomyelitis often requires a 4–6 weeks intravenous antibiotics,[2] so considering the practical clinical needs, we sampled the release medium at various time intervals for 46 days and did not reach to 100%. But evaluating by the parameters of cumulative release, daily release, burst effect, and the duration, the beads with 45 : 15 weight ratio was considered to be the suitable one for the purpose of treating or preventing osteomyelitis, because it can sustainably and effectively release LFX for at least 46 days with a comparatively thorough cumulative release and an acceptable burst effect.

Osteomyelitis is a bone disease caused by bacterial infection of the bone tissue. When it occurs, local vascular channels are compressed and obliterated by the inflammatory process, and this ischemia leads to bone necrosis, creating regions of the bone where there is insufficient antibiotic penetration. *S. aureus* is the most common pathogen isolated in osteomyelitis, while *E. coli* and *P. aeruginosa* are also occasionally encountered.[1] The significant advantage of the antibiotics incorporated into the biodegradable bead is that the local antibiotic concentrations are much higher than the minimum inhibitory concentration (MIC) for most pathogens commonly isolated in orthopedic infections.[24] This overcomes the drawbacks of the systemic antibiotic use.[14,27] The fluoroquinolones are potent antibacterial agents that have been used successfully to control a variety of bacterial pathogens. Unfortunately, *S. aureus* readily develops resistance to the derivatives such as ciprofloxacin.[28] The mutant prevention concentration (MPC) is defined as the MIC of the least susceptible single-step mutant. For bacterial populations that already contain mutations that lower susceptibility, the MPC is equivalent to the MIC of the least-susceptible next single-step mutant.[29] The increasing prevalence of antimicrobial resistance makes MPC important, because by using drug concentrations greater than the MPC the agents directly attack mutant bacteria. The MPC of LFX for *S. aureus* which causes osteomyelitis is 2 μg/mL while its MIC is 0.18 μg/mL for the same bacterium.[11] The daily concentrations of LFX eluted from the beads in this research were much greater than the MPC within 6 weeks, thus theoretically even for the mutated *S. aureus*, local LFX concentration resulted from the sustained release from the beads is high enough to eliminate the bacterium. The results of *in vitro* bacteriostasis in this research demonstrated that the beads can take bacteriocidal effects to *S. aureus*, *E. coli*, and *P. aeruginosa* constantly for 46 days *in vitro*.

Our future researches are going to investigate whether and/or how the biodegradable antibiotic beads PLA–LFX have an effect of anti-infections in animal models, especially in osteomyelitis animal models.

CONCLUSIONS

In conclusion, the blended biodegradable beads of polylactide and levofloxacin with 45 : 15 weight ratio can sustainably and effectively release levofloxacin for at least 46 days *in vitro*. The beads may have

potential value for use as a local antibiotic delivery system for treating or preventing osteomyelitis.

References

1. Chihara, S.; Segreti, J. Dis Mon 56, 5.
2. Lipsky, B. A. Clin Infect Dis 39 Suppl 2004, 2, S104.
3. Hayes, J. D.; Wolf, C. R. Biochem J 272, 2811990.
4. Bunetel, L.; Segui, A.; Cormier, M.; Langlais, F. Clin Pharmacokinet 1990, 19, 333.
5. Kanellakopoulou, K.; Giamarellos-Bourboulis, E. J. Drugs 2000, 59, 1223.
6. Bodmeier, R.; Chen, H. G. J Pharm Sci 1989, 78, 819.
7. Takahashi, M.; Onishi, H.; Machida, Y. J Control Release 2004, 100, 63.
8. Cheow, W. S.; Hadinoto, K. Colloids Surf B.; Biointerfaces 85, 214.
9. Mo, S. M.; Oh, I. J. J Nanosci Nanotechnol 11, 1795.
10. Cirpanli, Y.; Yerlikaya, F.; Ozturk, K.; Erdogar, N.; Launay, M.; Gegu, C.; Leturgez, T.; Bilensoy, E.; Calis, S.; Capan, Y. Pharmazie 65, 867.
11. Zhao, X.; Eisner, W.; Perl-Rosenthal N.; Kreiswirth, B.; Drlica, K. Antimicrob Agents Chemother 2003, 47, 1023.
12. Chen, L.; Wang, H.; Wang, J.; Chen, M.; Shang, L. J Biomed Mater Res B Appl Biomater 2007, 83, 589.
13. Wei, G.; Jin, L.; Xu, L.; Liu, Y.; Lu, W. Int J Pharm 398, 123.
14. Castro, C.; Sanchez, E.; Delgado, A.; Soriano, I.; Nunez, P.; Baro, M.; Perera, A.; Evora, C. J Control Release 2003, 93, 341.
15. Kumari, A.; Yadav, S. K.; Pakade, Y. B.; Kumar, V.; Singh, B.; Chaudhary, A.; Yadav, S. C. Colloids Surf B. Biointerfaces 82, 224.
16. Guerrero, S.; Muniz, E.; Teijon, C.; Olmo, R.; Teijon, J. M.; Blanco, M. D. J Pharm Sci 2008, 97, 3153.
17. Kakinoki, S.; Taguchi, T.; Saito, H.; Tanaka, J.; Tateishi, T. Eur J Pharm Biopharm 2007, 66, 383.
18. Gupta, H.; Jain, S.; Mathur, R.; Mishra, P.; Mishra, A. K.; Velpandian, T. Drug Deliv 2007, 14, 507.
19. Wang, K.; Jia, Q.; Han, F.; Liu, H.; Li, S. Drug Dev Ind Pharm 36, 1511.
20. Kobayashi, H.; Shiraki, K.; Ikada, Y. J Biomed Mater Res 1992, 26, 1463.
21. Moll, F.; Koller, G. Arch Pharm (Weinheim) 1990, 323, 887.
22. Steendam, R.; van der LaanA.; Hissink, D. J Control Release 2006, 116, e94.
23. Cohen, J.; Siegel, R. A.; Langer, R. J Pharm Sci 1984, 73, 1034.
24. Wang, G.; Liu, S. J.; Ueng, S. W.; Chan, E. C. Int J Pharm 2004, 273, 203.
25. Wang, F. J; Wang C. H. J Biomater Sci Polym Ed 2003, 14, 157.
26. Soriano, I.; Evora, C. J Control Release 2000, 68, 121.
27. Laurencin, C. T.; Gerhart, T.; Witschger, P.; Satcher, R.; Domb, A.; Rosenberg, A. E.; Hanff, P.; Edsberg, L.; Hayes, W.; Langer, R. J Orthop Res 1993, 11, 256.
28. Acar, J. F.; Goldstein, F. W. Clin Infect Dis 24 Suppl 1997, 1, S67.
29. Li, X.; Zhao, X.; Drlica, K. Antimicrob Agents Chemother 2002, 46, 522.