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Novel solvent-free fabrication of biodegradable poly-lactic-glycolic acid (PLGA) capsules for antibiotics and rhBMP-2 delivery

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Abstract

Osteomyelitis has been one of the most common causes of post-operative problems and complications despite the advances in surgical techniques and the availability of newly developed antibiotics. Local antibiotic and growth factor delivery devices for treatment of various surgical infections have been studied recently, especially in the case of orthopedic infections. The report was to develop novel solvent-free biodegradable capsules for antibiotics and growth factors delivery. To fabricate a biodegradable capsule, polylactide–polyglycolide copolymers were pre-mixed with vancomycin. The mixture was then compression molded and sintered to form a cylinder with a cover of 8 mm in diameter. After the addition of 1 and 10 μg recombinant bone morphogenetic protein (rhBMP-2) into the core, an ultrasonic welder was used to seal the capsules. An elution method was employed to characterize the in vitro release characteristics of the antibiotics and the rhBMP-2 over a 30-day period. The HPLC analysis and the bacterial inhibition test showed that biodegradable capsules released high concentrations and activity of vancomycin (well above the minimum inhibition concentration) in vitro for the period of time needed to treat bone infection; i.e. 4–6 weeks. In addition, the results of ELISA and ALP tests also suggested that the capsules released active rhBMP-2 for up to 30 days. By adopting this novel technique, we will be able to fabricate biodegradable capsules of various medicines for long-term drug deliveries.

Keywords: Osteomyelitis; Polylactide–polyglycolide; Vancomycin; rhBMP-2; Capsules; In vitro elution; Release characteristics

1. Introduction

Osteomyelitis is a difficult infection to treat and eradicate (Waldvogel et al., 1970; Waldvogel and Vasey, 1980). Long-term parental antibiotics with multiple surgical debridements are often required for effective therapy. Following debridement surgery, the dead space created by osteomyelitic tissue removal must be managed. Delivering an effective antimicrobial at sufficiently high concentrations to the area of infection in combination with surgery is a recognized treatment for bone infection (Buchholz et al., 1981; Hill et al., 1977; Elson et al., 1977; Calhoun and Mader, 1989; Ueng et al., 1997). It is usually performed with the use of polymethylmethacrylate (PMMA) bone cement beads in combination with standard treatments for bone infection. The success rate varies from 40% to 90%. The disadvantages associated with using the PMMA beads include a necessary secondary surgery to remove the beads and a less than optimal antibiotic elution profile since only 50% of the antibiotic is eluted from the bead after 4 weeks.

Bone healing is a complex physiological process that is initiated and controlled by growth factors, such as bone morphogenetic protein-2 and -7 (BMP-2, BMP-7) and transforming growth factor-β1 (TGF-β1). The importance of growth factors for bone healing has instigated the development of new treatment strategies, which consist of the local delivery of growth factors to the defect site with help of carrier materials. To date, collagen from animal sources has been utilized as a carrier material for rhBMPs in the clinic (Govender et al., 2002; Burkus et al.,

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2003). Nevertheless, animal-derived collagen has some limitations including the potential risk for disease transfer and the low efficacy of collagen in retaining rhBMPs in the release process (Ruhe et al., 2005).

An ideal drug delivery system for bone infection treatment should provide: (1) an adequate antimicrobial concentration and bioactive factors at the target site, (2) a slow and constant release of antimicrobial and bioactive factors over a prolonged period, and (3) be biodegradable so that a second operation is not required. Based on this concept, numerous groups have been focusing on developing a drug delivery system for antibiotics (Nie et al., 1995; Setterstrom et al., 1984; Calhoun and Mader, 1997; Shinto et al., 1992; Garvin et al., 1994) and growth factors (Bessho et al., 2002; Kokubo et al., 2002; Mori et al., 2000; Oldham et al., 2000; Rai et al., 2005; Kato et al., 2006).

In previous studies, we have developed various techniques for the fabrications of biodegradable antibiotics (Liu et al., 1999a, 2002, 2005; Wang et al., 2004) (including gram-positive vancomycin and gram-negative gentamicin) beads/capsules and investigated the release characteristics of the antibiotics from these devices. It was found that the developed biodegradable devices could release high concentration of active antibiotics for a period of 30 days in vitro and up to 56 days in vivo.

This current paper proposes a novel solvent-free method of manufacturing biodegradable capsules for both antibiotics and growth factors delivery. In comparison with other investigators (Nie et al., 1995; Setterstrom et al., 1984; Calhoun and Mader, 1997; Shinto et al., 1992; Garvin et al., 1994; Bessho et al., 2002; Kokubo et al., 2002; Mori et al., 2000; Oldham et al., 2000; Rai et al., 2005; Kato et al., 2006), we have adopted compression sintering and ultrasonic welding techniques to manufacture biodegradable polymer capsules that can simultaneously release vancomycin and rhBMP-2. Capsules were evaluated by an in vitro elution method. An HPLC analysis and a bacterial activity test were employed to evaluate the release rate of vancomycin from the biodegradable capsules, while an enzyme-linked immunosorbent assay (ELISA) and an alkaline phosphatase (ALP) tests were used to evaluate the release characteristics of the BMP-2. The final goal of this research was to develop a biodegradable system for the delivery of antibiotics and growth factors and to provide an improved method for surgical infection treatment.

2. Materials and methods

2.1. Materials

The polymers used were poly(D,L)-lactide-*co*-glycolide with a ratio of 50:50 and an intrinsic viscosity of 0.4. All polymers were available in powder form with particle size ranges from 100 to 200 μm. A DuPont model TA-2000 differential scanning calorimeter was used to characterize the thermal properties of the polymer. The measured results suggested that the polymers' glass transition temperature was in the range of 45–50 °C. The antibiotics used were commercial grade vancomycin powder with a particle size of 100 μm (Abbott Lab., USA). Recombinant human bone morphogenetic protein-2 (rhBMP-2) (R&D Systems, USA) was available in powder form of 10 μg. It was dissolved in the sterilized 4 mM HCl containing 0.1% bovine serum albumin. Two concentrations of 10 and 100 μg/ml were used.

2.2. Fabricate of antibiotics capsules

To fabricate the biodegradable capsules, polylactide–polyglycolide copolymers were pre-mixed with vancomycin. The mixture was then compression molded to form a cylinder with a cover of 8 mm in diameter (Fig. 1). The wall thickness of the cylinder and the cover was 1 mm. Table 1 lists the contents of the materials in the capsules. The compressed cylinder and cover with the mold were then placed in an oven for sintering. The sintering temperature was set at 55 °C, which was higher than the 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 materials. After sintering, 0.1 ml of BMP-2 of two different concentrations, 10 and 100 μg/ml (or equivalently 1 and 10 μg BMP-2 loadings), was added into the core of the cylinder and a 300 W portable ultrasonic welder was used to seal the capsule. During ultrasonic welding, high frequency and low amplitude mechanical vibrations were applied to the capsules. This resulted in cyclical deformation of the parts and of any surface roughness. The ultrasonic energy is converted into heat through the intermolecular friction within the polymer. The generated heat, which is highest at the weld interface due to asperities, is sufficiently high to melt polymer and fusion bond the parts. To ensure a complete sealing of the capsules, the amplitude of vibration and the weld time used for the welder were 20 μm and 3 s, respectively (Liu et al., 2005). For improved control of the heating and melting at the surface, an energy director is molded onto the surface of the cover to be joined (Fig. 1). The energy director, which has the smallest cross section and therefore the highest strain, heats, melts, and flows to fill the interface with molten polymer and to fusion joint the capsules (Liu et al., 1998, 1999b). Fig. 2 shows schematically the fabrication process of the biodegradable capsules.

2.3. In vitro elution

An in vitro elution method was employed to determine the release characteristics of antibiotics and growth factors from the capsules. A phosphate buffer, 0.15 mol/l (pH 7.4), was used as the dissolution medium. The capsules were placed in glass test tubes with a volume of 2 ml phosphate buffer. All tubes

Table 1
Weight, size and composition of the antibiotic capsules

Wall (polymer + vancomycin)				Core rhBMP-2 (μg/ml)
Thickness 1.0 mm	Polymer to vancomycin ratio	Weight of polymer (mg)	Weight of vancomycin (mg)	
Cylinder	5:1	229.1	36.7	10 or 100
Cover	5:1	83.5	12.8	

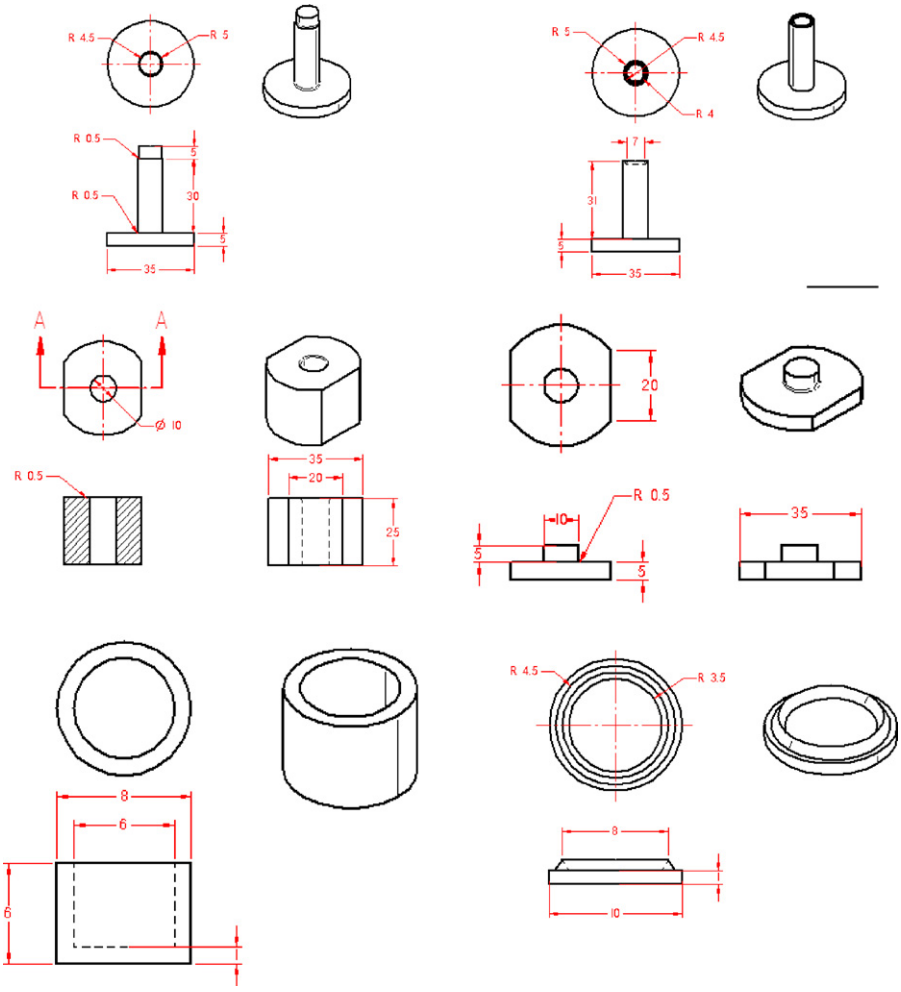

Fig. 1. Dimensions of the biodegradable capsules and the mold.

were incubated at 37 °C. The dissolution medium was collected for subsequent analyses at every 24 h interval. Fresh phosphate buffer (2 ml) was then added for the next 24 h period and this procedure was repeated until the capsule was fully dissolved.

2.4. HPLC analysis

The antibiotic concentrations in buffer for the elution studies were determined by a high-performance liquid chromatography

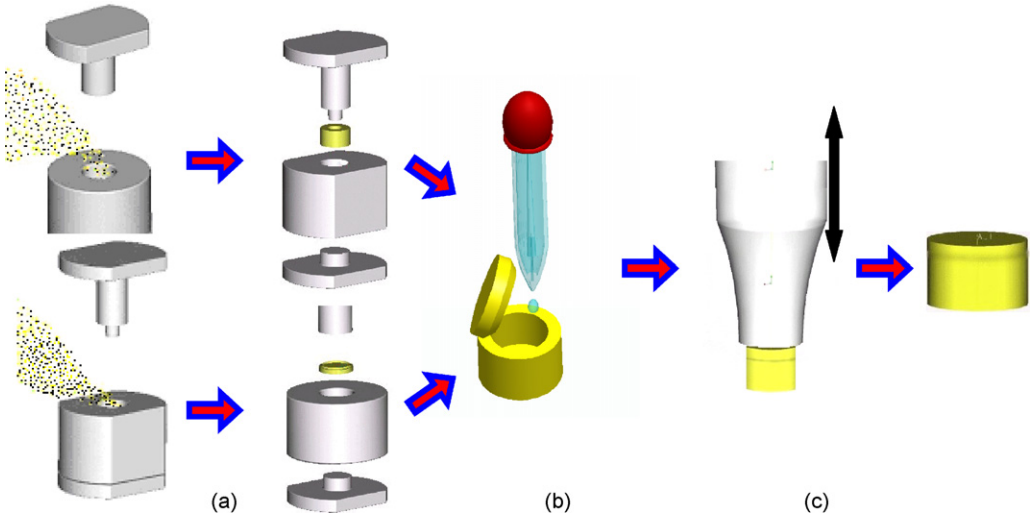

Fig. 2. Schematically, the fabrication process of the biodegradable capsules: (a) compression sintering, (b) addition of core rhBMP-2, and (c) ultrasonic vibration sealing.

(HPLC) assay standard curve for vancomycin. The HPLC analyses were conducted on a Walters 600 Multisolvent Delivery System. The column used for separation of the antibiotics was a SYMMETRY C_8, 3.9 cm × 150 mm HPLC column (Waters). The mobile phase contained 0.01 mol heptanesulphonic acid (Fisher Scientific UK Ltd.) and acetonitrile (Mallinckrodt, USA) (85/15, v/v). The absorbency was monitored at 280 nm and the flow rate was 1.4 ml/min. All samples were assayed in triplicate and sample dilutions were performed to bring the unknown concentrations into the range of the assay standard curve. A calibration curve was made for each set of the measurements (correlation coefficient >0.99). The elution product can be specifically identified and quantified with high sensitivity using the HPLC system (Khalfi et al., 1996).

2.5. Activities of released antibiotics

The relative activity test of vancomycin to Staphylococcus aureus (ATCC65389) was determined using an antibiotic disk diffusion method (Liu et al., 1999a) in Nutrient Broth (beef extract, peptone, Difco Laboratories). The eluent of the capsules was tested up to 30 days. Based on the HPLC measurement result, each sample was first diluted or concentrated to 50 μg/ml. Eight microliters of the buffer sample from each daily buffer sample was pipetted onto 6 mm absorption disks. The disks were placed on the nutrient agar plates that were seeded with a layer of Staphylococcus aureus, and the zones of inhibition were measured with a micrometer after 16–18 h of incubation at 35 °C. The relative activity of the released antibiotics was defined as:

$$\text{Relative activity (\%)} = \frac{\text{diameter of sample inhibition zone}}{\text{diameter of maximum inhibition zone}} \quad (1)$$

where the diameter of maximum inhibition zone is the diameter for the standard 50 μg/ml concentration of vancomycin.

The minimum inhibitory concentration of vancomycin to Staphylococcus aureus (ATCC65389) was determined using an antibiotic tube dilution method in Cation supplemented Mueller–Hinton Broth (Difco Laboratories). Vancomycin was diluted serially twofold in tubes containing 0.5 ml of the Cation Supplemented Mueller–Hinton Broth.

2.6. BMP-2 detection by enzyme-linked immunosorbent assay (ELISA)

The amount of rhBMP-2 in the collected medium was determined by enzyme linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA) (Anon., 2006) in accordance with the manufacture's instructions. Briefly, a monoclonal antibody specific for BMP-2 had been pre-coated onto a 96-well microplate. Standards and samples were pipetted into the wells and any BMP-2 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for BMP-2 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of BMP-2 bound in the initial step. The reaction was stopped with sulfuric acid and the optical density change recorded at 450 nm. Included in the enzyme linked immunosorbent assay were negative control wells containing just media and positive control wells containing known concentrations of rhBMP-2 facilitating construction of a standard curve. Bone morphogenetic protein-2 production in medium was determined by comparison to this standard curve. More details can be found in the instruction manual in Anon. (2006).

2.7. ALP assay

Since alkaline phosphatase is a cell-surface enzyme, alkaline phosphatase activity is measured in living cultures. New Zealand rabbits weighing 3 kg were anesthetized by an intravenous injection of 5 ml of ketamine hydrochloride (Ketalar, Parke Davis, Taiwan) and Rompum (Bayer, Leverkusen, Germany) mixture. Under sterile conditions, 10 ml of bone marrow aspirated from the iliac crest was collected into a syringe containing 6000 units of heparin to prevent clotting. The marrow sample was washed with Dulbecco's phosphate-buffered saline (DPBS), disaggregated by passing it gently through a 21-gauge intravenous catheter and syringe to create a single cell suspension. Cells were recovered after centrifugation at 600 × *g* for 10 min. Up to 2×10^8 nucleated cells in 5 ml of DPBS were loaded onto 25 ml of Percoll cushion (Pharmacia Biotech) of a density of 1.073 g/ml in a 50 ml conical tube. Cell separation was accomplished by centrifugation at 1100 × *g* for 40 min at 20 °C. The nucleated cells were collected from the interface, diluted with two volumes of DPBS, and collected by centrifugation at 900 × *g*. The cells were resuspended, counted, and plated at 2×10^5 cells/cm^2 in T-75 flasks (Falcon). The cells were maintained in Dulbecco's Modified Eagle's Medium-Low Glucose (DMEM-LG; Gibco) containing 10% fetal bovine serum (FBS) and antibiotics (mixture of 100 units/ml of penicillin and 100 μg/ml of streptomycin; Gibco) at 37 in a humidified atmosphere of 5% CO_2 and 95% air. After 4 days of primary culture, the non-adherent cells were removed by changing the medium; medium was changed every 3 days thereafter. MSCs grew as symmetric colonies and were subcultured at 10–14 days by treatment with 0.05% trypsin and 0.53 mM EDTA for 5 min, rinsed from the substrate with serum-containing medium, collected by centrifugation at 800 × *g* for 5 min, and seeded into fresh flasks at 5000–6000 cells/cm^2. All animal procedures received institutional approval and all studied animals were cared for in accordance with the regulations of the National Institute of Health of the Republic of China (Taiwan), under the supervision of a licensed veterinarian.

Cultures were incubated in a humidified atmosphere of 5% CO_2/95% air until cell confluence. The medium was withdrawn a 10 ml aliquot of alkaline phosphatase substrate buffer (50 mM glycine, 1 mM $MgCl_2$, pH 10.5), containing the soluble chromogenic alkaline phosphatase substrate (2.5 mM *p*-nitrophenyl phosphate), was added at room temperature. During incubation, cell-surface alkaline phosphatase converted *p*-nitrophenyl phosphate into *p*-nitrophenol, which then took on a yellow color. Twenty minutes after substrate addition, 1 ml of the buffer was

removed from the culture and mixed with 1 ml of 1N NaOH to stop the reaction. The absorbance of the mixture was read in triplicate on an ELISA plate-reader (MRX, Dynatech Labs) at 405 nm and compared to serially diluted standards. Enzyme activity was expressed as nmol *p*-nitrophenol/min.

3. Results

All capsules were manufactured by a compression-sintering and ultrasonic welding method. An in vitro elution method was employed to determine the release curves of vancomycin and BMP-2 from the polymer capsules. Three specimens were performed for each test. The release characterization of vancomycin was performed by an HPLC system and a bacteria activity test, while the release curve of rhBMP-2 was determined by the ELISA and ALP assays.

3.1. Release characteristics of antibiotics

The HPLC results for vancomycin standard curves with six different standard concentrations are showed in Fig. 3. The calibration fitting for the curve is:

$$Y = 1,565,830(X) - 510,180.6, \qquad R = 0.99999 \qquad (2)$$

Both the cylinder and the cover of the capsule were composed of poly(D,L)-lactide-*co*-glycolide/vancomycin while the core contained rhBMP-2. After the addition of BMP-2 into the core, the capsules were sealed by the ultrasonic welder. The HPLC analysis results in Fig. 4 from the in vitro elution test showed that the capsules released high concentration of vancomycin (well above the minimum inhibition concentration) for up to 30 days. In addition, the percentages of vancomycin released at days 7, 14, 21 and 28 were 54%, 78%, 89% and 93%, respectively.

The relative activity test of eluted vancomycin to Staphylococcus aureus (ATCC65389) was determined by using an antibiotic disk diffusion method in the Nutrient Broth. The disk diffusion assay results for vancomycin standard curves with five different standard concentrations are showed in Fig. 5a.

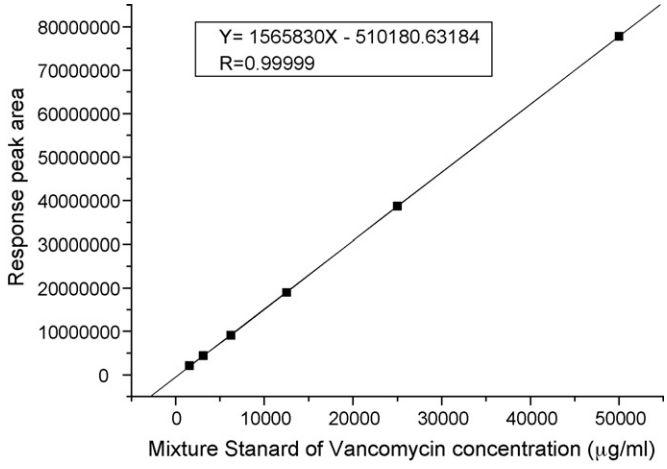

Fig. 3. HPLC calibration curve for vancomycin.

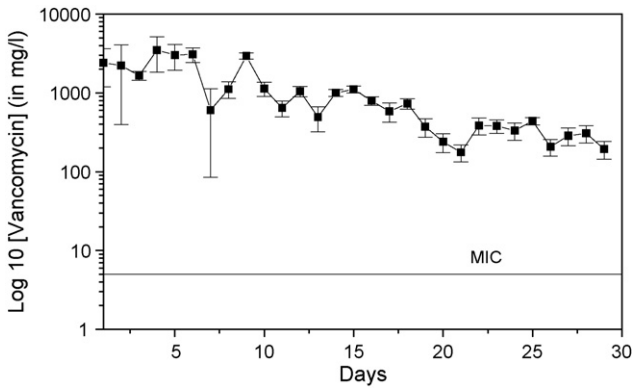

Fig. 4. Release curves of vancomycin from the biodegradable capsules (solid line is the minimum inhibition concentration).

The calibration fitting for the curve is:

$$Y = 4.2158\log(X) + 0.262, \qquad R = 0.98861 \qquad (3)$$

The results with relative activity and the diameter of the inhibition zone are shown in Fig. 5b. The diameters of sample inhibition zone ranged from 6 to 16 mm and the relative activity of vancomycin ranged from 37.5% to 100%.

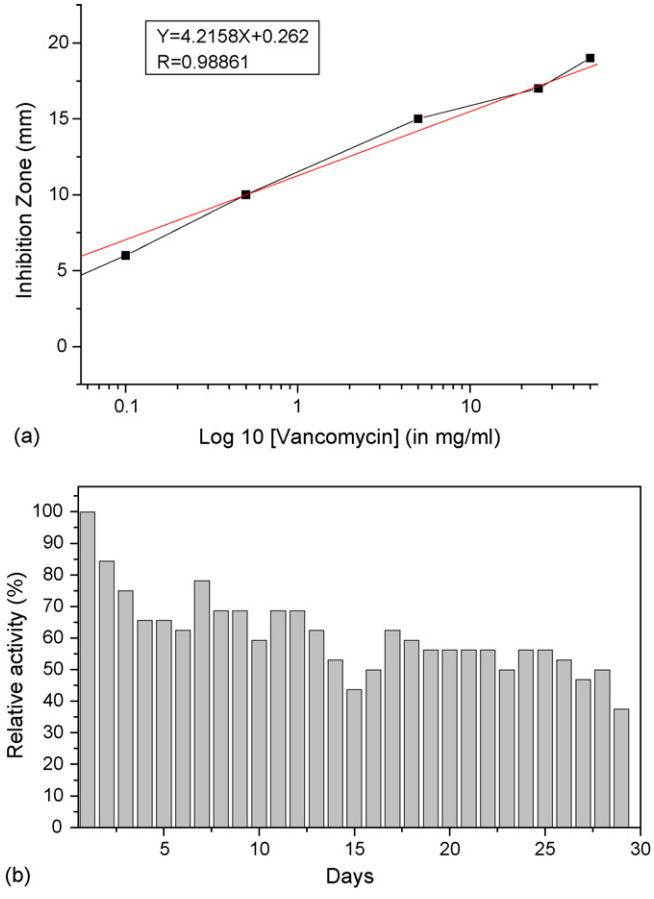

Fig. 5. (a) Calibration curve for the disk diffusion assay of vancomycin and (b) relative activity of eluted vancomycin.

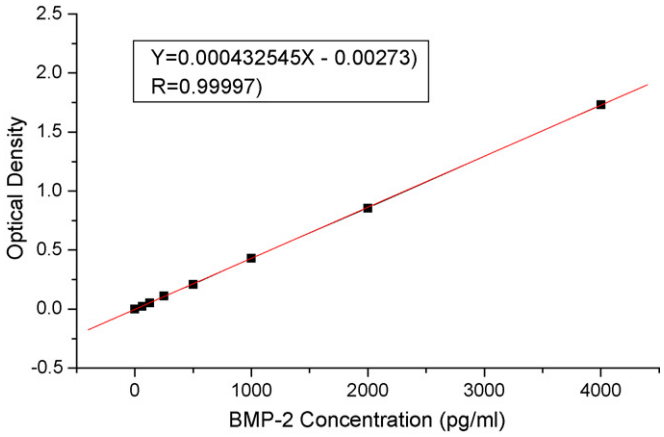

Fig. 6. ELISA calibration curve for BMP-2 concentration.

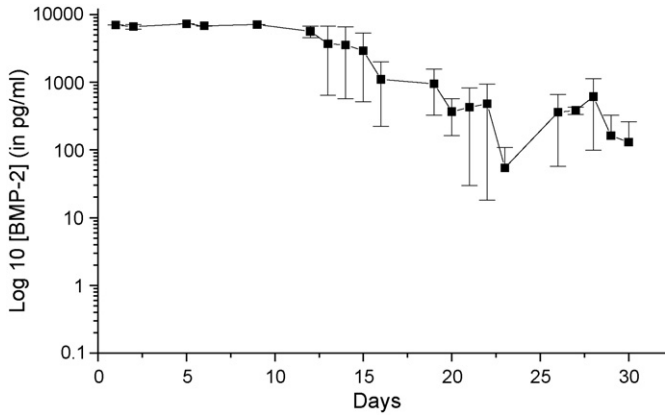

Fig. 8. Release curves of BMP-2 with 100 μg/ml loading at the core of the biodegradable capsules.

3.2. Release of BMP-2 from the biodegradable capsules

The ELISA assays for BMP-2 standard curves with eight different standard concentrations are showed in Fig. 6. The calibration fitting for the curve is:

$$Y = 0.000432545X - 0.00273, \qquad R = 0.99997 \tag{4}$$

Two different concentrations of 10 and 100 μg/ml were used loaded into the capsules. Fig. 7a illustrates the amount of rhBMP-2 over a period of 30 days for the capsules loaded with 10 μg/ml BMP-2, as quantified by ELISA. The bioactivity of the released BMP-2 was determined by the ALP assay. Fig. 7b shows that the released BMP-2 is still bioactive up to 30 days. In addition, Fig. 8 shows the release characteristic of rhBMP-2 for the capsules loaded with 100 μg/ml concentration. Clearly, the capsules loaded with high BMP-2 concentration (100 μg/ml) can release a higher amount of BMP-2 during the elution process.

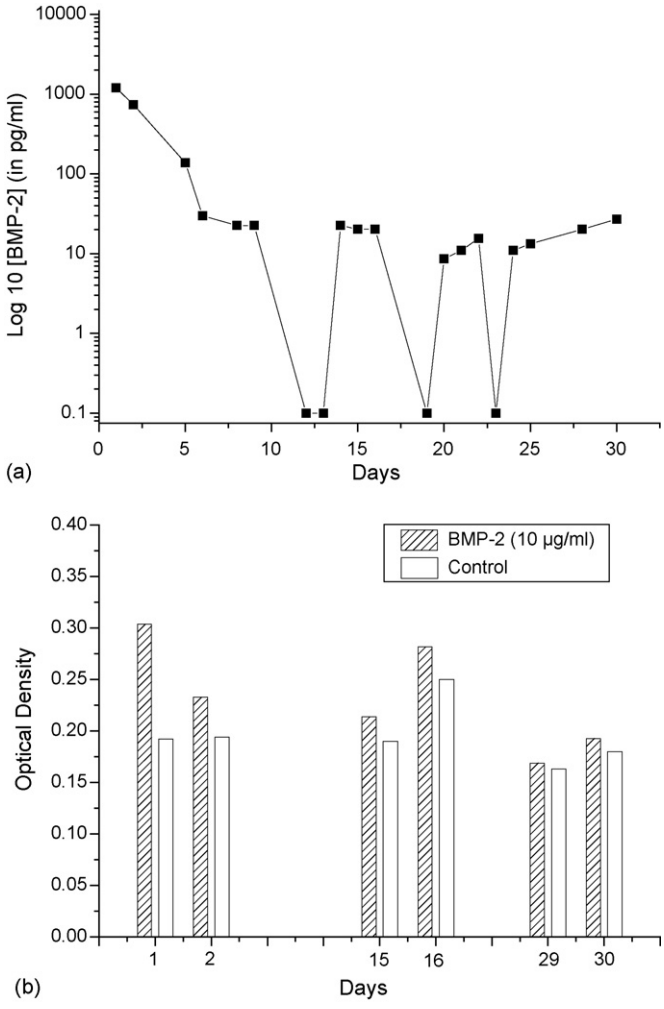

Fig. 7. (a) Release curves of BMP-2 with 10 μg/ml loading, and (b) bioactivity of released BMP-2 at various days.

4. Discussion

Various types of biomaterials were used based on their ability to achieve a sustained bacterial concentration of antibiotics and growth factors. Poly(lactic acid) is one of the most promising biodegradable bio-materials (Williams, 1982; Miller and Williams, 1984; Ali et al., 1993). It is non-toxic, elicits a minimal inflammatory response and can be eventually absorbed without any accumulation in the vital organs (Ali et al., 1993; Kobayashi et al., 1992). The release rate and duration from the polymeric devices depend upon the requirement of each application. In order to achieve this purpose, various techniques which may be employed are as follows: (1) lactic acid may be copolymerized with glycolic acid. The degradation rate can be varied with the percentage of glycolic acid in the copolymer—the higher the ratio of the lactic acid the longer dissolution time for the poly(D,L)-lacitde-*co*-glycolide beads; (2) the rate of diffusion across PLA beads can be adjusted by varying the molecular weight of the PLA—the lower the molecular weight, the faster the degradation rate; (3) the PLA derived from D,L-lactic acid is less ordered than that from L(+) lactic acid. The former will degrade faster than the latter. By adopting a higher molecular weight or a higher PLA ratio polymer in the experiments, the release rate of the biodegradable devices can be slowed.

In most previous studies, the antibiotic was microencapsulated in a poly(D,L)-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 antibiotic loading dosage (e.g. 50%) and was manufactured with the use of various solvents (Nie et al., 1995; Setterstrom et al., 1984; Calhoun and Mader, 1997; Garvin et al., 1994; Zhang et al., 1994; Jacob et al., 1991). 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 antibiotic release rate, but also its release mechanism.

Among the plethora of bioactive factors available, the bone morphogenetic protein-2 (BMP-2) is reportedly the strongest osteoinductive factor administered therapeutically to restore form and function to bone. Nevertheless, currently used carriers for BMP-2, mainly collagen and ceramics, are less than ideal and the development of a carrier for both antibiotics and bioactive factors is highly desired from a clinical point of view. Numerous groups are working on drug delivery systems for bone engineering. Problems inherent in the currently used delivery systems for BMP-2 include slow release, unfavorable architecture for vascularization and lack of mechanical strength. BMP-2 is endogenously produced within a few days after fracture, suggesting that BMP is necessary for bone formation within a few days after post-trauma.

In this study, a novel method was proposed to manufacture the biodegradable capsules that can release antibiotics and rhBMP-2 simultaneously: we chose low molecular weights (e.g. MW 5000), low antibiotics loading dosages (e.g. 20%), a low melt processing temperature (55 °C), as well as compression sintering and ultrasonic welding techniques to form biodegradable antibiotic/BMP-2 capsules. In addition, no solvent was needed during the manufacturing process, providing the advantage of avoiding the problems associated with the use of solvents.

During the manufacture of polymer capsules, 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 as densification (Liu, 1998). The antibiotic is then encapsulated by the polymer to form a composite wall for the capsules.

After the compression sintering, ultrasonic welding is used to seal the capsule made of PLA/PGA copolymer, which is a type of thermoplastic. In ultrasonic welding of thermoplastics, heat is generated when the plastic is subjected to cyclic strain. The power dissipated depends upon the loss modulus of the polymer and the cyclic strain amplitude (Liu et al., 1998):

$$Q = \frac{\omega \varepsilon_o^2 E''}{2} \tag{5}$$

$$E^* = E' + iE'' \tag{6}$$

where Q is the average power dissipated, ω the frequency, and ε_o is the strain amplitude. E^*, E' and E'' are the complex, storage and loss modulus, respectively (Liu et al., 1998, 1999b). In the experiments, the polylactide–polyglycolide used was an amorphous polymer and had a high stiffness and therefore a higher storage modulus E' and a lower loss modulus E''. It then vibrated in phase with the horn, and more energy was transferred to the energy director by vibration to seal the capsules. No seal break of the capsules was observed for the entire elution of the capsules in the experiments. The ultrasonic welding could provide a good seal at the weld interface.

For a water-soluble antibiotic in a hydrophobic polylactide matrix, the release mechanisms are controlled by channel diffusion, osmotic pressure, and polymer degradation. Firstly, when antibiotic loading is low, 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. With an increase in antibiotic loading, antibiotic particles will connect together to form channels leading to the surface of the bead. This antibiotic will be released by channel diffusion (Seigel and Langer, 1990). Secondly, if the polymer matrix surrounding the isolated particles remains intact during the release, antibiotic will not be released from these clusters. However, water will be taken up by a water-soluble antibiotic with a high osmotic pressure through the polymer, causing swelling of the particle. The polymer matrix may break under this swelling to form openings for antibiotic release. Finally, when the polymer molecular weight decreases sufficiently, loss of polymer begins. The antibiotic will then be released along with this polymer loss (Seigel and Langer, 1990).

The mechanism of release of rhBMP-2 from the capsules is also an important consideration. Drugs formulated in polymeric devices are usually released by diffusion through the polymer barrier, by degradation of the polymer materials, or a combination of both mechanisms. In this study, the slow degradation of PLGA materials makes diffusion through the capsules the only possible mechanism of drug release (Rai et al., 2005). As the loading of rhBMP-2 was low, the capsules did not release high enough concentration during the elution process (Fig. 7a). This might be due to the fact that during elution the rhBMP-2 particles may attach to the wall of the capsules or entrapped within the capsules. The released rhBMP-2 concentration is thus low. By increasing the loading of rhBMP-2 in the capsules, we can thus increase the released concentration of eluted rhBMP-2 (Fig. 8). In addition, the ALP assay conceded that the rhBMP-2 released still maintained its bioactivity at all time points. This implies that the aggregation of rhBMP-2 observed upon loading onto biodegradable capsules in the present study did not result in inactivation of the factor. In addition, at day 30, release study supernatants from biodegradable capsules loaded with 100 μg/ml of rhBMP-2 showed significant ALP activity.

This suggests that sufficient intact rhBMP-2 molecules were still present to enhance the osteoblastic phenotype and that rhBMP-2 can maintain its bioactivity in vitro for up to 30 days.

The bactericidal effects of the antibiotics and rhBMP-2 incorporated into the biodegradable capsules far outweigh any negative inherent effects of the device itself. A significant advantage of the biodegradable capsule 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 vancomycin eluted from the beads were much greater than the minimum inhibitory concentration for up to 30 days (Fig. 4). The capsules also released high concentration of rhBMP-2 for bone formation after post-trauma for a period of 30 days (Fig. 8). In addition, the activities of both the antibiotics and rhBMP-2 were still high after the fabrication process.

It should be noted that although this study has investigated the in vitro dissolution rate of the capsules, an extended study will be done in future works to determine the in vivo release pattern of the capsules and whether it is equivalent to the in vitro one.

5. Conclusions

This paper has proposed a novel solvent-free method of processing the biodegradable polymers as antibiotic capsules for a long-term drug release. An elution method was employed to characterize the in vitro release rates of the antibiotics and the rhBMP-2 from the capsules over a 30-day period. The HPLC analysis and the bacterial inhibition test showed that biodegradable capsules released high concentrations and activity of vancomycin (well above the minimum inhibition concentration) in vitro for the period of time needed to treat bone infection; i.e. 4–6 weeks. In addition, the results of ELISA and ALP tests also suggested that the capsules released high concentrations of active rhBMP-2 for up to 30 days. The bioactivities of the released antibiotics and rhBMP-2 were still high after the manufacturing process. By adopting this novel technique, we will be able to fabricate biodegradable capsules of various medicines for long-term drug deliveries.

Further studies being conducted in our laboratory are investigating the biodegradable antibiotic/rhBMP-2 capsules in animal model, such as a joint arthroplasty infection and localized osteomyelitis models. Eventually biodegradable antibiotic capsules may be used in humans for the treatment of various surgical infections.

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References

Ali, S.A.M., Doherty, P.J., Willliams, D.F., 1993. Mechanisms of polymer degradation in implantable devices. 2: Poly(D,L-lactic acid). J. Biomed. Mater. Res. 27, 1409–1418.

Anon. (2006). Quantikine, BMP-s Immunoassay, Catalog No. DBP200 R&D Systems, Minneapolis, MN, USA.

Bessho, K., Carnes, D.L., Cavin, R., Ong, J.L., 2002. Experimental studies on bone induction using low-molecular-weight poly(D,L-lactide-*co*-glycolide) as a carrier for recombinant human bone morphogenetic protein-2. J. Biomed. Mater. Res. 61, 61–65.

Buchholz, H.W., Elson, R.A., Engelbrecht, E., Lodenkamper, H., Rottger, J., Siegel, A., 1981. Management of deep infection of total hip replacement. J. Bone Joint Surg. Br. 63, 342–353.

Burkus, J.K., Dorchak, J.D., Sanders, D.L., 2003. Radiographic assessment of interbody fusion using recombinant human bone morphogenetic protein type 2. Spine 28, 372–377.

Calhoun, J.H., Mader, J.T., 1989. Antibiotic beads in the management of surgical infections. Am. J. Surg. 157, 443–449.

Calhoun, J.H., Mader, J.T., 1997. Treatment of osteomyelitis with a biodegradable antibiotic implant. Clin. Orthop. 341, 206–214.

Elson, R.A., Jephcott, A.E., McGechie, D.B., Vereitas, D., 1977. Antibiotic-loaded acrylic cement. J. Bone Joint Surg. 59, 200–205.

Garvin, K.L., Miyano, J.A., Robinson, D., Giger, D., Novak, J., Radio, S., 1994. Polylactide/polyglycolide antibiotic implants in the treatment of osteomyelitis: a canine model. J. Bone Joint Surg. 76, 1500–1506.

Govender, S., Csimma, C., Genant, H.K., Valentin-Opran, A., Amit, Y., Arbel, R., et al., 2002. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J. Bone Joint Surg. Am. 84-A, 2123–2134.

Hill, J., Klenerman, L., Trustey, S., Blowers, R., 1977. Diffusion of antibiotics from acrylic bone-cement in vitro. J. Bone Joint Surg. 59, 197–199.

Jacob, E., Setterstrom, J., Bach, D., Heath, J., McNiesh, L., Cierny III, G., 1991. Evaluation of biodegradable ampicillin anhydrate microcapsules for local treatment of experimental staphylococcal osteomyelitis. Clin. Orthop. 267, 237–244.

Kato, M., Toyoda, H., Namikawa, T., Hoshino, M., Terai, H., Miyamoto, S., Takaoka, 2006. Optimized use of a biodegradable polymer as a carrier material for the local delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2). Biomaterials 27, 2035–2041.

Khalfi, F., Dine, T., Gressier, B., Luyckx, M., Brunet, C., Ballester, L., Goudaliez, F., Cazin, M., Cazin, J.C., 1996. Compatibility and stability of vancomycin hydrochloride with PVE infusion material in various conditions using stability indicating high-performance liquid chromatographic assay. Int. J. Pharm. 139, 243–247.

Kobayashi, H., Shiraki, K., Ikada, Y., 1992. Toxicity test of biodegradable polymers by implantation in rabbit cornea. J. Biomed. Mater. Res. 26, 1463–1476.

Kokubo, S., Fujimoto, R., Yokota, S., Fukushima, S., Nozaki, K., Takahashi, K., Miyata, K., 2002. Bone regeneration by recombinant human bone morphogenetic protein-2 and a novel biodegradable carrier in a rabbit ulnar defect model. Biomaterials 24, 1643–1651.

Liu, S.J., 1998. Sintering rheology of semi-crystalline polymers. Int. Polym. Proc. 13, 88–90.

Liu, S.J., Chang, B.C., Chien, L.H., Tsai, Y.H., 1998. Development of weldability diagram for ultrasonically welded thermoplastics. Plast. Rubber Compos. 27, 279–286.

Liu, S.J., Ueng, S.W.N., Chan, E.C., Lin, S.S., Tsai, C.H., Wei, F.C., Shih, C.H., 1999a. In vitro elution of antibiotics from biodegradable beads. J. Biomed. Mater. Res. (Appl. Biomater.) 48, 613–620.

Liu, S.J., Lin, W.F., Chang, B.C., Wu, G., Hung, S.W., 1999b. Optimizing the joint strength in ultrasonically welded thermoplastics. Adv. Polym. Technol. 18, 125–136.

Liu, S.J., Ueng, S.W.N., Lin, S.S., Chan, E.C., 2002. In vivo release of antibiotics from biodegradable beads. J. Biomed. Mater. Res. (Appl. Biomater.) 63, 807–813.

Liu, S.J., Tsai, Y.E., Ueng, S.W.N., Chan, E.C., 2005. A novel solvent-free method for the manufacture of biodegradable antibiotic-capsules for a long-term drug release using compression sintering and ultrasonic welding. Biomaterials 26, 4662–4669.

Miller, N.D., Williams, D.F., 1984. The in vivo and in vitro degradation of poly(glycolic acid) suture material as a function of applied strain. Biomaterials 5, 365–368.

Mori, M., Isobe, M., Yamazaki, Y., Ishihara, K., Nakabayashi, N., 2000. Restoration of segmental bone defects in rabbit radius by biodegradable capsules containing recombinant human bone morphogenetic protein-2. J. Biomed. Mater. Res. 50, 191–198.

Nie, L., Nicolau, D.P., Nightingale, C.H., Browner, B.C., Quintiliani, R., 1995. In vitro elution of ofloxacin from a bioabsorbable polymer. Acta Orthop. Scand. 66, 365–368.

Oldham, J.B., Lu, L., Zhu, X., Porter, B.D., Hefferan, T.E., Larson, D.R., Currier, B.L., Mikos, A.G., Yaszemski, M.J., 2000. Biological activity of rhBMP-2 released from PLGA microspheres. J. Biomech. Eng. 122, 289–292.

Rai, B., Teoh, S.H., Hutmacher, D.W., Cao, T., Ho, K.H., 2005. Novel PCL-based honeycomb scaffolds as drug delivery systems for rhBMP-2. Biomaterials 26, 3739–3748.

Ruhe, P.Q., Boerman, O.C., Russel, F.G.M., Spauwen, P.H.M., Mikos, A.G., Jansen, J.A., 2005. Controlled release of rhBMP-2 loaded poly(D,L-lactic-*co*-glycolic acid)/calcium phosphate cement composites in vivo. J. Control. Release 106, 162–171.

Seigel, R.A., Langer, R., 1990. Mechanistic studies of macromolecular drug release from macroporous polymers. II: Models for the flow kinetics of drug release. J. Control. Release 14, 153–167.

Setterstrom, J.A., Tice, T.R., Myers, W.E., 1984. Development of encapsulate antibiotics for topical administration to wounds. In: Kim, S.W. (Ed.), Recent Advances in Drug Delivery Systems. Plenum, New York.

Shinto, Y., Uchida, A., Korkusuz, F., Araki, N., Ono, K., 1992. Calcium hydroxyapatite ceramic used as a drug delivery system for antibiotics. J. Bone Joint Surg. 74, 600–604.

Ueng, S.W.N., Wei, F.C., Lee, S.S., Shih, C.H., 1997. Management of large infected tibial defect with antibiotic beads local therapy and staged fibular osteoseptocutaneous free transfer. J. Trauma 43, 268–274.

Waldvogel, F.A., Medoff, G., Swartz, M.N., 1970. Osteomyelitis: a review of clinical features, therapeutic considerations and unusual aspects. N. Engl. J. Med. 282, 198–206, 260–266, 316–322.

Waldvogel, F.A., Vasey, H., 1980. Osteomyelitis: the past decade. N. Engl. J. Med. 303, 360–370.

Wang, G., Liu, S.J., Ueng, S.W.N., Chan, E.C., 2004. The release of cefazolin and gentamicin from biodegradable PLA/PGA beads. Int. J. Pharm. 273, 203–212.

Williams, D.F., 1982. Biodegradation of surgical polymers. J. Mater. Sci. 17, 1233–1246.

Zhang, X., Wyss, U.P., Pichora, D., Goosen MFA, 1994. Biodegradable controlled antibiotic release devices for osteomyelitis: optimization of release properties. J. Pharm. Pharmacol. 46, 18–24.