Polymer Degradation and Stability 97 (2012) 715–720

Contents lists available at SciVerse ScienceDirect

Polymer Degradation and Stability

journal homepage: www.elsevier.com/locate/polydegstab

The influence of γ irradiation and ethylene oxide treatment on the release characteristics of biodegradable poly(lactide-*co*-glycolide) composites

Chao-Ying Hsiao [a], Shih-Jung Liu [a,*], Steve Wen-Neng Ueng [b], Err-Cheng Chan [c]

[a] Department of Mechanical Engineering, Biomaterials Lab, Chang Gung University, 259, Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan
[b] Department of Orthopedic Surgery, Chang Gung Memorial Hospital, Taiwan
[c] School of Medical Technology, Chang Gung University, Taiwan

ARTICLE INFO

Article history:
Received 31 October 2011
Received in revised form
9 February 2012
Accepted 17 February 2012
Available online 24 February 2012

Keywords:
Biodegradable composites
Sterilization
γ irradiation
Ethylene oxide
Release characteristics
Vancomycin

ABSTRACT

This study evaluated the influence of γ irradiation and ethylene oxide sterilization on the release characteristics of vancomycin from biodegradable poly[(d,l)-lactide-*co*-glycolide] (PLGA) composite beads. Biodegradable composites incorporating vancomycin were prepared using a compression-sintering method. They were then subjected to various doses of γ irradiation and ethylene oxide treatment. After sterilization, the composites were placed in 3 ml of phosphate buffered saline and incubated at 37 °C. An in-vitro elution method and a high-performance liquid chromatography (HPLC) were used to characterize the release rates of the antibiotics over a 30-day period. A bacterial inhibitory test was also employed to examine the bioactivity of released antibiotics. All sterilizations were found to result in a decrease of the crystallinity of the polymeric materials, as well as the total release period of antibiotics. The ethylene oxide treatment led to a significant change of the morphology of the composites. Furthermore, the results suggest that the biodegradable composites can release high concentrations of antibiotics (well above the minimum inhibitory concentration) in-vitro for up to 28 days after γ irradiation of less than 25 kGy.

1. Introduction

A recognized treatment for infections such as osteomyelitis [1–5] has been delivering an effective and adequately high concentration of antimicrobial to target sites, along with surgery. Local antibiotics have the advantage of delivering high drug concentrations to the precise area required, and the total dosage of antibiotic applied locally is not normally sufficient enough to produce toxic systemic effects. Antibiotic-eluting composite beads [6] made out of biodegradable polymers have advantages in several ways. First, biodegradable composites provide bactericidal concentrations of antibiotics for the prolonged time needed to completely treat the particular infection. Second, variable biodegradability from weeks to months may allow many types of infections to be treated. Third, because the biodegradable composites dissolve, there is no need for surgical removal. Lastly, because the biodegradable composites dissolve slowly, the soft tissue or bone defect will slowly fill with tissue, thus eliminating the need for reconstruction.

Prior to their surgical implantation, all antibiotic-loaded composite beads must be sterilized to minimize the potential risk of infections and associated complications. Although heating provides the most reliable way to rid objects of all transmissible agents, it is not always appropriate, due to the potential damage inflicted on heat-sensitive materials like biodegradable polymers. The sterilization of medical devices made of polymeric materials is usually done by irradiation [7–13] or ethylene oxide (EO) treatment [7,9,14]. However, sterilizations can significantly alter the mechanical and physical properties of PLGA devices and leave harmful residues on these materials, causing them to fail in vivo. Furthermore, they may also influence the release behavior of pharmaceuticals from these devices. The specific effects of different techniques are determined by the sterilization parameters, the method used for fabrication, and the polymeric materials themselves [9].

Drug makers have sterilized pharmaceuticals by γ irradiation for more than four decades. γ rays have high penetrating ability and show a large depth of penetration into matter, but the degree of interaction is rather low. Although sterilization doses of radiation

* Corresponding author.
E-mail address: shihjung@mail.cgu.edu.tw (S.-J. Liu).

0141-3910/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymdegradstab.2012.02.015

usually are on the order of 25 kGy, high-energy γ irradiation is used mainly in the healthcare industries to sterilize disposable medical equipment, such as syringes, needles, cannulas and IV sets [15,16]. Pharmaceutical companies now also sterilize drugs such as ophthalmic preparations, topical ointments, veterinary products, etc. by irradiation. Sterilization by irradiation is based on ionized atoms. The freed electrons interact with DNA and kill microorganisms. γ ray sterilization is a non-polluting, environmentally friendly process, and since it is a continuous process, the results are more uniform than gas or high temperature sterilization, which are essentially batch processes. Sterilization by irradiation with γ rays may however, in some cases, affect material properties.

Ethylene oxide (EO) gas, on the other hand, is commonly used to sterilize objects sensitive to temperatures greater than 60 °C and/or radiation, such as plastics, optics, and electrics [17]. Ethylene oxide treatment is generally carried out between 30 °C and 60 °C, with relative humidity above 30% and a gas concentration between 200 and 800 mg/l, and typically lasts for at least 3 h. Ethylene oxide penetrates well, moving through paper, cloth, and some plastic films and is highly effective. EO can kill all known viruses, bacteria, and fungi, including bacterial spores, and is compatible with most materials (e.g. medical devices), even when repeatedly applied. However, ethylene oxide is recommended only when it is pharmaceutically absolutely necessary, mainly due to the known potential of EO for genotoxic carcinogenicity [7]. Despite the risks, EO continues to be the most common sterilization method, used for over 70% of total sterilizations and for 50% of all disposable medical devices, specifically in the case of collagen products [18]. Furthermore, EO is also utilized for the sterilization of commercially available suture material and wound dressings.

Like all methods of sterilization, EO and irradiation involve a compromise between inactivation of the contaminating microorganisms and damage to the product being sterilized. The imparted energy does not always differentiate between molecules of the contaminating microorganism and those of the pharmaceutical substrate, which in turn changes the release behavior of pharmaceuticals from the medical devices and their bioactivities. An integral part of the sterilization process validation is the determination of an appropriate technique and the dose for sterilization. Any deviation from the selected dose could either compromise the sterility of the product or damage the product. Presently, various efforts have been made to study the effects of γ irradiation and EO treatment on the material property changes of drug-loaded microspheres [7,8,11–14], but no research has investigated the influence of these sterilization techniques on the release behavior of antibiotics from the biodegradable composite beads.

In our previous studies [19,20], we had successfully developed biodegradable antibiotic composite beads that provide a sustained release of effective vancomycin for 32 days in-vitro. Before the beads can be adopted for clinical applications, they must be sterilized. This paper examined the influence of sterilizations on the in-vitro release characteristics of vancomycin from the poly[(d,l)-lactide-*co*-glycolide] (PLGA) beads. Vancomycin is effective against infections caused by methicillin-resistant *staphylococci* and *S epidermis* [36], and it is also useful in mixed infections involving *streptococci* and *enterococci*. Furthermore, unlike the penicillin or cephalosporin, vancomycin has a low occurrence of allergic reactions. A compression-sintering technique was employed to manufacture composites with vancomycin. Fabricated composites were then sterilized by γ irradiation and ethylene oxide (EO) treatment. After sterilizations, the composites were evaluated by an elution method and a bacterial inhibitory test. Material property changes of the composites due to sterilization were also examined.

2. Materials and methods

2.1. Composite materials and manufacturing

Antibiotic-polymer composites were fabricated in this study. The antibiotic used was commercial grade vancomycin powder with particle size of 100 μm (Sigma–Aldrich, Saint Louis, MO, U.S.A.). The polymeric materials used were poly[(d,l)-lactide-*co*-glycolide] (PLGA) with a ratio of 50:50 and an intrinsic viscosity of 0.4 (RG 503, Resomer, Boeringer Engelheim, Germany). The polymer and vancomycin were mixed by a lab scale dry mixer with the ratio of 5 to 1 (polymer to antibiotics), i.e. 200 mg of PLGA and 40 mg of vancomycin [12]. The mixture was compressed into cylindrical composite beads of 8 mm in diameter and 3.5 mm in height by a stainless mold. The compressed composites in the mold were then placed in an oven for sintering. The sintering temperature was set at 65 °C and the sintering time used was 30 min in order to attain an isothermal sintering of the composites. Fig. 1 shows photographically the fabricated biodegradable antibiotic composites.

2.2. Sterilization of antibiotic composites

Two sterilization methods, γ irradiation and ethylene oxide, were employed. The γ irradiation was performed courtesy of China Biochemical Co. (Taiwan) with a Cobalt-60 source at five different doses, including 15, 20, 25, 30, and 35 kGy. For the EO treatment, an EO sterilizer (Model YTM-EOG, Taiwan) with 1500 L chamber volume was used. The chamber was filled with pressurized air at 500 mmHg, then a vacuum of 0.0 mmHg was applied, and EO at 500 mmHg and 55 °C was introduced for 7 h. Afterwards, EO was desorbed for 6 cycles over 7 h between pressures of 495 mmHg and 30 mmHg.

2.3. In-vitro release of vancomycin

An in-vitro elution method was employed to determine the release characteristics of vancomycin from the antibiotic composites. A phosphate buffer, 0.15 mol/L (pH 7.4), was used as the dissolution medium. Each of the biodegradable antibiotic composites after sterilization was incubated in 3 ml of phosphate buffered saline at 37 °C for 24 h. The dissolution medium was collected and analyzed at 24-h intervals. Fresh phosphate buffer

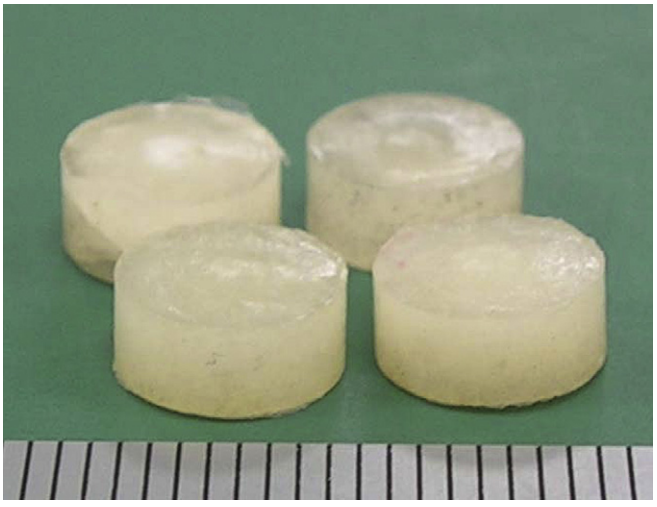

Fig. 1. Photograph of the biodegradable antibiotic composites.

(3 ml) was then added for the next 24-h period and this procedure was repeated until the composite was fully dissolved. The dissolution medium was collected and analyzed every 24 h.

The antibiotic concentrations in buffer solution for the elution studies were determined by a high-performance liquid chromatography (HPLC) assay. The HPLC analyses were conducted on a Walters 600 Multisolvent Delivery System. The column used for the 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 U.K. Ltd.) and acetonitrile (Mallinckrodt, U.S.A.) (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 measurements (correlation coefficient > 0.99). The elution product can be specifically identified and quantified with high sensitivity using the HPLC system.

2.4. Bioactivity of released antibiotics

Bioactivity of the released vancomycin on *Staphylococcus aureus* (ATCC65389) was determined by using an antibiotic disk diffusion method in a Nutrient Broth (beef extract, peptone, Difco Laboratories). Eight micro liters of solution from each daily buffer sample were pipetted onto 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 vancomycin was then determined by interpreting the curve. The bioactivities of the incubated vancomycin on *S. aureus* (ATCC65389) were determined by:

$$\text{Bioactivity}(\%) = \frac{\text{diameter of sample inhibition zone}}{\text{diameter of reference inhibition zone}} \qquad (1)$$

The minimum inhibitory concentration (MIC) of vancomycin on *S. aureus* (ATCC65389) was also determined using an antibiotic tube dilution method in the Cation supplemented Mueller-Hinton Broth (Difco Laboratories).

2.5. Differential scanning calorimetry

A DuPont model TA-2000 differential scanning calorimeter was used to characterize the thermal properties of the as-received polymers and the polymeric materials after sterilization by γ irradiation or EO gas. The scan temperature ranged from 30 to 70 °C. The heating rate of the materials was 10 °C/min.

2.6. SEM observations

A Hitachi Model S-3000N scanning electron microscopy was employed to observe the surface morphology of the biodegradable composites before and after sterilization. Prior to examination, the surfaces were covered with a layer of gold to make them conductive.

3. Results and discussion

3.1. Release of antibiotics from γ irradiated composites

In this study, the influence of irradiation on the release of antibiotic from biodegradable composites was investigated. Fig. 2 shows the release characteristics of biodegradable antibiotic composites subjected to various doses of γ irradiation. The experimental results from the in-vitro elution test showed that non-sterilized biodegradable composites released high concentrations of antibiotic (well above the MIC) for 30 days. No significant difference of the release curves was observed when low doses of irradiation (i.e., less than 20 kGy) were used to sterilize the composites. As the irradiation dose was increased to 25 kGy, the composites exhibited a more pronounced peak of release in the first few days of elution, and after that its release characteristic was similar to that of a non-sterilized composite (Fig. 2). In addition, the composites could provide a sustained release of vancomycin for 28 days.

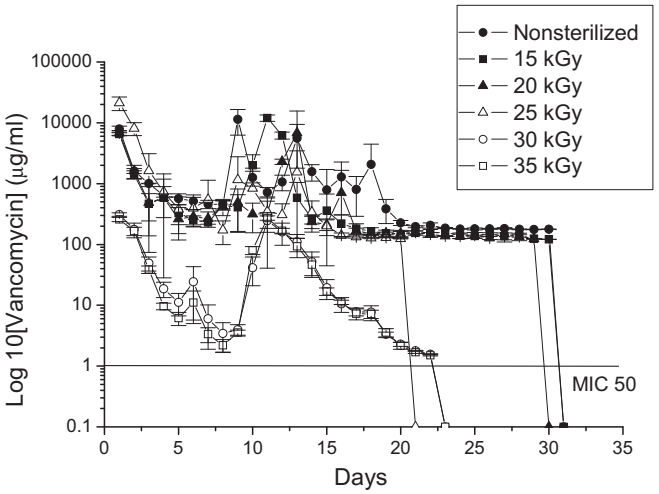

Fig. 2. Effects of γ irradiation on the release characteristics of antibiotic composites.

When the composites were sterilized by γ irradiation of 30 kGy and higher, the total release periods of vancomycin dropped to 22 days. Furthermore, the total amount of antibiotic released from the composites subjected to a high dose irradiation was less than the amount released from composites subjected to a lower dose of γ irradiation. The results suggest that the high dosage of γ rays might have deactivated the pharmaceuticals during irradiation. The total period of effective drug release thus decreased. Furthermore, while Friess and Schlapp [7] reported that their study showed no changes regarding the gentamicin release profile from γ sterilized micro-particles, the results of this study suggested that the irradiation had a profound influence on the release behavior of antibiotic-loaded composite beads.

3.2. Release of antibiotics from EO treated composites

The release characteristics of vancomycin from EO gas sterilized biodegradable composites were investigated and the results are shown in Fig. 3. While the non-sterilized composites exhibited an initial burst of release, the composites sterilized by EO gas did not show a burst of release for the first few days. Rather, the daily release was relatively stable until day 7, where a minor peak was observed. Nevertheless, the total period of drug release was reduced greatly to 17 days. The total amount of antibiotic released from the composites subjected to EO treatment was less than the amount released from non-sterilized composites. This suggests that the EO gas might have deactivated the antibiotics during the sterilization process. Friess and Schlapp [7] studied the sterilization of gentamicin loaded micro-particles and found that chemical changes of gentamicin after EO sterilization could be mainly due to

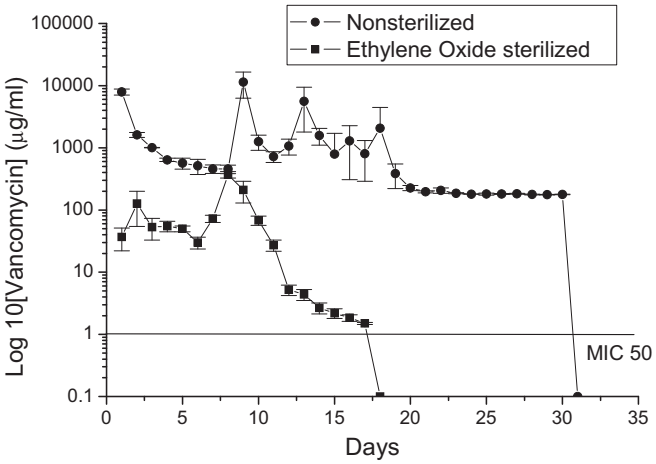

Fig. 3. Influence of ethylene oxide treatment on the release characteristics of antibiotic composites.

the EO attachment during sterilization. In addition, the total amount of antibiotic released from the EO treated composites was less than the amount released from composites subjected to γ irradiation (Fig. 2), which suggests that γ irradiation is a better choice of sterilization for the biodegradable antibiotic composites developed in this study.

3.3. Bioactivity of released antibiotics

The bioactivity of eluted vancomycin on *S. aureus* (ATCC65389) was determined by using an antibiotic disk diffusion method. Fig. 4a and b show the bioactivities of antibiotics released from composites subjected to γ irradiation and EO treatment, respectively. The bioactivity of vancomycin ranged from 33.6% to 100%, and from 27.8% to 100% (Fig. 4a), for the 25 kGy and 35 kGy irradiated composites, respectively, while the activity of antibiotics ranged from 30% to 100% (Fig. 4b) for the EO treated composites. When the γ irradiation dose was low (less than 25 kGy), the bioactivity of antibiotics remained unaffected, and when the dose was higher than 25 kGy, the bioactivity began to decrease after 17 days. The EO treatment, on the other hand, partially reduced the bioactivity of eluted antibiotics after 14 days [7]. Overall, the findings in this study reveal that the activities of the antibiotics are still high after the sterilization process.

3.4. Property changes of the biodegradable composites

Fig. 5 shows photographically the biodegradable composites after γ irradiation and EO gas treatment. While the biodegradable composites did not show any obvious exterior change after irradiation, the EO treated composites exhibited sponge-like surfaces. The scanning electron micrograph of the surfaces of the composites after sterilization is also shown in Fig. 6. Microscopically, the external surface of the γ irradiated composites was relatively smooth (Fig. 6b), while the EO treated composites showed foamed structures on their surfaces (Fig. 6c). While it has been reported that the sterilization showed no influence on the morphological change of micro-particles, the results of this study suggested that EO had a significant influence on the morphology of composite beads. The foamed structure of EO sterilized beads may lead to channel diffusion release of the antibiotics. The release rate of vancomycin from the composite beads increased and the total release period decreased accordingly, as suggested in Figs. 3 and 4.

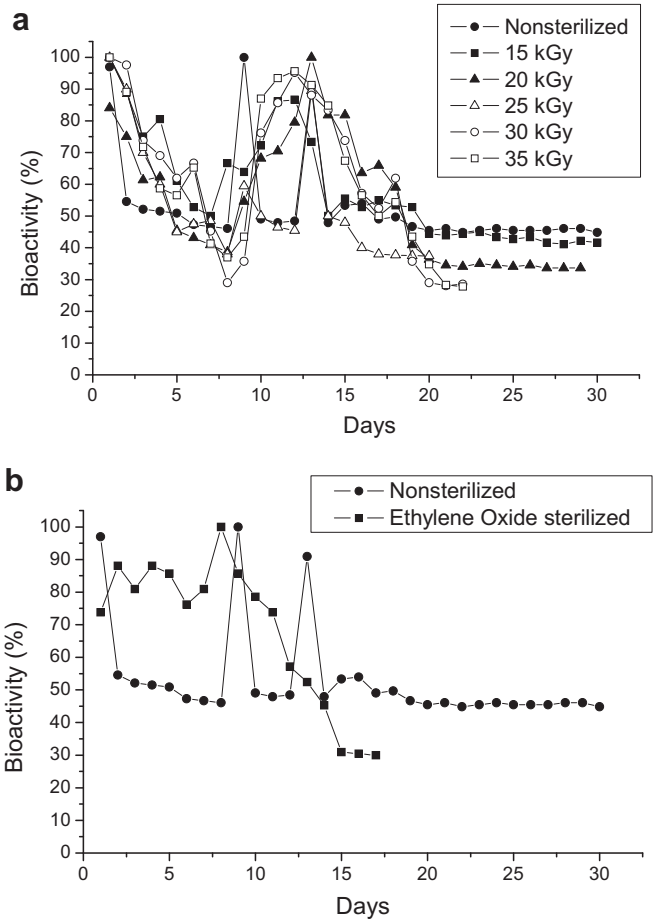

Fig. 4. Bioactivities of vancomycin released from composites subjected to (a) γ irradiation of various doses and (b) ethylene oxide treatment.

The results here suggest that the EO gas has a profound effect on the morphology of the biodegradable composites.

The results of the DSC analysis in Fig. 7 suggest that the sterilization, either γ irradiation or EO treatment, results in a decrease of the crystallinity and glass transition temperature of the polymeric materials. The rate of degradation of PLGA had been found to depend on its degree of crystallinity and its glass transition temperature (T_g) [21,22]. Loo et al. [10] studied the radiation effects on PLGA and found that the average molecular weight of PLGA decreased rapidly at low radiation dosages and remained relatively unchanged at high radiation dosages. Furthermore, the glass

Fig. 5. Photograph of antibiotic composites after γ irradiation (left) and ethylene oxide treatment (right).

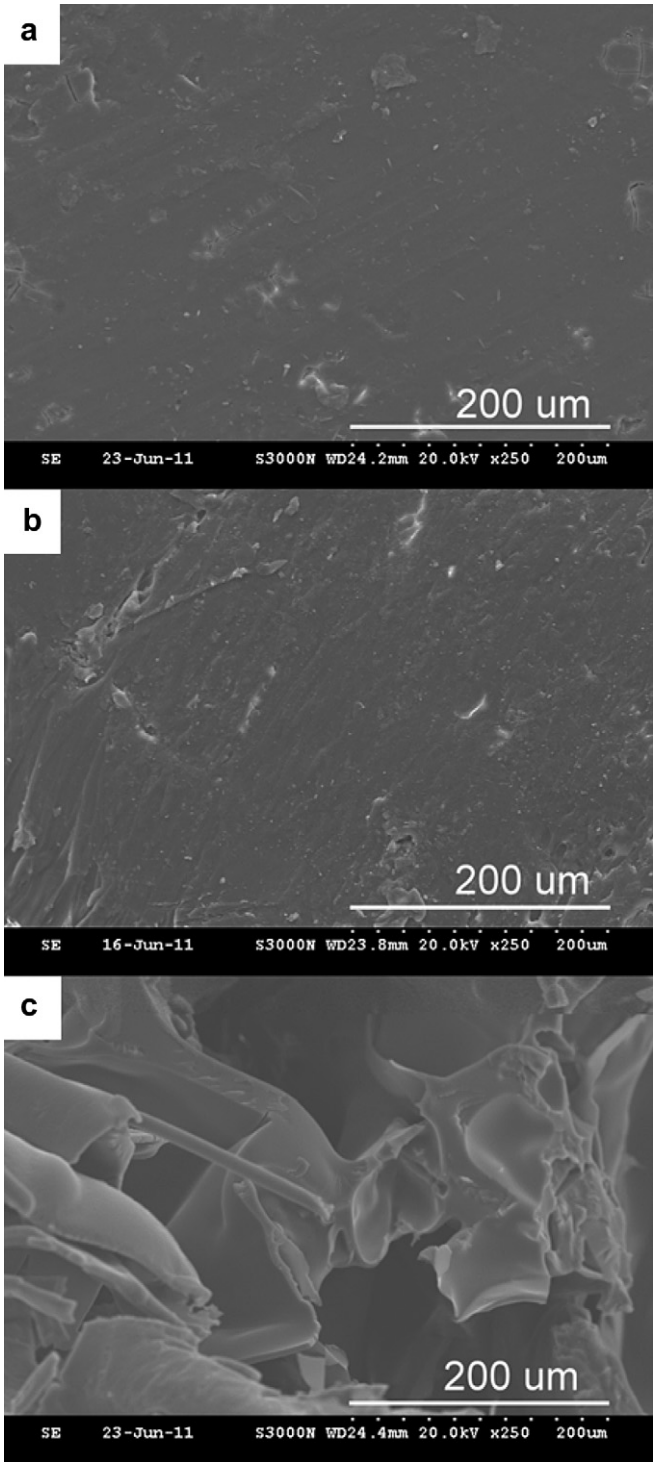

Fig. 6. SEM observations of a composite's surface (a) before sterilization, (b) after γ irradiation, and (c) after ethylene oxide treatment.

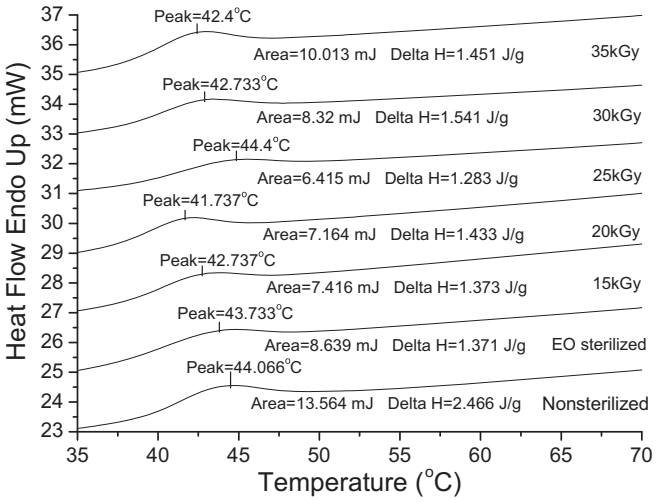

Fig. 7. Differential scanning calorimeter analysis of biodegradable composites subjected to different sterilization methods.

transition temperature of the polymeric materials decreased with increasing radiation doses, mainly due to the dominant effect of e-beam irradiation on molecular chain scission of the polymeric materials. On the other hand, Friess and Schlapp [7] studied the influence of EO sterilization and found that the EO treatment resulted in a decrease of molecular weight and glass transition temperature of the polymeric materials. The decrease in the degree of crystallinity increased hydrolytic degradation, which in turn accelerated the release rate of the antibiotic from the PLGA composites. The total release period of antibiotics decreased accordingly, as shown in Figs. 2 and 3.

The rate and duration of release of the antibiotics composites depend on the requirement of each application. For a water-soluble antibiotic in a hydrophobic PLGA matrix, the release mechanisms are controlled by channel diffusion, osmotic pressure, and polymer degradation [23]. 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 penetrate the polymer at an efficient rate. However, while the vancomycin particles could not be completely encapsulated by the polymeric materials during the manufacturing process, the antibiotic was released by channel diffusion, causing an initial burst of release, as shown in Figs. 2 and 3.

If the polymer matrix surrounding the isolated particles remains intact during the release, the 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. When the polymer molecular weight decreases sufficiently, loss of polymer begins [24]. The antibiotic will then be released along with this polymer loss. γ irradiation has been known to cause chain scission in PLGA polymers and result in a decrease of the molecular weight. During irradiation, radicals are formed, which can lead to chemical changes in drugs and excipients during sterilization [10]. Friess and Schlapp [7] studied the sterilization of gentamicin loaded PLGA/collagen micro-particles by using γ irradiation of 28.9 kGy and EO gas. They found that both irradiation and EO treatment resulted in a decrease of molecular weight and glass transition temperature of the polymeric materials. However, the irradiated polymers did not indicate changes in their chemical structures. In addition, no changes regarding the gentamicin release profile from γ irradiated materials was observed.

In this study, the bactericidal effects of the antibiotics incorporated into the biodegradable composite far outweigh any negative inherent effects of the composite itself. A significant advantage of the biodegradable antibiotic composite is that the local antibiotic concentrations are much greater than the minimum inhibitory concentration (MIC) for most pathogens commonly isolated in orthopedic infections. Furthermore, the experimental results suggest that the biodegradable composites could release high concentrations of antibiotic (well above the minimum inhibitory

concentration) in-vitro for up to 28 days after the γ irradiation of less than 25 kGy. The bactericidal power of the antibiotics is still high after the manufacturing process. This indicates that irradiation can be a potential sterilization method for the biodegradable antibiotic composites.

4. Conclusions

This study has evaluated the influence of γ irradiation and ethylene oxide sterilization on the release characteristics of vancomycin from biodegradable PLGA composites. The experimental results suggest that all sterilizations result in a decrease of the crystallinity of the polymeric materials, as well as the total release period of antibiotics. Ethylene oxide treatment led to a significant change of the morphology of the composites. When γ irradiations of less than 25 kGy were used, the biodegradable composites could release high concentrations of vancomycin (well above the minimum inhibitory concentration) in-vitro for 28 days. The total amount of antibiotics released from the EO treated composites was less than the amount released from composites subjected to γ irradiation, which suggest that γ irradiation is a better choice of sterilization for the biodegradable antibiotic composites developed in this study. The current research has identified the most appropriate sterilization method for the biodegradable composites so that long-term antibiotics are available for patients with osteomyelitis and various infections, such as thoracic, abdominal, and pelvic infections, as well as for the prophylaxis of these infections.

References

[1] Nandi SK, Kukherjee P, Roy S, Kundu B. Local antibiotic delivery systems for the treatment of osteomyelitis-a review. Mater Sci Eng C 2009;29:2478–85.
[2] Canale ST, Beaty JH. Campbell's operative orthopaedics. 11th ed. Philadelphia, Pennsylvania: Mosby Elsevier; 2008. 707–708.
[3] Keeling WB, Myers AR, Stone PA, Heller L, Widen R, Back MR, et al. Regional antibiotic delivery for the treatment of experimental prosthetic graft infections. J Surg Res 2009;157:223–6.
[4] Ueng SW, Wei FC, Shih CH. Management of large infected tibial defects with antibiotic beads local therapy and staged fibular osteoseptocutaneous free transfer. J Trauma 1997;43:268–74.
[5] Neut D, van de Belt H, Stokroos I, van Horn JR, van der Mei HC, Busscher HJ. Biomaterial-associated infection of gentamicin-loaded PMMA beads in orthopaedic revision surgery. J Antimicrob Chemother 2001;47:885–91.
[6] Zilberman M, Elsner JJ. Antibiotic-eluting medical devices for various applications. J Control Release 2008;130:202–15.
[7] Friess W, Schlapp M. Sterilization of gentamicin containing collagen/PLGA microparticles composites. Eur J Pharm Biopharm 2006;63:176–87.
[8] Dillen K, Weyenery W, Vandervoort J, Ludwig A. The influence of the use of viscosifying agents as dispersion media on the drug release properties from PLGA nanoparticles. Eur J Pharm Biopharm 2004;58:539–49.
[9] Holy CE, Cheng C, Davies JE, Shoichet MS. Optimizing the sterilization of PLGA scaffolds for use in tissue engineering. Biomaterials 2001;22:25–31.
[10] Loo SCJ, Ooi CP, Boey YCF. Radiation effects on poly(lactide-co-glycolide) (PLGA) and poly(L-lactide) (PLLA). Polym Degrad Stab 2004;83:259–65.
[11] Bittner B, Mader K, Kroll C, Borchert HH, Kessel T. Tetracycline-HCl loaded poly (D, L-lactide-co-glycolide) microspheres prepared by a spray drying technique: influence of γ-irradiation on radical formation and polymer degradation. J Control Release 1999;59:23–32.
[12] Yoshida S, Aso Y, Kojima S. Drug release from poly (D, L-lactide) microspheres controlled by γ-irradiation. J Control Release 1995;37:263–7.
[13] Jeong YI, Song JG, Kang SS, Ryu HH, Lee YH, Choi C, et al. Preparation of poly(DL-lactide-co-glycolide) microspheres encapsulating all-trans retinoic acid. Int J Pharm 2003;259:79–91.
[14] Choi Y, Kim SY, Moon MH, Kim SH, Lee KS, Byun Y. Poly(ethylene glycol)-poly(L-lactide) diblock copolymer prevents aggregation of poly(L-lactide) microspheres during ethylene oxide gas sterilization. Biomaterials 2001;22:995–1004.
[15] AAMI. AAMI technical information report, TIR 27, radiation sterilization—substantiation of 25 kGy as a sterilization dose—method VDmax. Arlington, VA: AAMI; 2001.
[16] Kowalski J, Tallentire A. Substantiation of 25 kGy as a sterilization dose: a rational approach to establishing verification dose. Radiat Phys Chem 1999;54:55–64.
[17] Athanasiou KA, Niederauer GG, Agrawal CM. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials 1996;17:93–102.
[18] Olde Damink LHH, Dijkstra PJ, Van Luyn MJA, Van Wachem PB, Nieuwenhuis P, Feijen J. Influence of ethylene oxide gas treatment on the in vitro degradation behavior of dermal sheep collagen. J Biomed Mater Res 1995;29:149–55.
[19] Liu SJ, Ueng SWN, Chan EC, Lin SS, Tsai CH, Wei FC, et al. In vitro elution of antibiotics from biodegradable beads. J Biomed Mater Res 1999;48:613–20.
[20] Liu SJ, Ueng SWN, Lin SS, Chan EC. In vivo release of antibiotics from biodegradable beads. J Biomed Mater Res 2002;63:807–13.
[21] Milicevic D, Trifunovic S, Galovic S, Suljovrujic E. Thermal and crystallization behavior of gamma irradiated PLLA. Radiat Phys Chem 2007;76:1376–80.
[22] Loo SCJ, Ooi CP, Wee SHE, Boey YCF. Effect of isothermal annealing on the hydrolytic degradation rate of poly(lactide-co-glycolide) (PLGA). Biomaterials 2005;26:2827–33.
[23] Seigel RA, Langer R. Mechanistic studies of macromolecular drug release from macroporous polymers. II. Models for the flow kinetics of drug release. J Control Release 1990;14:153–67.
[24] Ali SAM, Doherty PJ, Williams DF. Mechanisms of polymer degradation in implantable devices. 2. Poly(DL-lactic acid). J Biomed Mater Res 1993;27:1409–18.