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The release of cefazolin and gentamicin from biodegradable PLA/PGA beads

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Abstract

Infection has been one of the most common causes of problems and complications after the operation despite the advance in surgical techniques and the availability of newly developed antibiotics. Local antibiotic delivery beads for treatment of various surgical infections had been studied recently especially in osteomyelitis. This current paper used cefazolin sodium and gentamicin sulfate combined with biodegradable polymers (50:50 poly(DL-lactide):co-glycolide) as antibiotic beads for a long-term drug release. To manufacture an antibiotic bead, polylactide–polyglycolide copolymers were mixed with the antibiotics. The mixture was compressed and sintered at 55 °C to form beads of different sizes. The beads were placed in 3 ml of phosphate buffered saline and incubated at 37 °C. An elution method combined with a bacterial inhibitory test was employed to characterize the release rate of the antibiotics over a 30-day period. The results suggested that the biodegradable beads released high concentrations of antibiotic (well above the minimum inhibitory concentration) in vitro for the period of time needed to treat bone infection; i.e. 2–4 weeks. This provides advantages as a first line choice of long-term antibiotics for patients with osteomyelitis and various infections such as thoracic, abdominal, and pelvic infections, as well as for the prophylaxis of these infections.

Keywords: Local antibiotic delivery; Osteomyelitis; Cefazolin; Gentamicin; Polylactide–polyglycolide; Release rate

1. Introduction

Osteomyelitis is a difficult infection to treat and eradicate (Waldvogel et al., 1970). Surgery is the mainstay of treatment and prophylaxis of infection in soft tissue or bone and is required in virtually every case (with the exception of acute osteomyelitis in childhood). The necessity for antibiotics, either systemic antibiotics or local antibiotics, is well established in chronic infection. Delivering an effective antimicrobial at a sufficiently high concentration to the area of infection in combination with surgery is a recognized treatment for bone infection (Buchholz et al., 1981; Canner et al., 1984; Cierny and Mader, 1984; Carlsson et al., 1978, 1985; Cierny et al., 1985; Calhoun and Mader, 1989; Ueng et al., 1997).

Local antibiotics have the advantage of delivering high drug concentrations to the precise area required. It is usually performed with the use of polymethylmethacrylate (PMMA) bone cement beads in combination with standard treatments for bone infection. The total dose of antibiotic applied locally is not

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normally sufficient to produce toxic systemic effects. Polymethylmethacrylate beads have been wired together with gentamicin to form a string of beads. The success rate varies from 40 to 90%. The disadvantages associated with the PMMA beads include a necessary secondary surgery and a less than optimal antibiotic elution profile since only 50% of the antibiotic is eluted from the bead after 4 weeks (Hill et al., 1977; Elson et al., 1977; Welch, 1978; Neut et al., 2003; Flick et al., 1987; Hoff et al., 1981; Holm and Vejlsgaard, 1976; Wahlig et al., 1978; Wahlig and Dingeldein, 1980). An ideal drug delivery system should provide: (1) an adequate antimicrobial concentration at the target site, (2) a slow and constant release of antimicrobial over a prolonged period, and (3) be biodegradable so that a second operation is not required (Nie et al., 1995; Ali et al., 1993).

Antibiotic beads made out of biodegradable polymers have advantages over conventional polymethylmethacrylate beads and intravenous antibiotics in several ways. First, biodegradable beads provide bactericidal concentrations of antibiotics for the prolonged time needed to completely treat the particular orthopedic infection. Second, variable biodegradability from weeks to years may allow many types of infections to be treated. Third, because the biodegradable beads dissolve, there is no need for surgical removal; and fourth, because the biodegradable beads dissolve slowly, the soft tissue or bone defect will slowly fill with tissue, so there is no need for reconstruction.

Recent investigations have explored the use of biodegradable materials incorporating antibiotics for potential use in the treatment of infection of the musculoskeletal system. Setterstrom et al. (1984) described the development of biodegradable ampicillin loaded microspheres that provide continuous sustained release of medication for as long as 2 weeks in rats. Calhoun and Mader (1997) treated the localized osteomyelitis rabbits model with a biodegradable polylactic acid and poly(DL-lactide):co-glycolide antibiotic bead. Shinto et al. (1992) showed that the biodegradable gentamicin beads administered five times the minimum inhibitory concentrations for *Staphylococcus* species for at least 12 weeks in vitro. Garvin et al. (1994) described the polyglycolide beads loaded with gentamicin resulting in the effective treatment of tibial *Staphylococcus aureus* osteomyelitis in a canine model. Zhang et al. (1994) demonstrated that the release rate and duration from coated cylinders could be adjusted by cutting the cylinder into different lengths. This procedure offers a convenient method of adjusting the release rate to meet the specific antibiotic requirement of different patients. Jacob et al. (1993) evaluated the efficacy of local therapy with cefazolin microspheres for the prevention of infection in rabbits with contaminated open tibial fractures stabilized with internal fixation. Lin et al. (1999) proposed a compression sintering method of manufacturing biodegradable vancomycin beads. They also proposed (Ueng et al., 2002) that the release of vancomycin from biodegradable beads in rabbits and found that the beads released slower in vivo (8 weeks) than in vitro (4 weeks).

This paper studied the in vitro release characteristic of cefazolin and gentamicin from polylactide–polyglycolide (PLA/PGA) beads. A compression-sintering technique was employed to manufacture beads loaded with antibiotics of various sizes. Beads were evaluated by an elution method and a bacterial inhibitory test. The final goal of this research was to develop a biodegradable antibiotic delivery system so that long-term antibiotics would be available for patients with osteomyelitis and various infections such as thoracic, abdominal, and pelvic infections, as well as for the prophylaxis of these infections.

2. Materials and methods

2.1. Beads materials and manufacturing

Antibiotics–polymer beads were fabricated in this study. Commercial grade cefazolin sodium in powder form and standard reference grade gentamicin sulfate in gel-like form (Sigma, USA) were used. The polymeric materials used were poly(DL-lactide):co-glycolide with a ratio of 50:50 and an intrinsic viscosity of 0.4. A Dupont model TA-2000 differential scanning calorimeter was used to characterize the thermal properties of the polymers. The measured results in Fig. 1 showed that polymers' glass transition temperature was in the range of 40–55 °C. The polymer and cefazolin sodium and gentamicin sulfate were mixed by a lab scale dry mixer with the ratio of 5:1 (polymer:antibiotics). Table 1 lists the weight, size and composition of the antibiotic beads. The mixture

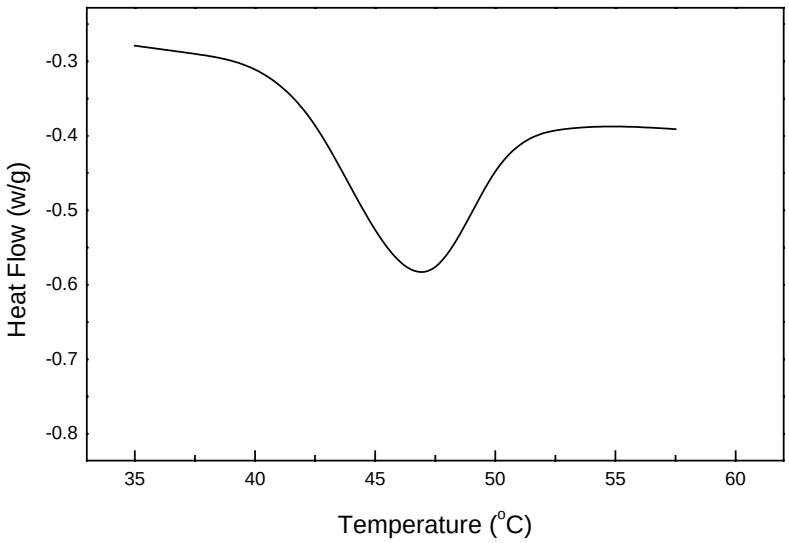

Fig. 1. Differential scanning calorimeter measurement of the biodegradable polymer.

was compressed into beads of different diameters (5, 8 and 10 mm) by a stainless mold. The compressed beads with the mold were then placed in an oven for sintering. Fig. 2 shows schematically the mold and the oven used to compression-sinter the antibiotics beads. The sintering temperature was set at 55 °C, which was higher than polymers' glass transition temperature, but low enough to avoid destroying the antibiotics. The sintering time used was 30 min in order to attain an isothermal sintering of the beads. Two-layer structured beads were also made. Beads of a small size (5 and 8 mm) were first manufactured. They were then coated with another thick layer of copolymer to form beads with outside diameters of 8 and 10 mm, respectively.

2.2. Thermal stability of antibiotics

In order to determine whether the heat might destroy the antibiotics during the manufacturing process of the biodegradable beads, thermal stability tests (Allocate et al., 2000) were performed. Twenty milligrams of cefazolin sodium was incubated in an oven for 30 min at various temperatures ranging from 40 to 100 °C, with a negative control that was not sintered. Each cefazolin sodium sample was dissolved in 20 ml of normal saline. The concentration equaled to 1 mg/ml. The relative activities of the incubated cefazolin sodium to *S. aureus* (ATCC65389) were determined by the antibiotic disk diffusion method. The equation and other relevant items for relative activity

Table 1
Weight, size and composition of the antibiotic beads

Layer (no. of beads)	Diameter × height, $D \times H$ (mm × mm)	Inner layer		Outer layer
		Weight of polymer (mg)	Weight of antibiotic (mg)	Weight of polymer (mg)
Single (5)	5 × 2.2	50	10	–
Single (8)	8 × 3.5	200	40	–
Single (10)	10 × 4.3	400	80	–
Double (5/8)	8 × 3.5	50	10	180
Double (5/10)	10 × 4.3	50	10	420
Double (8/10)	10 × 4.3	200	40	250

were:

$$\text{relative activity (\%)} = \frac{\text{the diameter of sample inhibition zone} - \text{the diameter of disk}}{\text{the diameter of maximum inhibition zone} - \text{the diameter of disk}}$$

The thermal stability of gentamicin was also performed with the same scheme against *Escherichia coli* (ATCC25922).

2.3. In vitro characterization

An in vitro elution method was employed to determine the release characteristics of cefazolin sodium from the antibiotic beads. A phosphate buffer, 0.15 mol/l (pH 7.4), was used as the dissolution medium. Each of the biodegradable antibiotic beads was incubated in 3 ml of phosphate buffered saline at 37 °C for 24 h. The dissolution medium was collected and analyzed at every 24 h interval. Fresh phosphate buffer (3 ml) was then added for the next 24 h period and this procedure was repeated until the bead was fully dissolved. The dissolution medium was collected and analyzed at every 24 h interval.

The released antibiotic concentration was characterized by a disk diffusion method. The released concentration of cefazolin to *S. aureus* (ATCC65389) was determined by using an antibiotic disk diffusion method in a nutrient broth (beef extract, peptone, Difco Laboratories). Eight microliters of solution from each daily buffer sample was pipetted on 6 mm disks. The disks were placed on nutrient agar plates (beef extract, peptone, agar, Difco Laboratories) and seeded with a layer of *S. aureus*, and the zones of inhibition were measured with a micrometer after 16–18 h of incubation at 35 °C. A calibration curve was first determined by six different standard concentrations (0, 0.01, 0.1, 1, 10, 100, 1000 mg/ml). The released concentration of cefazolin was then determined by interpreting the curve. The released concentration of gentamicin was also determined by the disk diffusion method the same as that of cefazolin, except that the bacteria used was *E. coli*.

The minimum inhibitory concentration of cefazolin to *S. aureus* (ATCC65389) was determined using an antibiotic tube dilution method in cation supplemented Mueller–Hinton broth (Difco Laboratories). Cefazolin was diluted serially twofold in tubes containing 0.5 ml of the cation supplemented Mueller–Hinton broth. The minimum inhibitory concentration of gentamicin to *E. coli* (ATCC25922) was also determined by the same scheme.

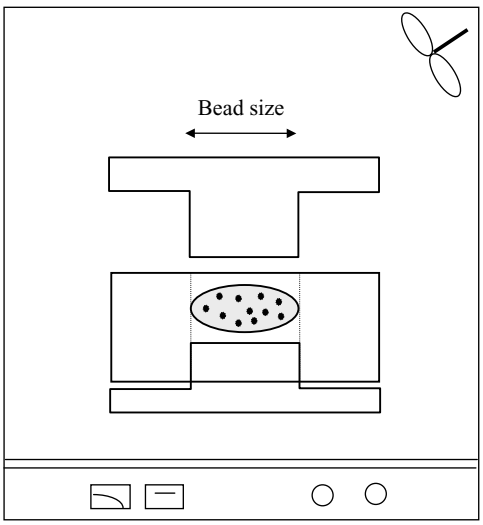

Fig. 2. Mold and the oven used to compression-sinter the antibiotics beads.

3. Results

The thermal stability results with cefazolin and gentamicin is shown in Fig. 3. The experimental results suggest that gentamicin was rather stable up to 100 °C, while cefazolin was stable up to 80 °C. This suggests that the antibiotics used in this study were not destroyed while we were manipulating the antibiotic beads (a sintering temperature of 55 °C).

The disk diffusion assay results for cefazolin and gentamicin standard curves with six different standard concentrations are showed in Fig. 4a and b, respectively. The calibration fittings for these curves are:

$$\text{[cefazolin]}: \quad Y = 8.6774 \log(X) + 22.447, \quad R^2 = 0.9796,$$

$$\text{[gentamicin]}: \quad Y = 5.993 \log(X) + 11.057, \quad R^2 = 0.97$$

Cefazolin beads of three different sizes (5, 8 and 10 mm) were manufactured and tested. Fig. 5 showed

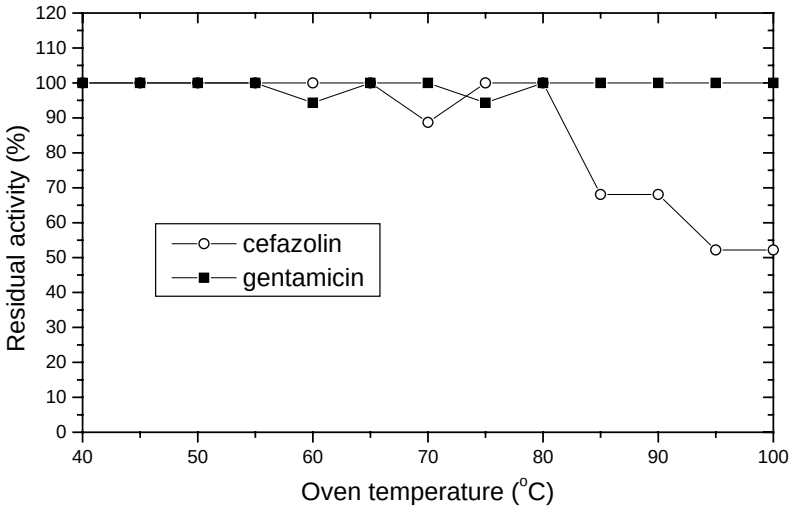

Fig. 3. Thermal stability of the antibiotics used in this study.

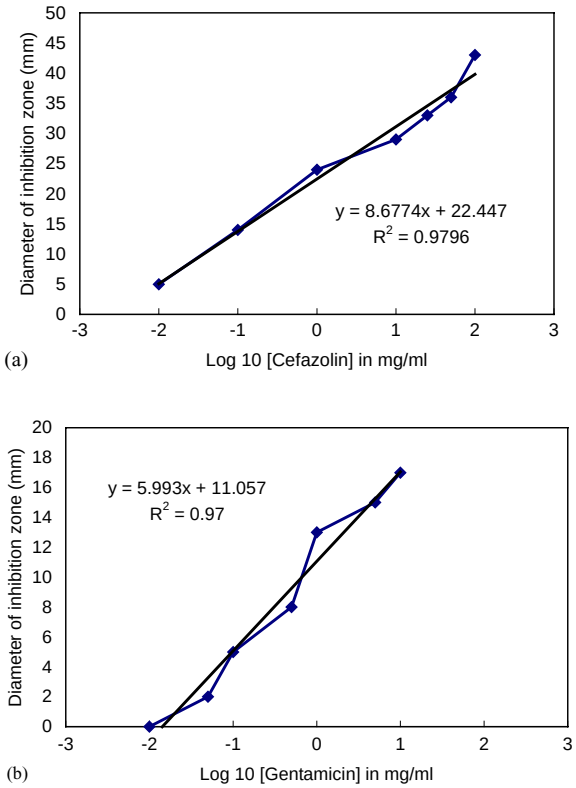

Fig. 4. Disk diffusion calibration curves of (a) cefazolin and (b) gentamicin.

the release curves of these beads. Obviously, the release rate decreases with the bead size. This is due to the fact that the weights of the beads are proportional to the cubic order of the bead size, while the dissolving rate is proportional to the surface area of the bead, which is proportional to the second order of the size, that is

$$\text{release rate} = \frac{L^2}{L^3} = \frac{1}{L}$$

where L is the nominal dimension of the bead. Therefore, the release from the larger beads was more gradual and sustained than that from the small beads.

The release characteristics of two-layer polymer–cefazolin beads were also studied. Polymer–cefazolin composite beads of 5 and 8 mm in diameter were first manufactured. They were then coated with another thick layer of copolymer to form beads with outside diameters of 8 and 10 mm, respectively. The release curves of the two-layer beads are shown in Fig. 6 (5/8 denotes 5 mm for the core bead and 8 mm for the outside diameter). Obviously, the release from the two-layer beads was much more gradual and sustained than that from the single layer beads. In addition, the manufactured 10 mm beads can release high concentration of cefazolin (higher than the minimum inhibitory concentration) for up to 4 weeks.

The in vitro characteristic of gentamicin was also studied. Fig. 7 showed the release rates of the

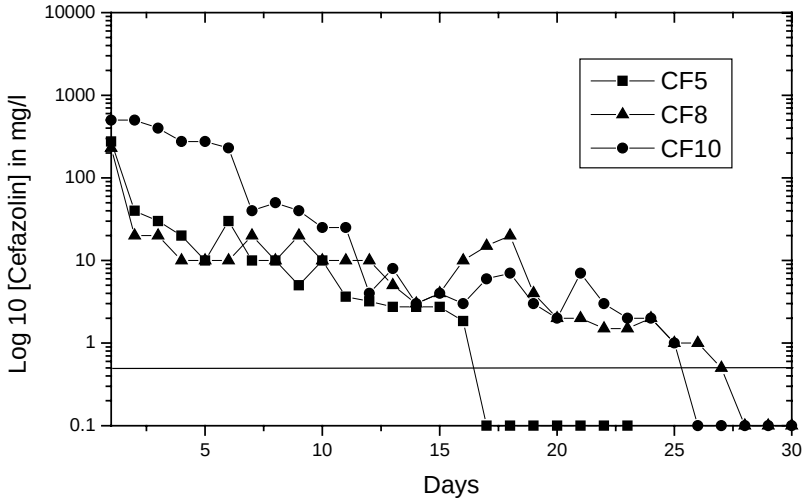

Fig. 5. Release curves of cefazolin from beads of different sizes (solid line is the MIC).

gentamicin beads with three different sizes (5, 8 and 10 mm). Again, the release rate decreases with the bead size. One can increase the total period of effective release by increasing the size of the beads. The 10 mm beads can release high concentration of gentamicin (well above the minimum inhibitory concentration) for up to 2 weeks.

The release characteristics of two-layered polymer–gentamicin beads were also studied. Fig. 8 showed the release curves of these beads (5/8, 5/10 and 8/10 for core diameter and outside diameter, respectively). It was very interesting to find that the total release period of the 8/10 double layer bead (Fig. 8) was shorter than that of the 10 mm single-layer bead (Fig. 7). This might be due to the fact the 8/10 double layer bead only contains 40 mg gentamicin at its core (see Table 1), which is less than that of 80 mg in the 10 mm single layer bead. The total release period of the 10 mm single layer gentamicin beads is thus longer.

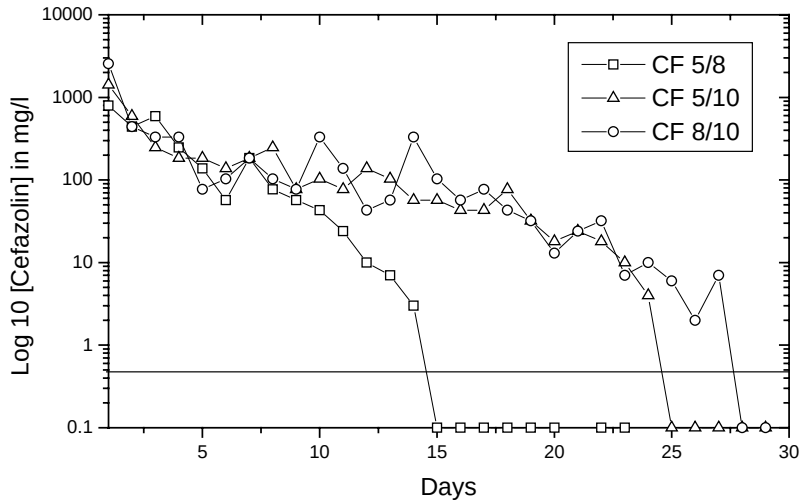

Fig. 6. Release curves of cefazolin from double-layered beads (solid line is the MIC).

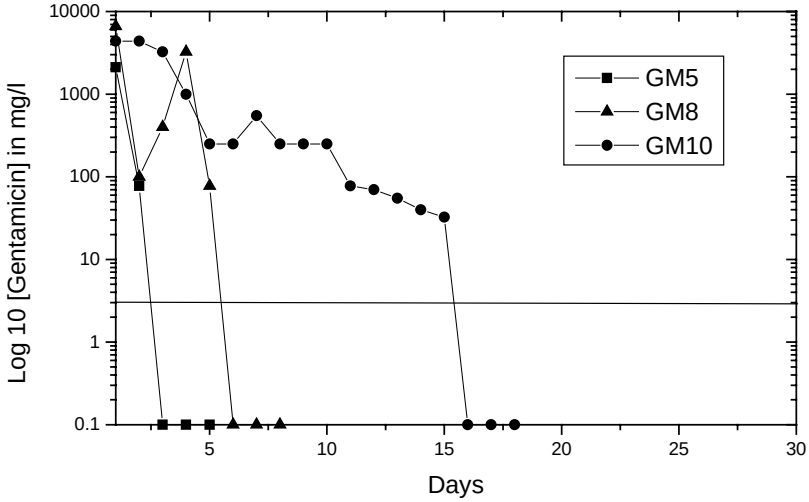

Fig. 7. Release curves of gentamicin from single-layer beads made of different sizes (5, 8, and 10 mm).

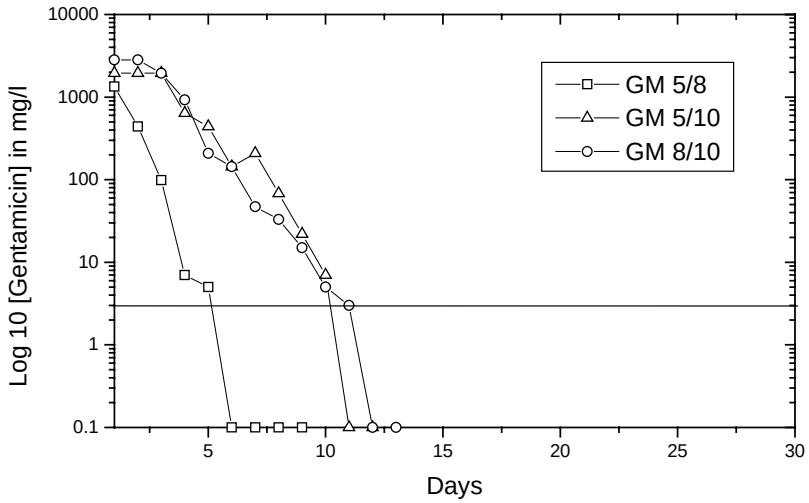

Fig. 8. Release curves of gentamicin from double-layered beads made of different inner and outer layer sizes.

4. Discussion

An ideal antibiotic delivery system for the treatment of osteomyelitis should be non-reactive in the body and stable for as long as antimicrobial therapy is desired. The most commonly used non-biodegradable matrix implant is gentamicin-loaded polymethylmethacrylate beads (Rushton, 1997; Song and Glenny, 1998; Weinstein, 1980; Hardman and Limbird, 1996). Despite their antibiotic release, these PMMA beads act as a biomaterial surface to which bacteria preferentially adhere, grow and potentially develop antibiotic resistance (Popham et al., 1991). A second operative surgery is usually necessary to remove the beads.

Since the initial work with PMMA, there has been a search to develop a biodegradable matrix implant. Poly(lactic acid) is one of the most promising biodegradable biomaterials. It is non-toxic, elicits minimal inflammatory response and can be eventually absorbed without any accumulation in the vital organs (Kobayashi et al., 1992; Ikada et al., 1985). Biodegradable materials are not only being used

as treatment methods, they have also been used to administer antibiotics (Wei et al., 1991). In most previous studies, the antibiotic was micro-encapsulated in a poly(DL-lactide):co-glycolide bead with a high molecular weight (e.g. MW 3.26×10^4), a high percentage of poly(lactic acid) ratio (e.g. 70:30), a high antibiotics loading dosage (e.g. 50%) and with high melt processing temperatures (e.g. 110 °C) (Calhoun and Mader, 1997; Shinto et al., 1992; Garvin et al., 1994; Zhang et al., 1994; Jacob et al., 1993). The release profile should have an initial high release rate to accommodate the possibility of infection just after an operation, followed by 2–4 weeks of a relatively constant release above the breakpoint sensitivity. Release kinetics was found to be influenced by the type of polymer utilized for microcapsule production. Differences in microcapsule degradability may influence not only the antibiotics release rate but also its release mechanism. In this study, we chose low molecular weights (e.g. MW 5000), low antibiotics loading dosages (e.g. 20%), and a low melt processing temperature (55 °C) to manufacture biodegradable antibiotic beads (Table 1).

During the processing of polymer beads, 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. This is referred to as sintering (Liu, 1998). 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. This is referred to densification (Liu, 1998). The antibiotic is then encapsulated by the polymer to form a composite bead. It has been suggested (Narkis and Rosenzwig, 1995) 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. However, the buoyancy, capillary, and hydrodynamic forces that force the interior void away from the beads' free surface, are not strong enough to overcome the surface tension force required to pull the surface void away from the bead's surface and into a bubble. Small voids thus form because of the encapsulation of air pockets between powder particles of polymers and antibiotics. The higher the void fraction inside the beads, the faster the antibiotic will be released by channel diffusions.

The release rate and duration of the antibiotics beads depend upon the requirement of each application. For a water-soluble antibiotic in a hydrophobic polylactide matrix, the release mechanisms are controlled by channel diffusion, osmotic pressure, and polymer degradation (Seigel and Langer, 1990). Firstly, when the antibiotic is surrounded by the polymeric material, antibiotic particles will be isolated in the polymer matrix. These particles will not be able to permeate through the polymer at a practically useful rate. The cefazolin sodium used in this study was in powder form and could be completely surrounded by the polymeric matrix. The release rate was thus slower (Fig. 5). While the gentamicin sulfate was in gel-like form and could not be completely encapsulated by the polymeric materials during the manufacturing, the antibiotic would then be released by channel diffusion, which has a higher release rate as shown in Fig. 7. Secondly, if the polymer matrix surrounding the isolated particles remains intact during the release, antibiotic will not be released from these clusters. However, a water-soluble antibiotic will take up water with 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. Finally, when the polymer molecular weight decreases sufficiently, loss of polymer begins. The antibiotic will then be released along with this polymer loss.

In this study, it is desirable to be able to adjust the release rate and duration from the antibiotic beads can be adjusted by their diameter. By increasing the diameter of the bead, one can load more antibiotics into the beads and prolong the total elution period of the beads, as shown in Figs. 5 and 7. Since cefazolin sodium can be encapsulated by the polymer and is not able to permeate through the coated copolymer wall, device structure has a significant effect on the release profile. The release of the double layer beads (Fig. 6) was much more gradual and sustained than that from the single layer beads (Fig. 5). On the other hand, due to the channel diffusion release mechanism of the gel-like gentamicin during the elution process, the coated layer did not show significant effects on the release rate of the beads.

The bactericidal effects of the antibiotics incorporated into the biodegradable bead far outweigh any negative inherent effects of the bead itself. A significant advantage of the biodegradable antibiotic bead is that the local antibiotic concentrations are much greater than the minimum inhibitory concentration (MIC) for most pathogens commonly isolated in orthopedic infections. The concentrations of both cefazolin and gentamicin eluted from the beads were much greater than the minimum inhibitory concentration for more than 4 weeks (Fig. 5) and 2 weeks (Fig. 7), respectively. The bactericidal power of the antibiotics is still high after the manufacturing process.

5. Conclusions

This study used cefazolin sodium and gentamicin sulfate combined with biodegradable polymers (50:50 poly(DL-lactide):co-glycolide) as antibiotic beads for a long-term drug release. The experimental results suggested that one could prolong the total effectively release period of antibiotics from the beads by increasing the size of the beads or by adopting multi-layered beads. Biodegradable beads released high concentrations of antibiotic (well above the minimum inhibitory concentration) in vitro for the period of time needed to treat bone infection; i.e. 2–4 weeks.

Future researches should focus on investigating the biodegradable antibiotic beads in animal models, such as in joint arthroplasty infection and localized osteomyelitis models. Eventually biodegradable cefazolin and gentamicin antibiotic beads may be used as a first line drug of choice in humans for the treatment of various surgical infections.

Acknowledgements

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