ELSEVIER

Biomaterials 22 (2001) 25–31

Biomaterials

Optimizing the sterilization of PLGA scaffolds for use in tissue engineering

Chantal E. Holy[a], Chantelle Cheng[a], John E. Davies[a], Molly S. Shoichet[a,b,*]

[a]*Institute of Biomaterials and Biomedical Engineering, University of Toronto, 170 College Street, Toronto, Ont., Canada M5S 3E3*
[b]*Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ont., Canada M5S 3E5*

Received 9 February 2000; accepted 6 April 2000

Abstract

There are few suitable techniques available to sterilize biodegradable polyester three-dimensional tissue engineering scaffolds because they are susceptible to degradation and/or morphological degeneration by high temperature and pressure. We used a novel poly(lactide-co-glycolide) scaffold (Osteofoam™) to determine the optimal sterilization procedure — i.e. a sterile product with minimal degradation and deformation. Initial studies, found that an argon plasma created at 100 W for 4 min was optimal for sterilizing Osteofoam™ scaffolds without affecting their morphology. The RFGD plasma sterilization method was compared to two well-established techniques — ethylene oxide (ETO) and γ-irradiation (γ) - which were in turn compared to disinfection in 70% ethanol. Disinfection in 70% ethanol serves as a useful control because it affects neither the morphology nor the molecular weight of the polymer; yet, ethanol is unsuitable as a sterilization method because it does not adequately eliminate hydrophilic viruses and bacterial spores. The three sterilization techniques, ETO, γ and RFGD plasma, were compared in terms of their immediate and long-term effects on the dimensions, morphology, molecular weight and degradation profile of the scaffolds. Scaffolds shrank to $\sim$ 60% of their initial volume after ETO sterilization whereas their molecular weight (Mw) decreased by $\sim$ 50% after γ-irradiation. Thus, both ETO and γ-irradiation posed immediate problems as sterilization techniques for 3-D biodegradable polyester scaffolds. During the in vitro degradation study, all sterilized samples showed advanced morphological and volume changes over time relative to ethanol (EtOH) disinfected samples, with the greatest changes observed for γ-irradiated samples. ETO, RFGD plasma sterilized and EtOH disinfected samples showed similar changes in Mw and mass over the 8-week time frame. Overall, of the three sterilization techniques studied, RFGD plasma was the best.

Keywords: Plasma; Biodegradables; PLGA scaffolds

1. Introduction

Polyester scaffolds for tissue-engineering must be either manufactured aseptically or sterilized after processing. For economical and practical reasons, the latter strategy has been employed with polyester pins and plates intended for in vivo use, and is considered a more realistic approach to achieve sterile biomedical devices [1]. Nevertheless, the challenge remains to determine an efficient and non-destructive sterilization procedure for polyester scaffolds that preserves their 3-D structure.

Biodegradable α-hydroxy polyesters have been used for several years as screws, rods and pins; they have also been investigated as scaffolds for tissue-engineered skin and cartilage [2,3]. We have recently reported the development of a novel 3-D poly(lactide-co-glycolide) PLGA 75/25 scaffold for bone tissue engineering applications (Osteofoam™) [4]. This scaffold is characterized by a distinct 3-D morphology, similar to trabecular bone that is essential for 3-D cell colonization and bone tissue formation [5,6]. In the present study, we investigated different sterilization methods to determine which procedure would be least destructive to the polymer and its 3-D geometry.

Biomedical devices prepared from biodegradable polyesters are usually sterilized by ethylene oxide (ETO) because other sterilization procedures, such as irradiation, heat, steam or acid, cause extensive deformation of the devices and accelerated polymer degradation [1,7]. Disinfection in 70% EtOH for 30 min is often used in vitro and is shown to produce no morphological and/or

* Correspondence address: Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ont., Canada M5S 3E5. Tel.: +1-416-978-1460; fax: +1-416-978-8605.

0142-9612/01/$ - see front matter
PII: S0142-9612(00)00136-8

chemical damages to polyester scaffolds. However, while gram-positive, gram-negative, acid-fast bacteria and lipophilic viruses show high susceptibility to concentrations of ethanol in water ranging from 60 to 80%, hydrophilic viruses and bacterial spores are resistant to the microbial effects of ethanol [8]. Therefore, EtOH is considered as a chemical disinfectant instead of a sterilizing media and cannot be used for in vivo applications of biomedical devices. ETO sterilization has its limitations as well; these include accelerated degradation of the polymer, and residual ethylene oxide gas within the bulk of the sterilized device [9,10]. Another sterilization method, γ-irradiation, causes substantial degradation of polyester chains with increasing dosages of radiation. For example, at the standard 2.5 Mrad sterilization dose, considerable damage was observed on PGA sutures [11].

Recently, a low-temperature radio-frequency glow discharge (RFGD) plasma treatment was introduced as a sterilizing method for polyester devices [12]. While the RFGD plasma was shown to induce surface cross-linking or branching of the polymer, it did not affect polymer crystallinity, mechanical properties, or overall melting temperature [13]. The sterilization efficiency of plasma gas was recently demonstrated by a 10^5 reduction of bacteria, bacterial endospores, yeast and bacterial viruses within 90 s of exposure to an atmospheric uniform glow discharge plasma [14], indicative of a similar sterilization efficiency to that of ETO and γ.

In an attempt to determine the optimal sterilization procedure for Osteofoam™, we compared ETO, RFGD plasma and ^{60}Co γ-irradiation sterilization procedures for (1) morphological alteration (2) chemical damage and (3) long-term effect on polymer degradation. While the sterilization potency of all three methods was previously described [14], we confirmed sterility for ETO, ^{60}Co γ-irradiated and RFGD plasma-treated samples. Polymer degradation was determined by measuring changes in molecular weight and mass immediately after the sterilization process. The long-term effects of each sterilization method were monitored for degradation in vitro over an 8-week degradation study in a phosphate-buffered saline (PBS). Given the drawbacks of ETO and γ-irradiation, we focussed most of our efforts on optimizing the RFGD plasma sterilization, and particularly on determining the minimum power and time required for sterilization without compromising 3-D structure or sterility. The degradation rate of polymer scaffolds, sterilized with one of the three sterilization methods, was compared to that of non-degraded, EtOH-disinfected scaffolds. For tissue engineering applications, the degradation rate of a polyester scaffold is particularly important since it should be concurrent with that of new tissue formation.

2. Materials and methods

2.1. Preparation of the polymer foams

PLGA 75/25 (Birmingham Polymers, Inc. Birmingham, AL) had an intrinsic viscosity of 0.87 dl/g at 30°C in chloroform. The weight-average molecular weight (Mw) of the random copolymer was determined by gel permeation chromatography (GPC) to be 116,000 g/mol. PLGA 75/25 foams were prepared as previously described [4]. Briefly, glucose crystals were dispersed in a PLGA 75/25 solution in dimethylsulfoxide (DMSO, BDH, Toronto, Ont.). The polymer was precipitated and the glucose crystals were extracted from the precipitated polymer in deionized distilled water (ddH_2O), obtained from Milli-RO 10 Plus and Milli-Q UF Plus apparatus (Bedford, MA) and used at 18 MΩ resistance. Foams were then dried to constant mass (0.01 mmHg, 72 h). Dimensions of the foams immediately after processing were $\sim 6 \times 6 \times 20$ mm (720 mm^3).

2.2. ETO sterilization and γ-irradiation

Scaffolds for ETO sterilization and γ-irradiation were packed in self-seal sterilization pouches (saf-T-seal®, MD Industries). ETO sterilization was achieved in a 100% ethylene oxide atmosphere at 57°C for 2 h. Samples were then exposed to air for 15 h. Gamma-irradiation sterilization was achieved at a dose of 2.5 Mrad ^{60}Co at room temperature.

2.3. RFGD plasma treatment

Scaffolds were packed in sterilization pouches (as mentioned above) for treatment by RFGD argon plasma (model PDC-32G, Harrick Scientific, Ossining, NY). The sterilization pouches were placed in the chamber of the electrode-less, glow discharge apparatus, which was purged 3 times with argon gas for 2 min. The chamber was then evacuated to 1.0 Torr. Plasma was ignited by a radio-frequency excitation source and was maintained at one of two powers for the following times: (1) 33 W for either 2, 4, 5 or 10 min or (2) 100 W for either 2, 4, 5, 10 or 40 min.

2.4. Sterility testing

All scaffolds were tested for sterility immediately following sterilization, using a previously described procedure [12]. Briefly, samples were immersed in a soybean casein digest broth for cultivation of fastidious microorganisms (Sigma T8907) and maintained under agitation at 37°C for 5 days. Control, non-sterilized scaffolds were used as negative controls. Clouding of the broth after 5 days indicated contamination and inefficient sterilization, while a clear, uncontaminated

broth indicated efficient sterilization, producing a sterile product.

2.5. Polymer degradation

Newly processed scaffolds were prepared with a 3-D rectangular geometry, allowing an accurate measure of their initial dimensions and mass. Scaffolds were then divided into five groups, and each group treated by one of (1) ETO sterilization, (2) RFGD plasma sterilization at 100 W for 4 min, (3) ^{60}Co-γ-irradiation, (4) ethanol disinfection (70% EtOH, 30 min = controls) or (5) none of the above (= non-degrading controls). The latter were kept in an evacuated dessicator for the entire degradation study. Each treated sample was placed in a perforated Eppendorf tube and immersed in PBS (purchased from Gibco BRL) at 37°C and pH 7.4 for the length of the degradation study. The ratio of sample mass to buffer volume was 1:1000 (w/v). The pH of the buffer was checked routinely and the buffer was replaced whenever its pH dropped below 7.0, thereby eliminating accelerated degradation due to acidic product formation. Every week, for 8 weeks, 3 samples from each different treatment group and 3 samples from the degrading control group (EtOH) were removed from the buffer, washed repeatedly with ddH_2O and dried under vacuum ($P = 0.01$ mmHg, 72 h, room temperature). Dried samples were evaluated for changes in mass, molecular weight, dimensions and morphology, using techniques described above.

2.6. Changes in molecular weight

The changes in polymer molecular weight were determined at each degradation time. Since all polymer scaffolds were prepared from a single polymer batch, only one sample was analyzed by GPC per sterilization group. Specifically, 50 μl of a 0.1% PLGA 75/25 solution in tetrahydrofuran (THF) was injected onto and eluted through a series configuration of columns (American Polymer Standards 10^5, 10^4, 500 Å, cross-linked polystyrene/divinylbenzene) at a flow rate of 0.8 ml/min. The GPC (Waters 2690, operating temp. 35°C) was equipped with a refractive index detector (Waters 410, operating temp. 32°C) and calibrated with polystyrene standards (Aldrich, Ont., Canada). Data were analyzed using Millennium version 2.15.01 software.

2.7. Changes in mass

Mass loss was measured using a Sartorius MC5 microbalance (Göttingen, Germany). At each time point, three samples were weighed after drying and mass loss was calculated by comparing the initial mass (W_0) with that at a given time point (W_t). Measurements were made for samples sterilized by each of the three techniques, the results of which are presented as the mean ± standard deviation ($n = 3$), as calculated by

$$\text{Mass loss} = \frac{(W_0 - W_t)}{W_0} 100\%. \quad (1)$$

2.8. Change in morphology and dimensions

Scanning electron micrographs were taken on a Hitachi 2500 scanning electron microscope (SEM) at an acceleration voltage of 15 kV after sputter-coating the samples with gold under argon atmosphere (Polaron Instrument Inc., Doylestown, PA). Sterilized and degraded foams were documented for changes in gross (i.e. outer) foam dimensions ($n = 3$ at each time point and for each sterilization group). Outer dimensions of foams were measured with a Max-Cal digital caliper (Labcor, Anjou, Qué.).

3. Results

In order to determine which sterilization methodology (RFGD plasma, ETO or γ-irradiation) would minimize changes to polymer molecular weight and 3-D geometry while providing a sterile product, we first determined the optimal RFGD plasma conditions. In the initial screen, changes in scaffold dimensions were compared before and immediately after sterilization. Significant dimensional differences warranted further analysis by SEM.

3.1. Optimization of RFGD plasma treatment

Different RFGD plasma treatment times and intensities were tested, as summarized in Table 1. At the lower of the two powers investigated (33 W), 100% sterility was not achieved for any exposure time, even after 10 min. Longer treatment times at 33 W were not considered because after 10 min, 20% of the samples shrank considerably by 48% of their initial gross volume (from $\sim 6 \times 6 \times 20\,mm^3$ to $\sim 5 \times 5 \times 15\,mm^3$). Thus we concluded that sterility and dimensional integrity coul not be achieved using an RFGD plasma at 33 W. At the higher of the two powers investigated (100 W), 100% sterility was achieved for samples exposed to the argon plasma for 4 or more min. While no morphological change was observed at 4 min, after 5 min of treatment, 2 of the 10 samples lost ~30% of their initial volume (from $\sim 6 \times 6 \times 20\,mm^3$ to $\sim 6 \times 6 \times 15.5\,mm^3$). After 10 min of treatment all samples lost up to 50% of their initial volume. These results suggest that there is a narrow time frame within which samples can be sterilized without altering their 3-D morphology. For this reason, subsequent studies compared RFGD plasma sterilization of Osteofoam™ scaffolds at 100 W and 4 min.

Table 1
Effect of plasma treatment time and power on sterility and dimensions of scaffolds

Plasma treatment		% sterility ($n = 10$)	% dimensional change ($n = 10$)
Power	Time (min)		
33 W	2	60	0
	4	60	0
	5	60	0
	10	70	20[a]
100 W	2	60	0
	3	80	0
	4	100	0
	5	100	20[b]
	10	100	100[c]
	40	100	100[c]

[a] 2 of 10 samples shrank by ~48%.
[b] 2 of 10 samples shrank by ~30%.
[c] All samples shrank by ~50%.

3.2. Comparing sterilization techniques for changes in molecular weight and morphology

Samples sterilized by ETO, γ-irradiation and RFGD plasma were compared to those disinfected in EtOH in terms of changes in weight-average molecular weight (Mw) and dimensions, as summarized in Table 2. EtOH, γ-irradiation or RFGD plasma did not alter the dimensions of Osteofoam™; however, ETO-treated samples shrank considerably, losing approximately 50% of their initial volume. Qualitatively, it was observed that ETO-sterilized samples were stiffer and more brittle than all other sterilized samples. All samples changed from their initial Mw of 116,000 g/mol as a result of sterilization. The Mw of RFGD plasma-treated samples increased to 158,000, indicative of surface cross-linking of polymer chains, while the Mw of ETO and γ-irradiated samples decreased to 103,000 and 54,000 respectively, indicative of polymer degradation. Of the three techniques investigated, ETO affected polymer molecular weight the least; however, ETO affected scaffold dimensions dramatically, making it unsuitable for sterilization of Osteofoam™. Gamma-irradiation and RFGD plasma techniques both affected the polymer molecular weight substantially, yet neither affected the scaffold's dimensions. In order to evaluate the long-term effects of the sterilization procedures on the scaffold's properties, a degradation study was conducted comparing all sterilized samples to EtOH disinfected samples.

3.3. Degradation study

All samples were immersed in PBS at pH 7.4 and 37°C for 8 weeks. Samples were analyzed weekly for changes in Mw, morphology and mass. As shown in Fig. 1, the Mw of all samples decreased over the 8-week time period of the degradation study. Time zero is the time immediately following sterilization, prior to degradation. At that time point, the Mw of the RFGD plasma treated samples was much higher, as explained above, than that of all other treated samples. Despite the initial increase in Mw following sterilization, after one week in PBS, the Mw of the RFGD plasma sterilized sample decreased to a Mw similar to that of the EtOH disinfected controls. Furthermore, from weeks 1 to 8, the RFGD plasma, ETO and EtOH control samples all demonstrated a similar Mw rate loss of ~5800 g/mol per week. At week 8, all had a Mw of approximately 55,000 g/mol. Gamma-irradiated samples showed a significant initial reduction in Mw and degraded to 22,000 g/mol after 8 weeks. Thus, of the three techniques, ETO and RFGD plasma were less

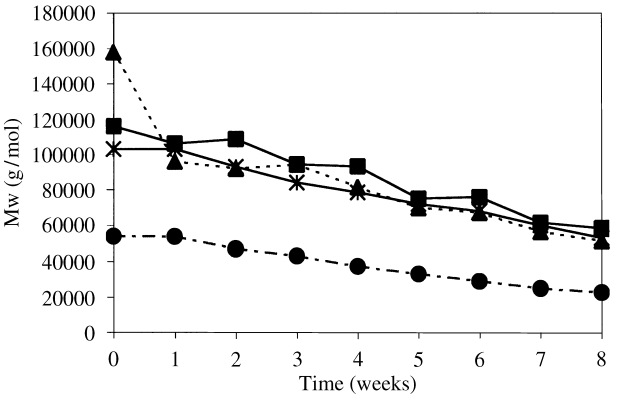

Fig. 1. Comparison of changes in Mw over the time course of the degradation study of PLGA scaffolds sterilized by either: (■) EtOH disinfection; (▲) plasma treatment; (●) γ-irradiation; and (∗) ETO treatment ($n = 1$).

Table 2
The effects of sterilization techniques were compared in terms of relative change in molecular weight and dimensions

Treatment	Change in molecular weight	Change in volume	Sterilization efficiency ($n = 10$)
ETO	12% loss	50% loss	100%
Gamma irradiation	54% loss	0%	100%
Plasma (4 min, 100 W)	36% gain	0%	100%
EtOH disinfection	0%	0%	Variable > 70%

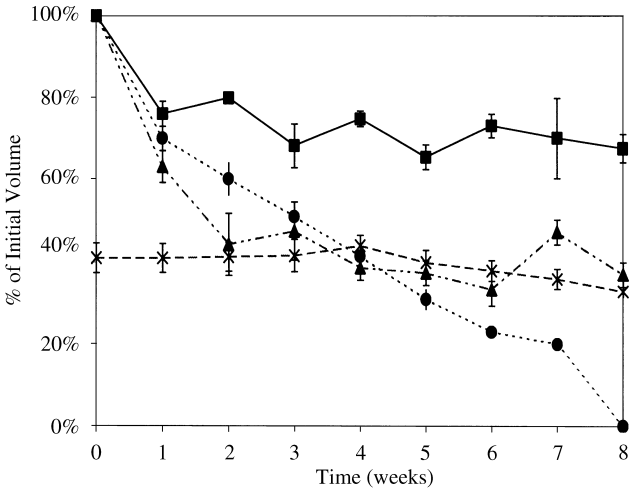

Fig. 2. Comparison of changes in volume over the time course of the degradation study of PLGA scaffolds sterilized by either: (■) EtOH disinfection; (▲) plasma treatment; (●) γ-irradiation; and (∗) ETO treatment. ($n = 3$, mean $\pm$ standard deviations are reported).

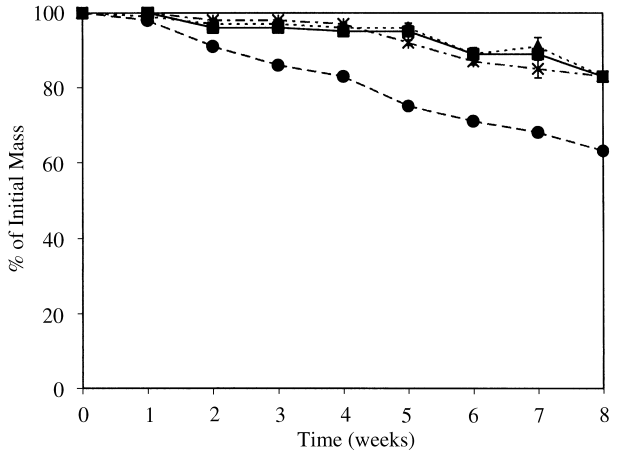

Fig. 3. Comparison of changes in mass over the time course of the degradation study of PLGA scaffolds sterilized by either: (■) EtOH disinfection; (▲) plasma treatment; (●) γ-irradiation; and (∗) ETO treatment ($n = 3$, mean $\pm$ standard deviations are reported).

destructive in terms of polymer molecular weight than γ-irradiation.

Dimensional changes, expressed as percent volume change in Fig. 2, were observed for all samples during the degradation study. Gamma-irradiated samples experienced the most significant change in volume during the degradation study; after 8 weeks in PBS, they became too fragile to be handled and broke into pieces. Control EtOH-disinfected scaffolds decreased to $\sim 78\%$ of their initial volume while ETO and RFGD plasma-sterilized scaffolds decreased to $\sim 40\%$ of their initial volume over the 8-week period. While the overall dimensional change of all sterilized samples decreased, their degradation profiles were distinct: as mentioned previously, ETO-sterilized scaffolds shrank considerably during the sterilization process, but remained at $\sim 40\%$ of their initial volume for the entire duration of the degradation study. RFGD plasma sterilized samples shrank to $\sim 40\%$ of their initial volume over the first two weeks, and then remained relatively constant for the duration of the degradation study. While γ-irradiated samples did not change shape during the sterilization process, they showed a constant rate of volume loss over the 8-week period. The dimensional changes observed over time indicate that γ-irradiation limits the lifetime of the scaffolds to < 8 weeks post-sterilization, making ETO and RFGD plasma treatments the preferred techniques for long-term use of the scaffolds.

The preference of ETO and RFGD plasma over γ-irradiation sterilization was confirmed with the change in mass loss over time. As shown in Fig. 3, RFGD plasma, ETO and EtOH scaffolds showed similar mass loss profiles and overall mass loss (of 15%) over the 8-week period. However, samples sterilized by γ-irradiation lost mass at an accelerated rate, losing 38% of their initial mass after 8 weeks. This was expected given that a significant loss in molecular weight is required prior to loss in mass for bulk-degrading polymers [15].

To gain a greater understanding of the changes observed in the 3-D structure of the scaffolds, overall porosity was examined, with particular attention paid to the macroporous morphology, and the presence of nanopores both within the bulk and in the polymer surface. As shown in Fig. 4, after 8 weeks of degradation, the macroporous morphology of control, non-degraded samples (cf. Fig. 4a) was similar to that of EtOH disinfected samples, degraded for 8 weeks in PBS (cf. Fig. 4b). However, numerous nanopores were observed in the latter (arrow) indicating bulk degradation within the polymer pore walls (cf. Fig. 4c).

Samples sterilized by either RFGD plasma or ETO had similar overall morphologies after 8 weeks of degradation as shown in Fig. 5. In both, ETO-sterilized samples (Fig. 5a) and RFGD plasma-treated samples (Fig. 5b) the macropore size was reduced by approximately 40–60% and the nanoporosity within the bulk of the pore wall was lower than that seen within control scaffolds (cf. Fig. 4c). The pore wall surface was smoother than control samples and the scaffolds had a melted appearance. As may be expected from the 100% volume decrease reported in Fig. 2, γ-irradiated scaffolds were too fragile for handling after 8 weeks of degradation. Interestingly, remnants of the 3-D morphology were still apparent in the small pieces of degraded scaffold, with pore walls and macropores still visible, as shown in Fig. 6a. The surface of the polymer pore wall contained abundant nanopores and had a very rough, wrinkled appearance, as shown in Fig. 6b. These morphological

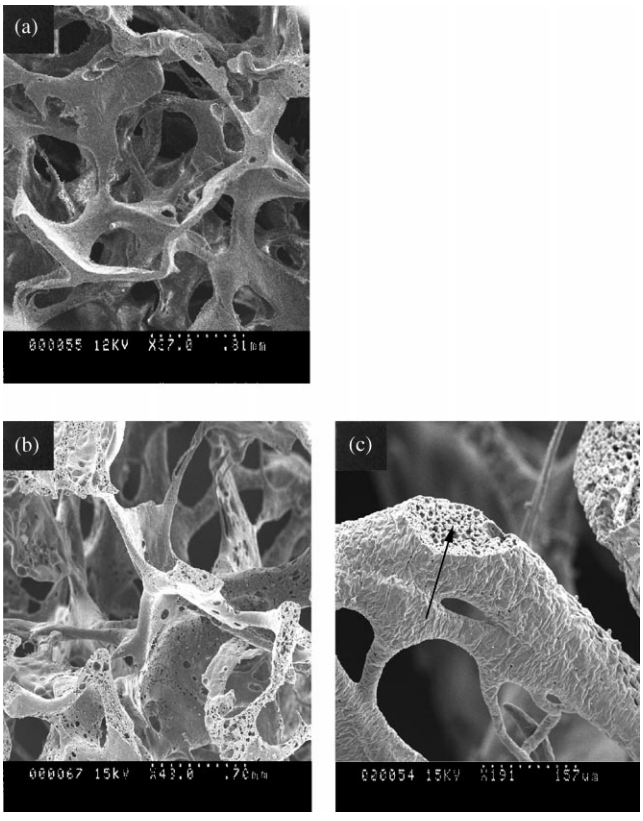

Fig. 4. Scanning electron micrographs of PLGA scaffolds. (a) Non-degraded controls have very open pore morphology with strut-like polymer scaffolding between the pores. Samples that were disinfected in ethanol after 8 weeks of degradation in PBS are observed at low and high magnification. At low magnification (b), the pore walls are well defined and the pore structure has not collapsed. At higher magnification (c), the polymer surface is wrinkled and a large number of nanopores (arrow) are visible in the bulk of the polymer (field width: *a*: 2.31 mm, *b*: 2.67 mm, *c*: 0.530 mm).

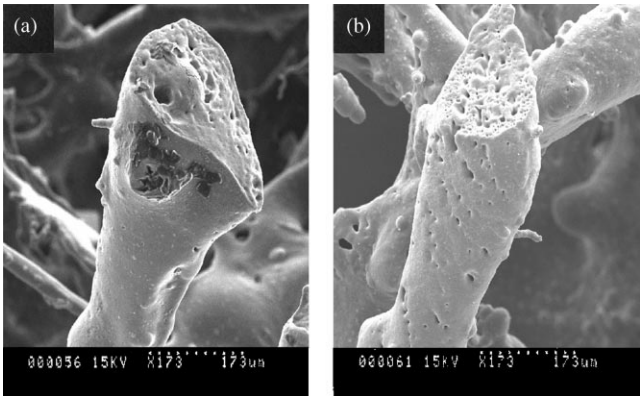

Fig. 5. Scanning electron micrographs of PLGA scaffolds that were sterilized by (a) ETO and (b) plasma after 8 weeks of degradation. The polymer surface has a smooth appearance. Fewer nanopores were visible in the bulk of the polymer than on EtOH disinfected samples (field width *a* and *b*: 0.583 mm).

observations confirm that ETO and RFGD plasma samples degraded more slowly than γ-irradiated samples during the 8-week degradation time.

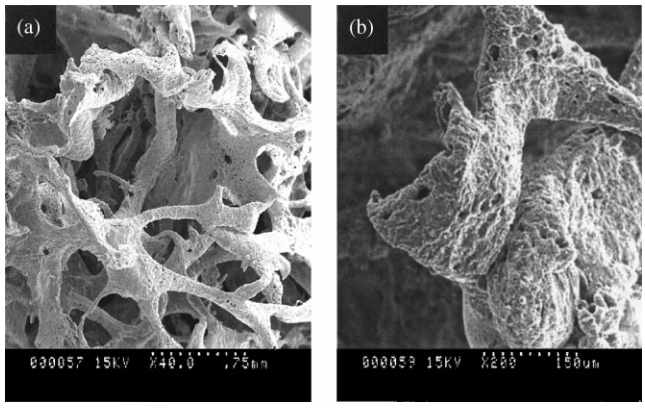

Fig. 6. Scanning electron micrographs of PLGA scaffolds that were sterilized by γ-irradiation after 8 weeks of degradation. The polymer skin is wrinkled, with numerous nanopores, indicating considerable degradation (field width: *a*: 0.254 mm; *b*: 0.506 mm).

4. Discussion

Our goal was to determine the optimal sterilization technique causing minimum damage to the delicate 3-D scaffold geometry, which is critical to successful tissue engineering. While ethanol treatment is an inappropriate sterilization technique, it affects neither the scaffold's morphology nor the polymer's chemical properties, and was therefore used as a control against which the other sterilization techniques were compared. ETO and γ-irradiation are common sterilization techniques but are destructive. Low-temperature RFGD plasma treatments have been proposed to overcome the limitations associated with ETO and γ-irradiation [12]. In this study, we first determined the appropriate conditions for RFGD plasma sterilization and then compared the morphological and chemical changes of low temperature RFGD plasma sterilized scaffolds to those of samples sterilized by ETO and γ-irradiation. Using an argon plasma for 4 min and at 100 W, we were able to prepare sterile scaffolds without overall dimensional change. Since it has been shown that greater than 10^5 bacteria and spores are destroyed by plasma gas after 90 s [14], we anticipated that most microorganisms would be killed after 4 min and confirmed this with a sterility test. Due to the complex morphology of our scaffolds, we were unable to accurately count bacterial numbers, but are confident with the reproducibility of the standard broth assay employed.

The Mw of plasma-treated samples increased immediately following RFGD plasma treatment, suggesting either surface crosslinking or branching. It is unlikely that the scaffold was crosslinked throughout because its degradation rate would have been considerably reduced as compared to that of EtOH disinfected samples. Despite this initial increase in Mw, the Mw decreased in

a similar manner to those of control and ETO samples between 1 and 8 weeks of degradation. After 1 week in buffer, RFGD plasma samples lost $\sim 40\%$ of their initial volume while after 8 weeks, both ETO and RFGD plasma samples lost $\sim 60\%$ of their initial volume. After 8 weeks, both ETO and RFGD plasma samples had similar morphologies: the polymer pore walls had a "melted" appearance, as if the nanopores had collapsed together to provide a more condensed scaffold. We previously observed similar "melted" morphologies on scaffolds that had been degraded for 18 weeks at pH 5.0 [15]. This compressed appearance of ETO and RFGD plasma-treated samples accounts for their reduced volumes relative to EtOH control samples. The change in mass of ETO and RFGD plasma treated samples was similar to that of EtOH control samples. Morphology comparisons indicated an advanced level of degradation of RFGD plasma (and ETO) sterilized scaffolds, relative to controls disinfected in EtOH. Thus, while ETO sterilization had a greater impact on the scaffold dimensions initially, after 8 weeks of degradation, scaffolds sterilized by ETO and RFGD plasma were indistinguishable.

Clearly, of the three methods compared, γ-irradiation was the most damaging. While the volume changed the least initially, after 8 weeks it fell precipitously to zero. The Mw of the γ-irradiated samples decreased by more than 50% of its original value following sterilization; the mass decreased to $\sim 62\%$ after 8 weeks of degradation; and samples were too fragile for handling after 8 weeks of degradation.

5. Conclusions

Our results show that low-temperature RFGD plasma sterilization is the most suitable method for sterilizing polyester devices for tissue-engineering applications because it does not alter the 3-D morphology of such scaffolds. While γ-irradiation is efficient in sterilizing and preserving the morphology of a 3-D scaffold, it dramatically decreases the polymer molecular weight and hence, accelerates degradation over time. Although ETO is less damaging than γ-irradiation in terms of Mw loss, it has a profound affect on scaffold dimensions (and thus morphology). Scaffolds treated by RFGD plasma showed no change in morphology and limited change in Mw. Furthermore, the degradation profile of RFGD plasma-treated samples most closely resembled that of controls. New methods that sterilize polyester scaffolds without affecting them chemically and morphologically would be optimal for in vivo use of 3-D scaffolds.

Acknowledgements

The authors acknowledge financial support from BoneTec Corporation and the Alternative Youth Centre for Employment (Ontario) for CC.

References

[1] Montanari L, Costantini M, Signoretti EC, Valvo L, Santucci M, Bartolomei M, Fattibene P, Onori S, Faucitano A, Conti B, Genta I. Gamma irradiation effects on poly(DL-lactide-co-glycolide) microspheres. J Control Rel 1998;56:219–29.

[2] Fauza DO, Fishman SJ, Mehegan K, Atala A. Videofetoscopically assisted fetal tissue engineering: skin replacement. J Ped Surg 1998;33:357–61.

[3] Rudert M, Hirschmann F, Wirth CJ. Growth of chondrocytes on different biomaterials Orthopade 1999;28:68–75.

[4] Holy CE, Davies JE, Shoichet MS. Bone tissue engineering on biodegradable polymers: preparation of a novel poly(lactide-co-glycolide) foam. In: Peppas NA, Mooney DJ, Mikos AG, Brannon-Peppas L, editors. Biomaterials, carriers for drug delivery, and scaffolds for tissue engineering. New York: A.I.Ch.E. Press, 1997. p. 272–4.

[5] Holy CE, Shoichet MS, Davies JE. Bone marrow cell colonization of, and extracellular matrix expression on, biodegradable polymers. Cells Mater 1997;7:223–34.

[6] Ishaug SL, Crane GM, Miller MJ, Yasko AW, Yaszemski MJ, Mikos AG. Bone formation by three-dimensional stromal osteoblast culture in biodegradable polymer scaffolds. J Biomed Mater Res 1997;36:17–28.

[7] Sintzel MB, Schwach-Abdellaoui K, Mader K, Stosser R, Heller J, Tabatabay C, Gurny R. Influence of irradiation sterilization on a semi-solid poly(ortho ester). Int J Pharm 1998;175:165–76.

[8] Gardner JF, Peel MM. Introduction to sterilization, disinfection and infection control. New York, NY: Churchill Livingstone, 1991. p. 91–137.

[9] Zislis T, Martin SA, Cerbas E, Heath JR, Mansfield JL, Hollinger JO. A scanning electron microscopic study of *in vitro* toxicity of ethylene-oxide-sterilized bone repair materials. J Oral Implant 1989;15:41–6.

[10] Volland C, Wolff M, Kissel T. The influence of terminal gamma sterilization on captopril containing poly(DL-lactide-*co*-glycolide) microsphere. J Control Rel 1991;31:293–305.

[11] Gilding D, Reed AM. Biodegradable polymers for use in surgery — polyglycolic/poly(lactic acid) homo- and copolymers. Polymer 1979;20:1459–64.

[12] Gogolewski S, Mainil-Varlet P, Dillon JG. Sterility, mechanical properties, and molecular stability of polylactide internal-fixation devices treated with low-temperature plasmas. J Biomed Mater Res 1996;32:227–35.

[13] Ayhan F, Ayhan H, Piskin E. Sterilization of sutures by low temperature argon plasma, J Bioactive Biocompat Polymers 1998;13(1):65–72.

[14] Kelly-Wintenberg K, Hodge A, Montie TC. Use of a one atmosphere uniform glow discharge plasma to kill a broad spectrum of microorganisms. J Vac Sci Technol A 1999;17(4):1539–44.

[15] Holy CE, Dang S, Davies JE, Shoichet MS. *In vitro* degradation of a novel poly(lactide-co-glycolide) 75/24 foam. Biomaterials 1999;20:1177–85.