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In vitro elution of vancomycin/amikacin/steroid from solvent-free biodegradable scleral plugs

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ABSTRACT

The purpose of this report was to develop solvent-free biodegradable scleral plugs for vancomycin, amikacin and dexamethasone delivery for endophthalmitis treatment. To fabricate a biodegradable plug, polylactide–polyglycolide copolymers were pre-mixed with the drugs. The mixture was then compression molded and sintered to form a scleral plug of 1.4 mm in diameter. An elution method was utilized to characterize the in vitro release characteristics of the antibiotics and the steroids over a 14-day period. The HPLC analysis and bacterial inhibition test showed that biodegradable scleral plugs released a high concentration resulting in significant activity of vancomycin and amikacin (well above the minimum inhibition concentrations) and dexamethasone in vitro, for the period of time needed to treat intraocular infection. A bacterial inhibition test was carried out to determine the relative activity of the released antibiotics. The activities of the eluted vancomycin and amikacin ranged from 69% to 89% and from 66% to 88%, respectively. In addition, the experimental result suggests that one will be able to reduce the drug release rate and prolong the total release period of the plugs by adopting a lower antibiotic/steroid to polymer ratio, increasing the sintering temperature, or increasing the compression pressures.

1. Introduction

Endophthalmitis is a serious, sight-threatening disease, and occurs when intraocular surgery, penetrating injury, a corneal ulcer, or periocular infection breaches the external ocular barriers and allows a pathogen access to the intraocular spaces. Delivering an effective antimicrobial at sufficiently high concentrations to the area of infection is a standard treatment for postoperative endophthalmitis (Graham and Peyman, 1974; Peyman and Herbst, 1974; Diamond, 1981; EVSG, 1995). This is currently done by trans pars plana intravitreal injection of both antibiotics directed against gram-positive and gram-negative microorganisms (Han et al., 1996; Benz et al., 2004; Aguilar et al., 1995; Haider et al., 2001; D'Amico et al., 1985; Campochiaro and Lim, 1994) and steroids (Das et al., 1999; Shah et al., 2000). However, injections by needles repeated every 3 days for up to 2 weeks may increase the risks of intraocular infection and hemorrhage as well as retinal detachment. Additionally, the level of pharmaceutical delivery into the vitreous chamber is less than optimal.

An ideal drug delivery system for endophthalmitis treatment 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. Based on this concept, a biodegradable scleral plug (Yasukawa et al., 2001) (Fig. 1) incorporating various types of pharmaceuticals can be a good alternative for drug delivery into the intraocular spaces.

This paper was to develop solvent-free biodegradable scleral plugs for the delivery of antibiotics and steroids. We have adopted a compression sintering technique to manufacture biodegradable polymer plugs that can simultaneously release vancomycin, amikacin and dexamethasone. Scleral plugs were evaluated by an in vitro elution method. An HPLC analysis and a bacterial activity test were utilized to evaluate the release characteristics of vancomycin, amikacin and steroids from the biodegradable plugs. The final goal of this research was to develop a biodegradable system for the delivery of antibiotics and steroids, and to provide an improved method for endophthalmitis treatment.

2. Materials and methods

2.1. Materials

The polymers used were polylactide–polyglycolide with a ratio of 50:50 and a molecular weight of approximately 30,000. All poly-

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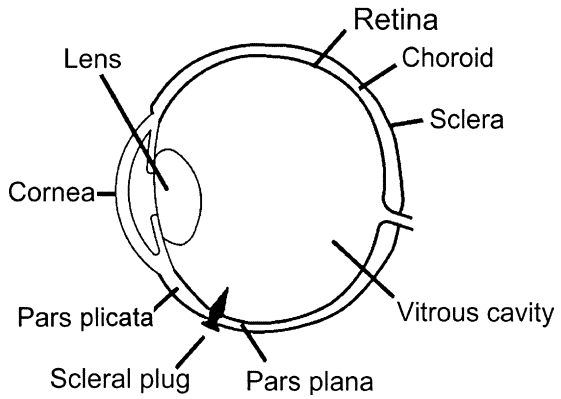

Fig. 1. Schematically, the biodegradable scleral plug for intraocular drug delivery.

mers 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 polymer' glass transition temperature was in the range of 45–50 °C. The antibiotics used were commercial grade vancomycin (Abbott Lab., USA) and amikacin (Federal Chem., Taiwan), while the steroid used was dexamethasone (Sigma, USA). All pharmaceutics were available in powder form with a particle size of approximately 100 μm.

2.2. Fabrication of antibiotics/steroid plugs

To fabricate the biodegradable plugs, polylactide–polyglycolide copolymers were pre-mixed with the drugs. Two drug/polymer ratios were used, i.e., 1:2 and 1:3. The corresponding weight ratios of vancomycin, amikacin and steroids of the drug combination are 1:0.4:0.4 and 0.8:0.3:0.3, respectively. Table 1 lists the contents of the mixtures used in the plugs. The mixture was then compression molded (Liu et al., 1999; Wang et al., 2004) to form a scleral plug of 1.4 mm in diameter (Fig. 2(a)). The compressed plugs 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 degrading the antibiotics and the steroids. The sintering time used was 30 min in order to attain an isothermal sintering of the materials. Fig. 2(b) shows schematically the mold and the biodegradable plugs.

2.3. Activities of released antibiotics

The relative activity test of vancomycin to *Staphylococcus aureus* (ATCC65389) was determined using an antibiotic disk diffusion method in Nutrient Broth (beef extract, peptone, Difco Laboratories). The eluent of the plugs was tested up to 14 days. Each sample was first diluted or concentrated to 50 μg/ml. A buffer sample of 8 μL collected daily was pipetted onto 6 mm absorption disks. The disks were placed on nutrient agar plates that were 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. The relative activity

Table 1
Composition of the biodegradable scleral plugs.

Drug/polymer ratio	Weights of drugs (mg)			Weight of polymer (mg)
	Vancomycin	Amikacin	Dexamethasone	
1:2	1	0.4	0.4	4
1:3	0.8	0.3	0.3	4.5

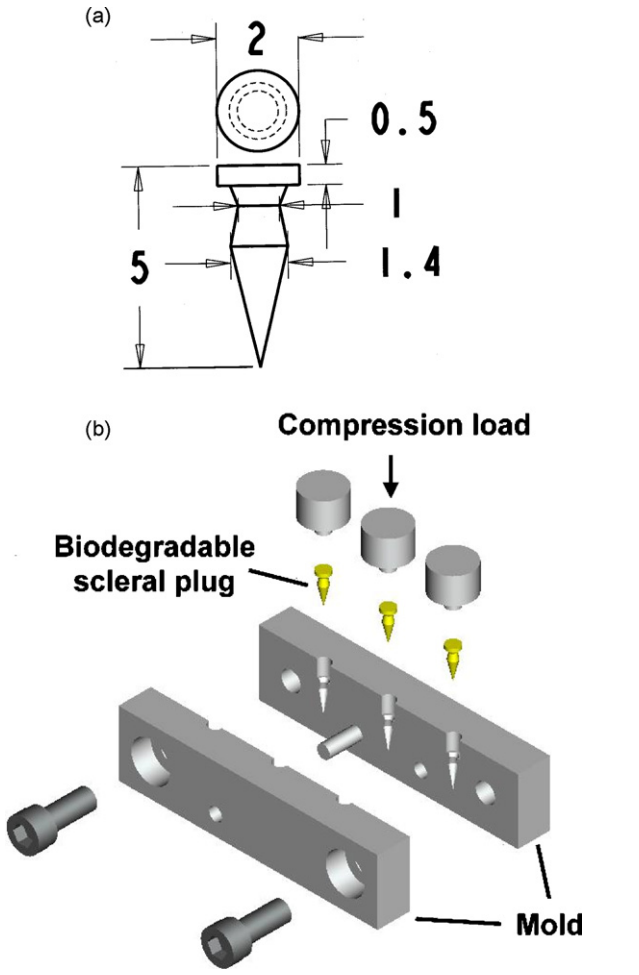

Fig. 2. (a) Dimensions of the scleral plug (unit: mm), and (b) the mold for the manufacture of the plugs.

of the released antibiotics was defined as:

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

The released concentration of amikacin was also determined by the disk diffusion method the same as that of vancomycin, except that the bacteria used was *Escherichia coli*.

2.4. Thermal stability of antibiotics and steroids

In order to determine whether the heat might degrade the antibiotics during the manufacturing process of the biodegradable plugs, thermal stability tests were performed. A 20 mg sample of vancomycin hydrochloride was incubated in an oven for 120 min at various temperatures ranging from 37 to 80 °C, with a negative control that was not sintered. The relative activities of the incubated vancomycin hydrochloride to *S. aureus* (ATCC65389) were determined by the antibiotic disk diffusion method. The thermal stability of amikacin was also performed with the same scheme against *E. coli* (ATCC25922).

The thermal stability of the dexamethasone was determined by the Fourier Transform Infrared (FTIR) spectrometry to examine whether the chemistry and orientation of material structures varied with temperatures. The FTIR analysis was conducted on a Bruker Tensor 27 spectrometer. Dexamethasone was considered stable if there was no obvious structural change due to temperature.

2.5. In vitro elution

An in vitro elution method was utilized to determine the release characteristics of antibiotics and steroid from the plugs. A balance salt solution (BSS) buffer was used as the dissolution medium. The plugs were placed in glass test tubes with 2 ml of BSS buffer. All tubes were incubated at 37 °C. The dissolution medium was collected for subsequent analyses at every 24-h interval. Fresh BSS buffer (2 ml) was then added for the next 24-h period and this procedure was repeated for 14 days.

2.6. HPLC analysis

The antibiotics and steroids concentrations in buffer for the elution studies were determined by a high-performance liquid chromatography (HPLC) assay (Liu et al., 1999; Milojevic et al., 2002). The HPLC analyses were conducted on a Hitachi L-2200 Multisolvent Delivery System. 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.

3. Results

Biodegradable scleral plugs that can deliver various types of pharmaceuticals into the intraocular spaces were successfully manufactured by using the compression-sintering (Liu et al., 1999; Wang et al., 2004) mold shown in Fig. 2(b), which includes two mold-halves and compression plungers.

Before manufacturing the scleral plugs, the thermal stability results with various pharmaceuticals were investigated. The experimental result in Fig. 3(a) suggests that vancomycin was rather stable up to 80 °C. On the other hand, despite the stability of amikacin decreased somewhat with oven temperature, the activity of amikacin was still high (up to 85%) at 55 °C. Meanwhile, no obvious structural change was observed due to temperature increase of up to 80 °C (Fig. 3(b)) for dexamethasone. The steroids used for the experiments were thus thermally stable. All these suggest that the pharmaceuticals used in this study were not degraded while we were manipulating the antibiotic/steroid plugs (at a sintering temperature of 55 °C).

3.1. Release characteristics of antibiotics/steroids

The HPLC calibrations for vancomycin and amikacin standard curves with five different standard concentrations are

$$[\text{Vancomycin}] \quad Y = 20591.8X + 58544.9 \quad R = 0.9997 \qquad (2)$$

$$[\text{Amikacin}] \quad Y = 384.5X + 925.0 \quad R = 0.99874 \qquad (3)$$

On the other hand, the HPLC calibration results for dexamethasone standard curves with five different standard concentrations can be described by

$$[\text{Dexamethasone}] \quad Y = 10089.1X + 44651.6 \quad R = 0.99522 \qquad (4)$$

The HPLC analysis results in Fig. 4 from the in vitro elution test showed that the plugs released a high concentration of vancomycin and amikacin (well above the minimum inhibition concentration) and dexamethasone for up to 14 days.

In this study, it is desirable to be able to adjust the release rate and duration from the antibiotic/steroid plugs by adopting various manufacturing parameters. The effect of the drug mixture/polymer ratio was studied. The result in Fig. 4(a) shows that the release rate of vancomycin decreases with the antibiotic/steroid to polymer ratio. On the other hand, Fig. 5 shows the release curves of the plugs subjected to different sintering temperatures. The experimental result suggests that the drug release is higher when the sintering temperature is lower. Furthermore, two different compression pressures (278 and 556 MPa), which were obtained by dividing the applied forces to the plug's cross-sectional area, were used in the experiments. Fig. 6 shows the release curves of vancomycin, amikacin and dexamethasone from the plugs subjected to different compression pressures during processing. The result also suggests that the release rate of the plugs decrease with the compression pressure. Overall the experimental results from the in vitro elution test showed that the plugs released a high concentration of vancomycin and amikacin (well above the minimum inhibition concentration) and dexamethasone for up to 14 days.

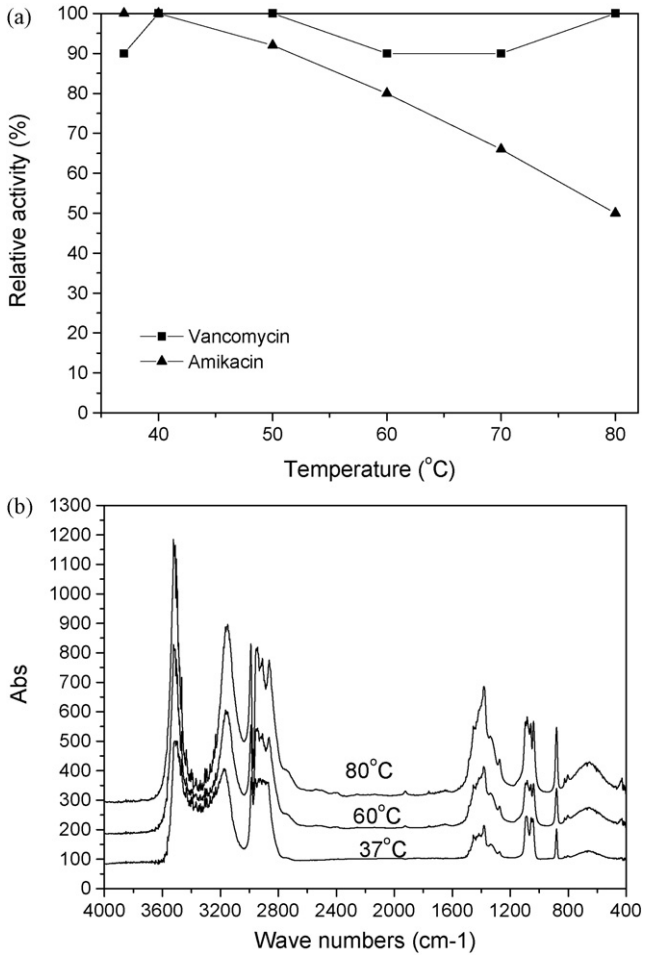

Fig. 3. Thermal stability of (a) the antibiotics, and (b) steroid used in this study.

3.2. Relative activities of eluted antibiotics

The relative activity test of eluted vancomycin to *S. aureus* (ATCC65389) was determined by using an antibiotic disk diffusion method in the Nutrient Broth. The relative activity of eluted amikacin to *E. coli* (ATCC25922) was also determined by the same scheme. The results with relative activity of vancomycin and amikacin are shown in Fig. 7. The activities of the eluted vancomycin and amikacin ranged from 69% to 89% and from 66% to 88%, respectively.

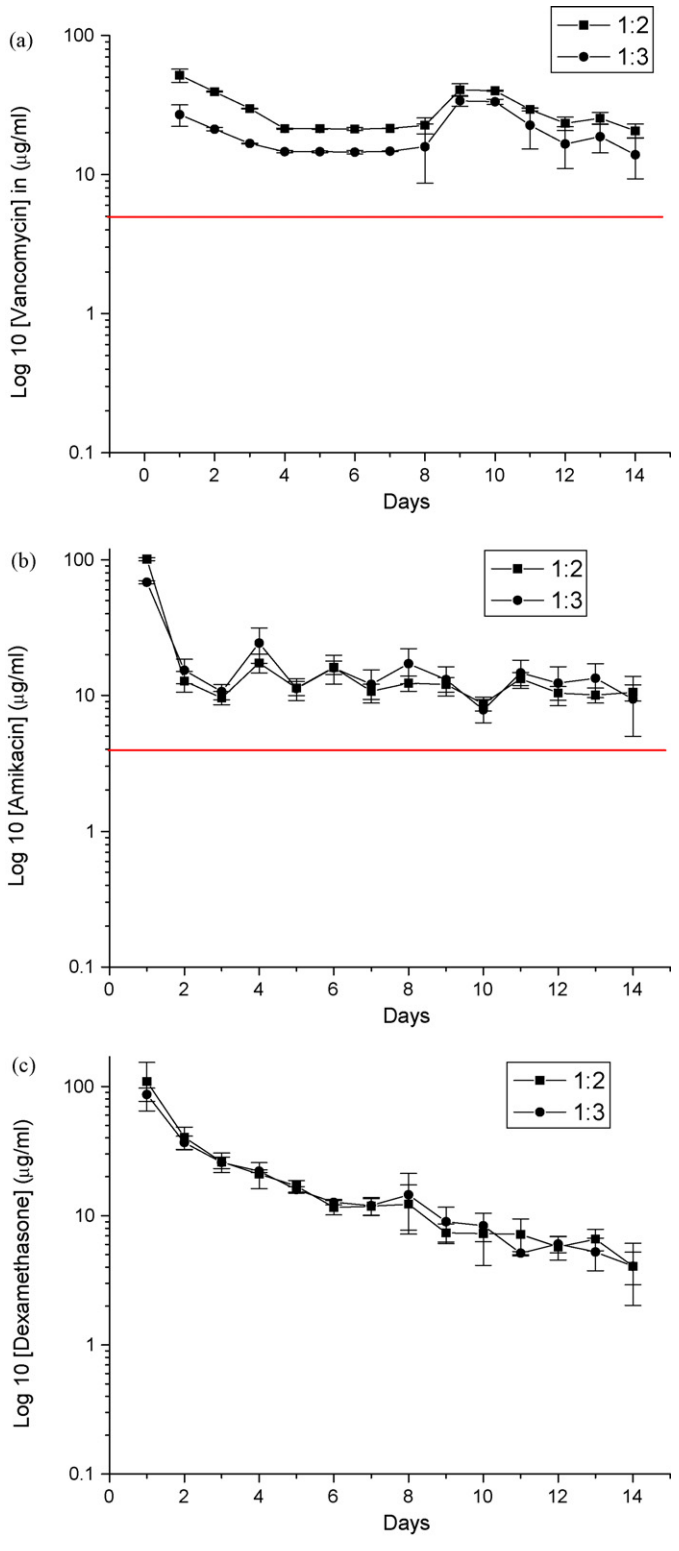

Fig. 4. Release curves of (a) vancomycin, (b) amikacin, and (c) dexamethasone, from biodegradable scleral plugs of different drug mixture to polymer ratios (the oven temperature is 55 °C and the pressure is 278 MPa, solid lines are the minimum inhibition concentrations for antibiotics).

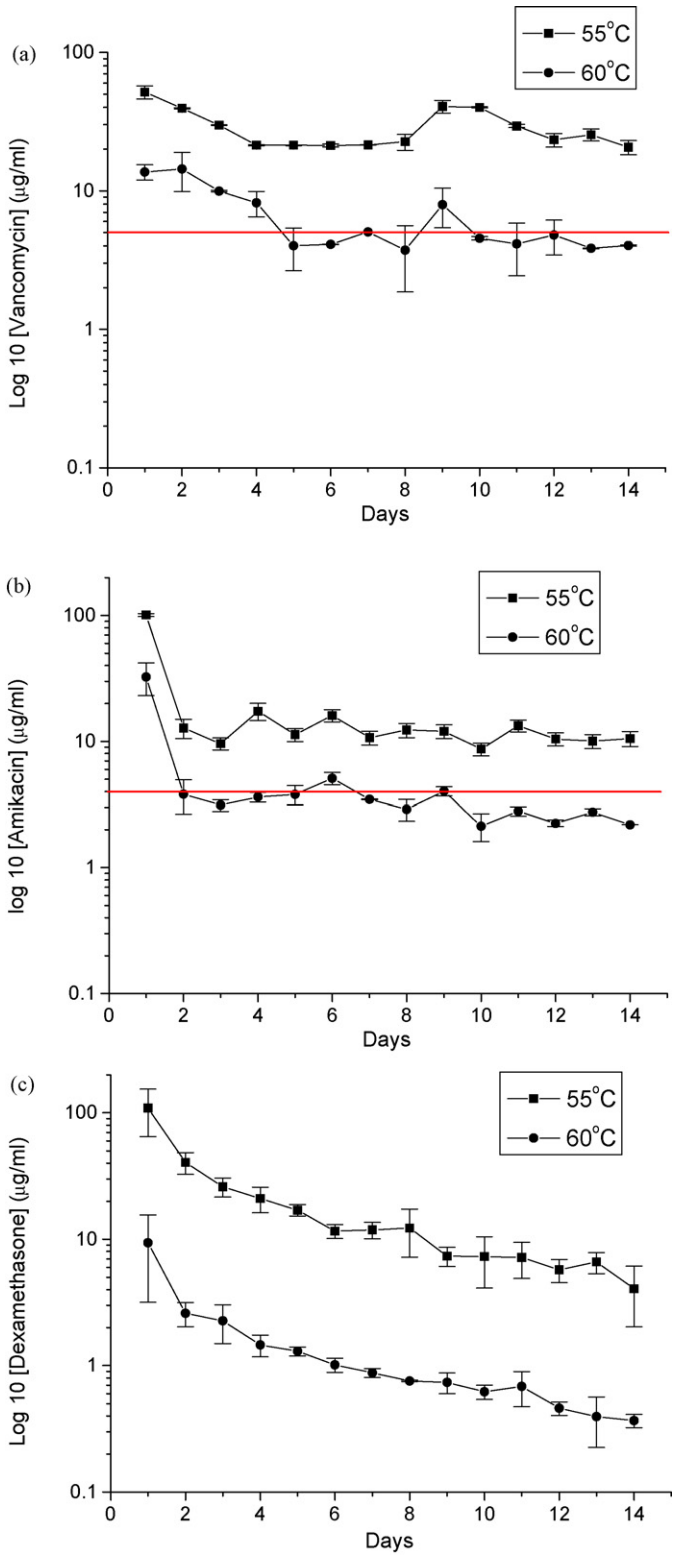

Fig. 5. Release curves of (a) vancomycin, (b) amikacin, and (c) dexamethasone, from biodegradable scleral plugs subjected to different oven temperatures (the drug mixture to polymer ratio is 1:2 and the pressure is 278 MPa, solid lines are the minimum inhibition concentrations for antibiotics).

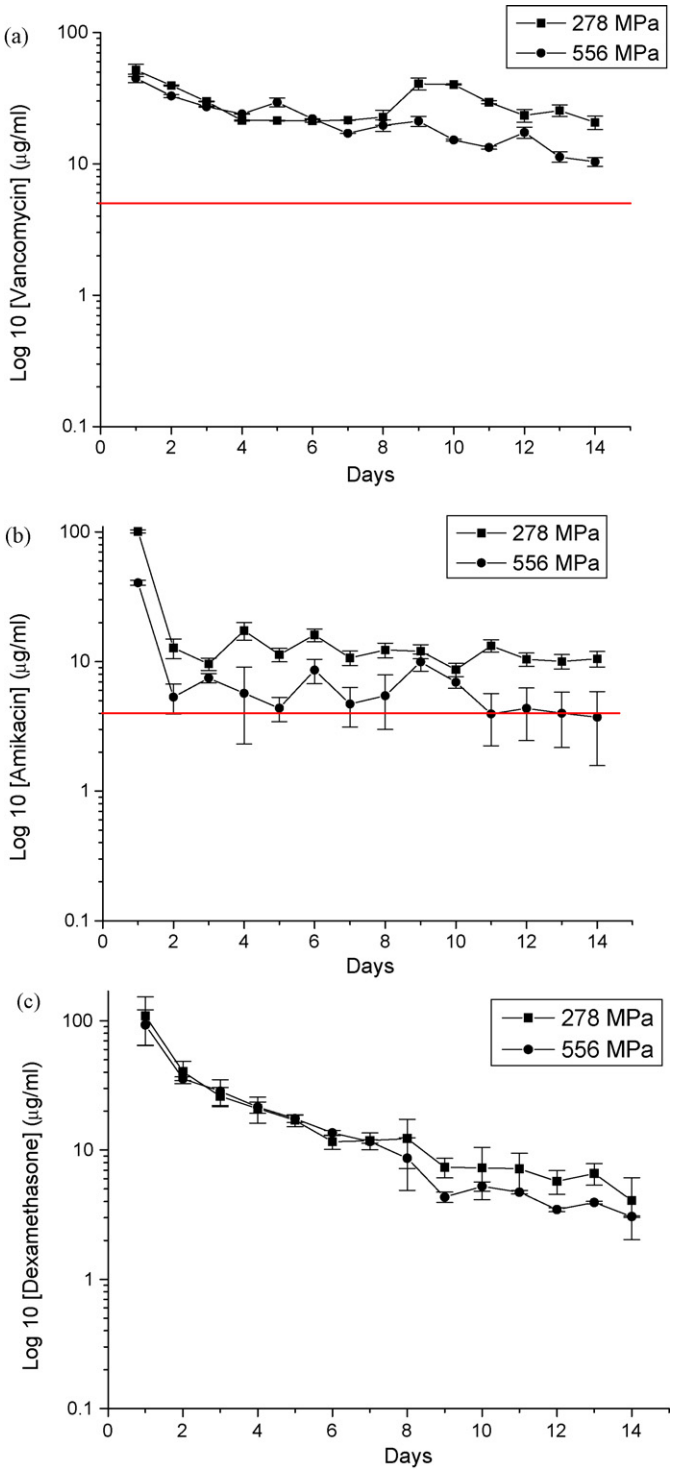

Fig. 6. Release curves of (a) vancomycin, (b) amikacin, and (c) dexamethasone, from biodegradable scleral plugs subjected to different compression pressures (the drug mixture to polymer ratio is 1:2 and the oven temperature is 55 °C, solid lines are the minimum inhibition concentrations for antibiotics).

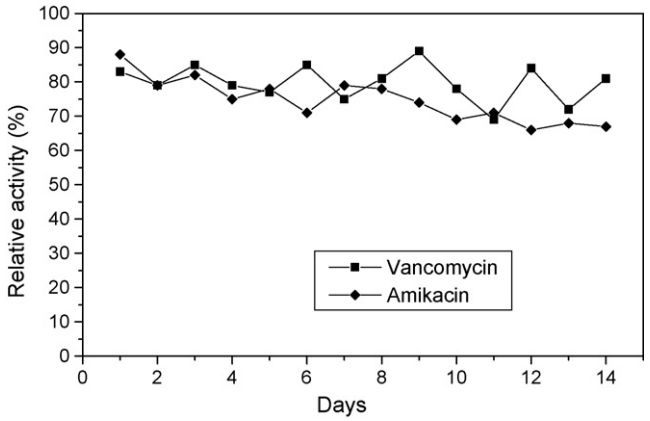

Fig. 7. The relative activity of eluted antibiotics.

4. Discussion

In considering antibiotic treatment of endophthalmitis, it is important to recognize that no single antibiotic provided coverage for all of the microbes isolated from eyes with endophthalmitis (Han et al., 1996; Benz et al., 2004). Combination therapy is usually adopted as the initial empiric treatment of suspected sight-threatening bacterial endophthalmitis, despite small percentage loss of dexamethasone in BSS plus has been reported (Hui et al., 2007). Vancomycin is a glycopeptide that is highly active against gram-positive cocci. It is rapidly bactericidal for actively dividing organisms, inhibiting bacterial cell wall synthesis by binding with protein precursors of its structure (Aguilar et al., 1995; Haider et al., 2001). Essentially all gram-positive bacteria that frequently cause endophthalmitis are sensitive to vancomycin, including staphylococci, streptococci, and *Bacillus* species. On the other hand, gram-negative bacteria are resistant to many antibiotics, but are usually sensitive to aminoglycosides (D'Amico et al., 1985). Amikacin is an aminoglycoside antibiotic that has been widely used for the treatment of endophthalmitis and prophylaxis of ocular infection, mainly due to the lower toxicity of amikacin compared to other aminoglycoside antibiotics such as gentamicin and tobramycin (Campochiaro and Lim, 1994). On the other hand, corticosteroids are recommended as an important adjunct to antibiotics and vitrectomy in the management of infectious bacterial endophthalmitis (Das et al., 1999; Shah et al., 2000). Corticosteroids are known to limit the degree of inflammation caused by toxins released by the microorganisms. For patients where oral corticosteroids cannot be given for medical reasons, intravitreal corticosteroids could be beneficial.

In this study, a solvent-free method was used to manufacture biodegradable scleral plugs that can release antibiotics and steroids simultaneously: by choosing low molecular weights polymers (e.g., MW 30,000), a low melt processing temperature (55 °C), as well as compression sintering to form biodegradable antibiotic/steroid plugs. In addition, no solvent was needed during the manufacturing process, providing the advantage of avoiding the problems associated with the use of solvents.

For pharmaceutics in a hydrophobic polylactide matrix, the release mechanisms are controlled by channel diffusion, osmotic pressure, and polymer degradation (Seigel and Langer, 1990). When antibiotic/steroid loading is low, drugs 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 the drug mixture loading, antibiotic/steroid particles will connect together to form channels leading to the surface of the plug. This drug mixture will be released by channel diffusion, which has a higher release rate. On the other hand, if the polymer matrix surrounding the isolated particles remains intact during the release, antibiotic/steroid will not be released from these clusters. However, water will be taken up by a water-soluble antibiotic/steroid mixture 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/steroid release. Finally, when the molecular weight of the polymer decreases sufficiently,

loss of polymer begins. The antibiotic/steroid mixture will then be released along with this polymer loss.

During the manufacture of polymer plugs, 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 and steroid 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 pharmaceuticals. 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 and steroid are then encapsulated by the polymer to form a composite wall for the plugs. Therefore, by increasing the sintering temperature, one can slow down the dilution rate and prolong the total release duration of the polymer–antibiotics/steroids plugs as shown in Fig. 5. However, adopting very high temperatures of sintering may lead to the potential damage of the drugs. On the other hand, increasing the compression pressure squeezes the bubbles that have formed in the melt to slowly diminish in size. This decelerates the channel diffusion of the plugs. As the compression pressure is increased, the dissolving rate of the plugs decreased.

The bactericidal effects of the antibiotics and steroids incorporated into the biodegradable scleral plug far outweigh any negative inherent effects of the device itself. A significant advantage of the biodegradable plug is that the local antibiotic and steroid concentrations are high during the period of endophthalmitis treatment. The concentrations of vancomycin and amikacin eluted from the plugs were much greater than the minimum inhibitory concentrations for up to 14 days. The plugs also released high concentration of steroids for endophthalmitis treatment for a period of 14 days. Furthermore, the bactericidal power of the antibiotics is still high after the fabrication process.

5. Conclusions

This paper has developed solvent-free biodegradable antibiotic/steroids plugs for a long-term drug release. An elution method was utilized to characterize the in vitro release characteristics of the antibiotics and the steroids over a 14-day period. The followings can be drawn based on the results of this study:

1. The HPLC analysis and bacterial inhibition test showed that biodegradable scleral plugs released a high concentration resulting in significant activity of vancomycin and amikacin (well above the minimum inhibition concentrations) and dexamethasone in vitro, for the period of time needed to treat intraocular infection.
2. The activities of the eluted vancomycin and amikacin ranged from 69% to 89% and from 66% to 88%, respectively. The bioactivities of the released antibiotics were still high after the manufacturing process.
3. One will be able to reduce the drug release rate and prolong the total release period of the plugs by adopting a lower antibiotic/steroid to polymer ratio, increasing the sintering temperature, or increasing the compression pressures.

Further studies being conducted in our laboratory are investigating the biodegradable antibiotics/steroids plugs in animal endophthalmitis model. Eventually biodegradable plugs containing various types of pharmaceuticals may be used in humans for the treatment of intraocular diseases.

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