Polymer Degradation and Stability 96 (2011) 1625–1630

Contents lists available at ScienceDirect

Polymer Degradation and Stability

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

Stability of a salicylate-based poly(anhydride-ester) to electron beam and gamma radiation

Roselin Rosario-Meléndez [a], Linda Lavelle [b], Stanko Bodnar [b], Frederick Halperin [b], Ike Harper [b], Jeremy Griffin [c], Kathryn E. Uhrich [a,c,*]

[a] Rutgers, The State University of New Jersey, Department of Chemistry and Chemical Biology, 610 Taylor Road, Piscataway, NJ 08854, United States
[b] Sterile Process Technology, Johnson & Johnson, 930 East Route 202, Raritan, NJ 08869, United States
[c] Rutgers, The State University of New Jersey, Department of Biomedical Engineering, 599 Taylor Road, Piscataway, NJ 08854, United States

ARTICLE INFO

Article history:
Received 3 March 2011
Received in revised form
20 May 2011
Accepted 7 June 2011
Available online 16 June 2011

Keywords:
Polyanhydride
Sterilization
Drug release
Gamma irradiation
Electron beam
Stability

ABSTRACT

The effect of electron beam and gamma radiation on the physicochemical properties of a salicylate-based poly(anhydride-ester) was studied by exposing polymers to 0 (control), 25 and 50 kGy. After radiation exposure, salicylic acid release *in vitro* was monitored to assess any changes in drug release profiles. Molecular weight, glass transition temperature and decomposition temperature were evaluated for polymer chain scission and/or crosslinking as well as changes in thermal properties. Proton nuclear magnetic resonance and infrared spectroscopies were also used to determine polymer degradation and/or chain scission. *In vitro* cell studies were performed to identify cytocompatibility following radiation exposure. These studies demonstrate that the physicochemical properties of the polymer are not substantially affected by exposure to electron beam and gamma radiation.

1. Introduction

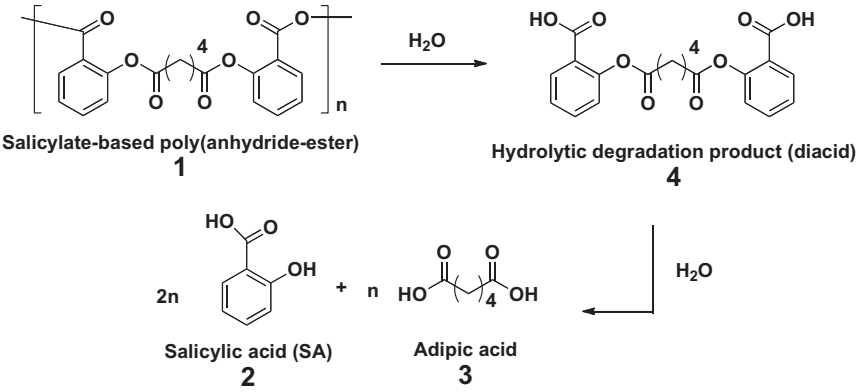
Salicylic acid (SA) is the active metabolite of aspirin and an anti-inflammatory, anti-pyretic, keratolytic and analgesic drug widely used to treat different health conditions [1,2]. A new mode of SA delivery is possible by the chemical incorporation of SA into a polymer backbone to yield salicylate-based poly(anhydride-esters) (PAEs) [3–5]. The physicochemical properties of such polyanhydrides have been investigated over the last decade [5–14]. The ability to formulate these polymers into different geometries such as powders [6], disks [5,9,15], fibers [7], microspheres [10,16], etc. contributes to their wide scope of applications. In addition, these polymers allow for moderate to high SA loading, ranging from 60 to 80% active drug content, because of the direct insertion of the bioactive molecule into the polymeric backbone [3]. Upon exposure to water, the PAEs undergo hydrolytic degradation, releasing SA at different rates as a function of polymer composition [5–7,11]. Temperature can also influence the polymer degradation rate; polymer **1** is relatively stable in powder form when stored at low temperatures under an inert atmosphere. Yet, the PAEs degrade faster at temperatures above 25 °C [15] and in the presence of water (Scheme 1). Further, the PAEs are shown to be non-toxic *in vitro* [14] and *in vivo* [9,16,17] and, therefore, have great potential in various biomedical applications [6,9,13,14,17]. However, the polymer should meet the pharmacopeial and commercial requirement of sterility [18–22].

To manufacture these polymers for *in vivo* administration, the physicochemical properties and the SA release profile must remain relatively unchanged after sterilization and processing. Common sterilization methods for medical devices and drugs include dry heat at 150–170 °C, saturated steam at 115–132 °C and ethylene oxide exposure at 35–60 °C [23,24]. All three options are not viable for sterilization of salicylate-based PAE, **1** because it is "designed to degrade", when placed in the body, which is an aqueous environment maintained at 37 °C.

As alternate sterilization methods, ionizing radiation such as electron beam and gamma do not involve high temperatures or the presence of excessive moisture. Electron-beam (or e-beam) is an ionizing radiation generated using electricity and magnetism to accelerate electrons to a high energy level, whereas gamma is

* Corresponding author. Rutgers, The State University of New Jersey, Department of Chemistry and Chemical Biology, 610 Taylor Road, Piscataway, NJ 08854, United States. Tel.: +1 732 445 0361; fax: +1 732 445 7036.
E-mail address: keuhrich@rutgers.edu (K.E. Uhrich).

0141-3910/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymdegradstab.2011.06.005



Scheme 1. Hydrolytic degradation of salicylate-based poly(anhydride-ester) (**1**).

electromagnetic radiation emitted by man-made isotopes ^{60}Co and ^{137}Cs [19]. Both e-beam and gamma radiation are also successfully employed for the sterilization of thermolabile medical devices and drug delivery systems [25,26]. However, ionizing radiation can induce polymer chain scission. For example, gamma radiation reduced the molecular weight of poly(DL-lactide-*co*-glycolide) (PLGA) [23], poly(L-lactide) (PLLA) [27], and biodegradable polyurethanes [18]. Similarly, e-beam radiation increased polymer degradation rate by chain scission of PLGA and PLLA [28]. E-beam and gamma radiations can also change the polymer properties by crosslinking the chains [19,20]. Both forms of ionizing radiation have been shown to cause polymer crosslinking of a PLLA copolymer [21] and the corresponding homopolymers, PLLA, and PLGA [27,29].

To investigate the effect that e-beam and gamma radiation have on the physicochemical properties of polymer **1**, samples were exposed to both ionizing radiation processes. Polymer was exposed to 25 and 50 kGy in each process, as a typical sterilization dose is 25 kGy [30,31] and 50 kGy is a typical maximum processing dose. After radiation exposure, SA release *in vitro* was monitored to assess potential changes to drug release profiles. Molecular weight (M_w), glass transition temperature (T_g) and decomposition temperature (T_d) were evaluated for polymer chain scissioning and/or crosslinking as well as changes in thermal properties. Proton nuclear magnetic resonance (^{1}H NMR) and Fourier-transform infrared (FT-IR) spectroscopies were also used to determine polymer degradation and/or chain scission. *In vitro* cell studies were also performed to assess cytocompatibility following radiation exposure.

2. Materials and methods

2.1. Materials

Acetic anhydride used to synthesize the polymer was purchased from Fischer (Fair Lawn, NJ). Fetal bovine serum was purchased from Atlanta Biologicals (Lawranceville, GA). All other chemicals and reagents were purchased from Sigma–Aldrich (Milwaukee, WI) and used as received.

2.2. Sample preparation

Polymer **1** was synthesized using previously reported methods [4,5]. Properties of the raw polymer were as follows: Color = off-white, M_w = 16,800 Da, T_g = 50 °C, T_d = 279 °C. Polymer **1** was ground into a powder using a mortar and pestle and placed (1.00 g) into BD Falcon 5 mL polystyrene round-bottom tubes (12 × 75 mm style; BD Bioscience Discovery Labware, Bedford, MA) and capped. Samples were sent to Sterile Process Technology (Johnson & Johnson) for radiation exposure. Samples were then analyzed within one week after exposure. A visual assessment for changes in color and texture was immediately performed following each radiation exposure. Material characterization studies were performed in triplicate.

2.3. Physicochemical characterization

^{1}H NMR spectra were obtained using a Varian 500 MHz spectrometer. Samples (10 mg) were dissolved in deuterated dimethyl sulfoxide (DMSO–d_6), which was used as an internal reference. Each spectrum was an average of 16 scans. Fourier transform infrared (FT-IR) spectra were obtained using a Thermo Nicolet/Avatar 360 FT-IR spectrometer. Polymer samples (10 mg/mL) were solvent-cast onto NaCl plates using dichloromethane under ambient air conditions. Each spectrum was an average of 32 scans.

2.4. Molecular weight

Gel permeation chromatography (GPC) was used to determine the weight-averaged M_w of polymer **1**. Samples were analyzed with a Perkin–Elmer LC system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 advanced sample processor. A Dell OptiPlex GX110 computer running Perkin–Elmer TurboChrom 4 software was utilized for data collection and control. The connection between the LC system and the computer was made using a Perkin–Elmer Nelson 900 Series Interface and 600 Series Link. Samples were dissolved in dichloromethane (10 mg/mL) and filtered through 0.45 μm polytetrafluoroethylene (PTFE) syringe filters (Whatman, Clifton, NJ) prior to elution through a Jordi divinylbenzene mixed-bed GPC column (7.8 × 300 mm) (Alltech Associates, Deerfield, IL) at a rate of 1 mL/min for a total run time of 30 min. Molecular weights were calculated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

Table 1
Molecular weight and thermal properties of polymer **1** (mean ± standard deviation). Melting point (T_m = 174–176 °C) of the degradation product (diacid **4**) was not observed in any sample.

Exposed sample	M_w (Da)	T_g (°C)	T_d (°C)
0 kGy	16,500 ± 200	51 ± 0	272 ± 1
25 kGy e-beam	16,300 ± 700	50 ± 1	273 ± 0
50 kGy e-beam	15,100 ± 400	48 ± 0	272 ± 0
25 kGy gamma	15,700 ± 70	50 ± 1	269 ± 1[a]
50 kGy gamma	14,700 ± 70[a]	48 ± 1	273 ± 1

[a] Result is statistically different compared to the unexposed sample (0 kGy).

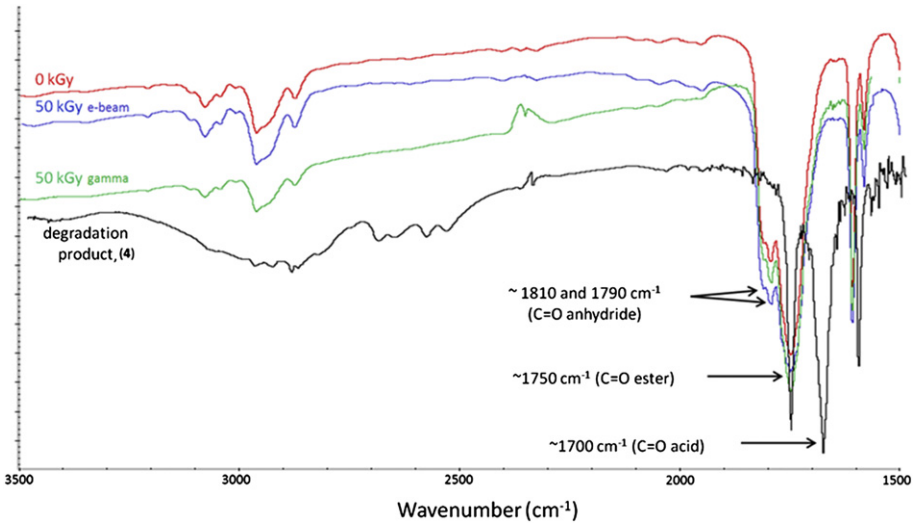


Fig. 1. Infrared spectra of representative samples including stretch bands that indicate polymer degradation. From top to bottom: (red) unexposed sample (0 kGy); (blue) sample exposed to 50 kGy e-beam; (green) sample exposed to 50 kGy gamma; and (black) hydrolytic degradation product (diacid **4**).

2.5. Thermal analysis

Thermal analysis was performed using differential scanning calorimetry (DSC) to obtain T_g and thermogravimetric analysis (TGA) to obtain T_d. DSC was performed using a Thermal Advantage (TA) DSC Q200 running on an IBM ThinkCentre computer equipped with TA Universal Analysis software for data collection and control. Samples (8 mg) were heated under nitrogen from −10 °C–200 °C at a ramp rate of 10 °C/min. A minimum of two heating/cooling cycles was used for each sample set. The T_g was calculated as half C_p extrapolated. TGA analysis was performed using a Perkin–Elmer TGA7 analyzer with TAC7/DX controller equipped with a Dell OptiPlex Gx 110 computer running Perkin–Elmer Pyris software. Samples (~10 mg) were heated under nitrogen at a rate of 10 °C/min from 25 to 400 °C. T_d was defined as the onset of decomposition and is represented by the beginning of a sharp slope on the thermogram.

2.6. Radiation exposure

Samples designated for gamma irradiation were exposed under ambient conditions using a MDS Nordion Gamma Cell 220 Research Irradiator with a Cobalt 60 source. The dose rate in gamma radiation processes was approximately 0.002 kGy/s for these studies. Temperatures during gamma exposure ranged from 30 °C to 37 °C, up to a maximum exposure time of 9 h. Samples slated for e-beam irradiation were processed under ambient conditions in the Mevex 5 MeV, 2 kW electron beam linear accelerator. Samples were placed upright in an ethafoam jig and presented single-sided to the beam. The dose rate for e-beam ranged from 12.5 kGy/s (25 kGy) to 25 kGy/s (50 kGy). The temperature ranged from 38 °C (25 kGy dose) to 55 °C (50 kGy) during the e-beam exposures. Notably, this temperature increase was an instantaneous spike, not a prolonged exposure. Samples designated as controls were not exposed to ionizing radiation.

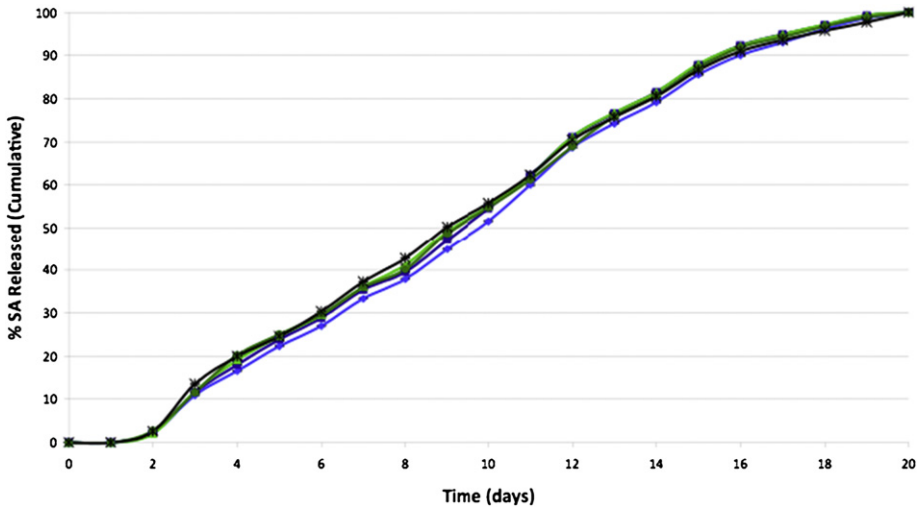


Fig. 2. *In vitro* salicylic acid release profiles from radiation exposed and unexposed polymer. 25 kGy gamma, 50 kGy gamma, 25 kGy e-beam, 50 kGy e-beam, and unexposed (0 kGy), all displayed similar release profiles.

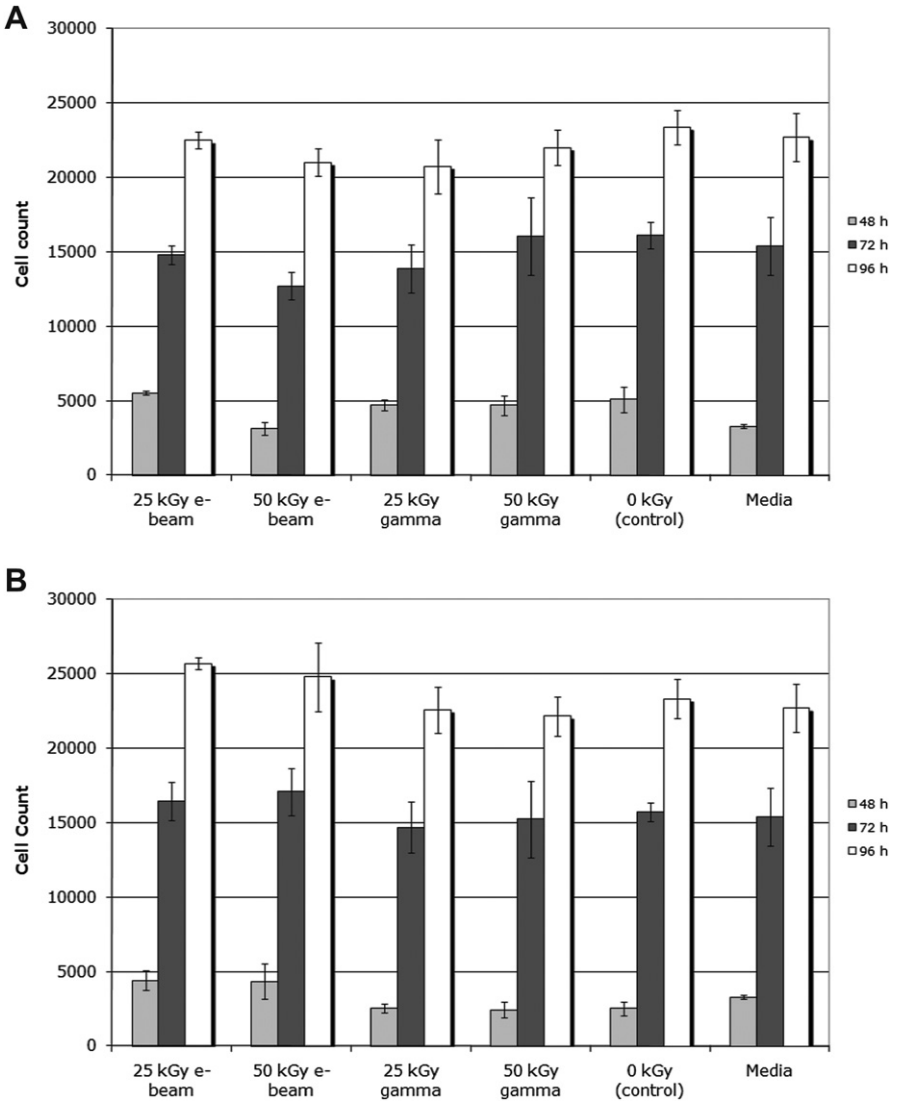


Fig. 3. Cell viability at 48, 72 and 96 h in culture media with polymers at concentration of (A) 0.10 mg/mL media and (B) 0.01 mg/mL media. Data represent mean and standard deviation of 3 samples. No significant differences against the media control were observed.

2.7. In vitro drug release

After radiation exposure, polymer **1** (150 mg, powder) was compressed into a disk (10 mm diameter × 1 mm thick) using a hydraulic press (Carver model M, Garfield, NJ) applying pressure (10,000 psi) for 10 min. Disks (triplicate) were placed into scintillation vials and 10 mL phosphate buffered saline (PBS) pH 7.4 added. Samples were incubated at 37 °C under constant shaking (60 rpm) in an Excella E25 Incubator Shaker (New Brunswick Scientific, New Brunswick, NJ). PBS was removed daily and replaced with fresh PBS. Samples were analyzed by UV/visible spectroscopy (Perkin–Elmer Lambda XLS) at $\lambda = 303$ nm.

2.8. Cell toxicity

Cytocompatibility of polymer **1** following radiation exposure was evaluated by culturing NCTC clone 929 strain L mouse areolar fibroblast cells (L929) (ATCC, Manassas, Virginia) in media containing the dissolved polymers. These L929 cells are a standard cell type for cytocompatibility testing as recommended by the American Society for Testing and Materials (ASTM) [32]. Each polymer sample was dissolved in 10 mg/mL dimethyl sulfoxide (DMSO) as a stock solution and serially diluted with cell culture media to two concentrations (0.01 mg/mL and 0.10 mg/mL), based on standard cytotoxicity protocols [33,34]. Cell culture media consists of Dulbecco's Modified Eagle's Medium, 10% v/v fetal bovine serum, 1% L-glutamate and 1% penicillin/streptomycin. The polymer-containing media was distributed into a 96-well plate (Fisher, Fair Lawn, NJ) and seeded at an initial concentration of 2000 cells per well (triplicate). The media containing the dissolved polymer was compared to two controls: DMSO-containing media and media only.

Cellular morphology was observed and documented at 10× original magnification using a light microscope (Olympus, IX81, Center Valley, PA) at 48, 72, and 96 h post seeding. Cell viability was determined by using a CellTiter 96®AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI). The MTS tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyph-enyl)-2-(4-7 sulfophenyl)-2H-tetrazolium, inner salt; MTS(a)] is bioreduced by cells into a colored formazan product that is soluble in the tissue culture medium. Following the appropriate incubation time, 20 μL of the MTS reagent was added to 100 μL of culture

medium and further incubated for 4 h. The absorbance was then recorded with a microplate reader (Model 680; Bio-Rad, Hercules, CA) at 490 nm. Cell numbers were calculated based upon a standard curve created 24 h after original cell seeding.

2.9. Statistical analysis

Statistical analysis was performed with Kaleida Graph (Synergy Software, Reading, PA). Differences were considered significant at $p < 0.05$ using ANOVA followed by pairwise comparison with Dunnett's post hoc test.

3. Results and discussion

To determine the effect of e-beam and gamma radiation (25 and 50 kGy) on the physicochemical properties of polymer **1**, samples were analyzed for changes in color/texture, M_w, T_g, T_d, ^{1}H NMR and FT-IR shifts. *In vitro* cell toxicity and SA release studies of polymer **1** were conducted following exposure.

Qualitatively, samples were visually analyzed for changes in color and texture (not shown). No color change was observed in the irradiated samples (25 and 50 kGy, e-beam and gamma) compared to the unexposed (0 kGy) polymer controls. Further, all samples remained as loose powders following irradiation.

Changes in M_w and T_g values are potential indicators of polymer degradation and/or crosslinking following radiation exposure. As shown in Table 1, sample M_w values were slightly reduced after exposure to e-beam and gamma radiation with samples exposed to gamma having a consistently lower M_w. This difference is possibly due to the differences in dose rate, as the time needed to deliver the desired dose is longer for the gamma process than in an e-beam process. Approximately 10% loss in the M_w was observed at the highest exposures (i.e., 50 kGy). The M_w of the sample exposed to 50 kGy gamma was the only statistically different sample compared to the unexposed control. Relatedly, polymer samples exposed to a 25 kGy dose displayed no change in T_g values whereas samples exposed to 50 kGy dose displayed a 3 degree drop in T_g (Table 1), corresponding to a 6% change from the unexposed polymer. Only one distinct T_g was observed and no melting points for the degradation product **4** (T_m = 174–176 °C) were noted. All T_d values remained relatively constant following radiation exposure (Table 1) with only one statistical significantly different sample (25 kGy gamma). The minimal decrease in M_w and T_g values as well as the absence of degradation product melting points indicate minimal polymer chain degradation occurs upon radiation exposure. ^{1}H NMR spectrometry and FT-IR spectroscopies were also used to monitor polymer degradation and crosslinking. The samples did not display the characteristic acid proton (COOH) signal at ~13 ppm that appears upon hydrolysis of the anhydride linkages. In the IR spectra, the anhydride carbonyl bond (C=O) stretches at ~1810 and ~1790 cm^{-1} would decrease with polymer **1** hydrolysis with a corresponding increase in C=O bond stretches for the carboxylic acid at ~1700 cm^{-1}. As shown in Fig. 1, the carboxylic acid stretches were not observed in the polymer samples (compared to the diacid, **4**). The C=O anhydride and ester stretches were clearly retained (1747 cm^{-1}) in all polymer samples.

The most critical criteria may be the maintained ability of polymer **1** to release SA *in vitro* after radiation exposure. Retention of the drug release profile after irradiation is important to determine polymer property changes. Fig. 2 shows the cumulative percent release of SA, where the SA was released over 20 days and all the samples (unexposed controls and exposed [25 and 50 kGy, e-beam and gamma]) displayed similar release profiles. The controlled degradation of polymer **1** to release SA remained unchanged after radiation exposure.

To ensure that radiation did not alter the cytocompatibilities of polymer **1**, cytotoxicity experiments were performed. Two polymer concentrations were chosen to mimic early-stage (0.01 mg/mL) and late-stage (0.10 mg/mL) polymer degradation [32,33]. Studies were performed over a 96 h time period, during which cell viability (Fig. 3) and morphology (Fig. 4) were evaluated. Cell viability for the polymer-containing media and the media control at 0.10 and 0.01 mg/mL at all three time points is shown in Fig. 3. Statistical analysis showed no significant differences at 95% confidence level for any sample compared to the media control at both concentrations used and all time points. Comparison between the polymer-containing samples and the media control indicate normal to higher cell viability suggesting that the polymer remained non-cytotoxic after radiation exposure. Fig. 4 shows representative light microscopy images, comparing the media control and the polymer-containing samples at 0.10 mg/mL and 96 h. For all conditions, the cell images demonstrate the typical proliferation and morphology expected to a healthy fibroblast cell line. After 96 h of culture, proliferating viable cell are visible with stellate morphology and extending filopodia.

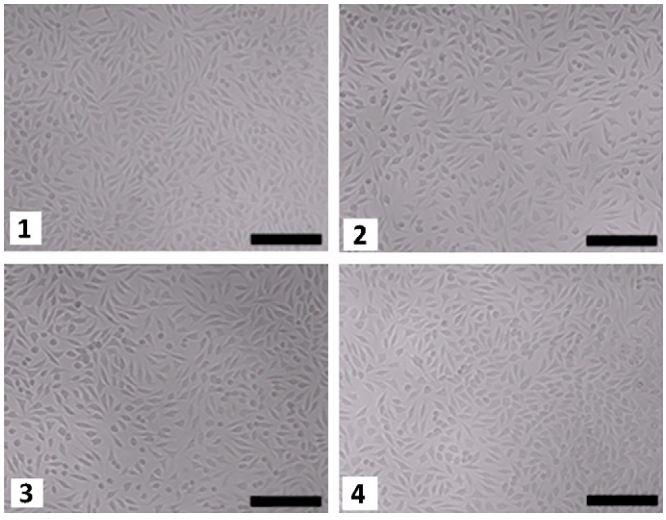


Fig. 4. Light microscope images (10× magnification) of L929 mouse fibroblasts after 96 h of culture in polymer **1** (0.10 mg/mL) exposed under various conditions: (1) 50 kGy e-beam, (2) 50 kGy gamma, (3) unexposed polymer control, (4) media control. Scale bar is 200 μm in all images.

4. Conclusion

PAEs such as polymer **1** are designed to hydrolytically degrade, releasing SA in a controlled fashion. For clinical use, however, the polymers must withstand sterilization and processing methods. This study demonstrated that exposure to e-beam and gamma radiation (25 and 50 kGy) did not substantially affect polymer composition, molecular weight, thermal properties, degradation characteristics, or cytocompatibility of polymer **1**. Therefore, e-beam or gamma radiation doses up to 50 kGy are suitable as a sterilization dose. For potential future products consisting of salicylate-based PAE, the minimum and maximum sterilization doses will need to be determined on a per product basis.

Acknowledgements

The authors thank the National Institutes of Health (NIH 5 R01DE0132070-09 and NIH 1 R01DE019926-01) and the Graduate

Assistance in Areas of National Need (GAANN) Fellowship for financial support. Dr. Bryan Langowski, Roberto Delgado, and Denise Cullerton (Rutgers, Department of Chemistry & Chemical Biology) are thanked for intellectual discussion.

References

[1] Hersh EV, Moore PA, Ross GL. Over-the-counter analgesics and antipyretics: a critical assessment. Clin Ther 2000;22(5):500–48.
[2] O'Neil MJ, Heckelman PE, Koch CB, Roman KJ. The Merck index. NJ: Merck Whitehouse Station; 2006.
[3] Erdmann L, Uhrich KE. Synthesis and degradation characteristics of salicylic acid-derived poly (anhydride-esters). Biomaterials 2000;21(19):1941–6.
[4] Schmeltzer RC, Anastasiou TJ, Uhrich KE. Optimized synthesis of salicylate-based poly (anhydride-esters). Polym Bull 2003;49(6):441–8.
[5] Prudencio A, Schmeltzer RC, Uhrich KE. Effect of linker structure on salicylic acid-derived poly (anhydride-esters). Macromolecules 2005;38(16):6895–901.
[6] Carbone AL, Uhrich KE. Design and synthesis of fast-degrading poly (anhydride-esters). Macromol Rapid Commun 2009;30(12):1021–6.
[7] Whitaker-Brothers K, Uhrich K. Poly (anhydride-ester) fibers: role of copolymer composition on hydrolytic degradation and mechanical properties. J Biomed Mater Res A 2004;70(2):309–18.
[8] Whitaker-Brothers K, Uhrich K. Investigation into the erosion mechanism of salicylate-based poly (anhydride-esters). J Biomed Mater Res A 2006;76(3):470–9.
[9] Bryers JD, Jarvis RA, Lebo J, Prudencio A, Kyriakides TR, Uhrich K. Biodegradation of poly (anhydride-esters) into non-steroidal anti-inflammatory drugs and their effect on *Pseudomonas aeruginosa* biofilms in vitro and on the foreign-body response in vivo. Biomaterials 2006;27(29):5039–48.
[10] Yeagy BA, Prudencio A, Schmeltzer RC, Uhrich KE, Cook TJ. Characterization and in vitro degradation of salicylate-derived poly (anhydride-ester microspheres). J Microencapsul 2008;23:643–53.
[11] Schmeltzer RC, Uhrich KE. Synthesis and characterization of salicylic acid-based poly (anhydride-ester) copolymers. J Bioact Compat Polym 2006;21(2):123.
[12] Schmeltzer RC, Johnson M, Griffin J, Uhrich K. Comparison of salicylate-based poly (anhydride-esters) formed via melt-condensation versus solution polymerization. J Biomater Sci Polym Ed 2008;19(10):1295–306.
[13] Rosenberg L, Carbone A, Römling U, Uhrich K, Chikindas M. Salicylic acid-based poly (anhydride esters) for control of biofilm formation in *Salmonella enterica* serovar typhimurium. Lett Appl Microbiol 2008;46(5):593–9.
[14] Johnson ML, Uhrich KE. Concurrent release of admixed antimicrobials and salicylic acid from salicylate-based poly (anhydride-esters). J Biomed Mater Res A 2009;91(3):671–8.
[15] deRonde BM, Carbone AL, Uhrich KE. Storage stability study of salicylate-based poly (anhydride-esters). Polym Degrad Stab 2010;95:1778–82.
[16] Harten RD, Svach DJ, Schmeltzer R, Uhrich KE. Salicylic acid-derived poly (anhydride-esters) inhibit bone resorption and formation in vivo. J Biomed Mater Res A 2005;72(4):354–62.
[17] Reynolds MA, Prudencio A, Aichelmann-Reidy ME, Woodward K, Uhrich KE. Non-steroidal anti-inflammatory drug (NSAID)-Derived poly (anhydride-esters) in bone and periodontal regeneration. Curr. Drug Deliv 2007;4(3):233–9.
[18] Gorna K, Gogolewski S. The effect of gamma radiation on molecular stability and mechanical properties of biodegradable polyurethanes for medical applications. Polym Degrad Stab 2003;79(3):465–74.
[19] Razem D, Katusin-Razem B. The effects of irradiation on controlled drug delivery/controlled drug release systems. Radiat Phys Chem 2008;77(3):288–344.
[20] Çalis S, Bozda S, Ka HS, Tunçay M, Hincal AA. Influence of irradiation sterilization on poly (lactide-co-glycolide) microspheres containing anti-inflammatory drugs. Il Farmaco 2002;57(1):55–62.
[21] Williams HE, Huxley J, Claybourn M, Booth J, Hobbs M, Meehan E, et al. The effect of [gamma]-irradiation and polymer composition on the stability of PLG polymer and microspheres. Polym Degrad Stab 2006;91(9):2171–81.
[22] Edlund U, Albertsson AC, Singh S, Fogelberg I, Lundgren B. Sterilization, storage stability and in vivo biocompatibility of poly (trimethylene carbonate)/poly (adipic anhydride) blends. Biomaterials 2000;21(9):945–55.
[23] Odelius K, Plikk P, Albertsson AC. The influence of composition of porous copolyester scaffolds on reactions induced by irradiation sterilization. Biomaterials 2008;29(2):129–40.
[24] Gogolewski S, Mainil-Varlet P. Effect of thermal treatment on sterility, molecular and mechanical properties of various polylactides: 2. Poly (D, L-lactide) and poly (DL-lactide). Biomaterials 1997;18(3):251–5.
[25] Benson RS. Use of radiation in biomaterials science. Nucl Instrum Meth B 2002;191(1–4):752–7.
[26] Igartua M, Hernández RM, Rosas JE, Patarroyo ME, Pedraz JL. [gamma]-Irradiation effects on biopharmaceutical properties of PLGA microspheres loaded with SPf66 synthetic vaccine. Eur J Pharm Biopharm 2008;69(2):519–26.
[27] Nugroho P, Mitomo H, Yoshii F, Kume T. Degradation of poly (L-lactic acid) by [gamma]-irradiation. Polym Degrad Stab 2001;72(2):337–43.
[28] Loo SCJ, Ooi CP, Boey YCF. Radiation effects on poly (lactide-co-glycolide)(PLGA) and poly (L-lactide)(PLLA). Polym Degrad Stab 2004;83(2):259–65.
[29] Phong L, Han ESC, Xiong S, Pan J, Loo SCJ. Properties and hydrolysis of PLGA and PLLA cross-linked with electron beam radiation. Polym Degrad Stab 2010;95(5):771–7.
[30] Martínez-Sancho C, Herrero-Vanrell R, Negro S. Study of gamma-irradiation effects on aciclovir poly (D, L-lactic-co-glycolic) acid microspheres for intravitreal administration. J Control Release 2004;99(1):41–52.
[31] Yagoubi N, Peron R, Legendre B, Grossiord J, Ferrier D. Gamma and electron beam radiation induced physico-chemical modifications of poly (propylene). Nucl Instrum Meth B 1999;151(1–4):247–54.
[32] Duncan E, Lemons J, Mayesh J, Saha P, Saha S, Scrantron M. New products and standards in biomaterials science. New York: Elsevier Academics Press; 2004.
[33] Fischer D, Li Y, Ahlemeyer B, Krieglstein J, Kissel T. In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials 2003;24(7):1121–31.
[34] Biological evaluation for medical devices-part 5: tests for cytotoxicity in vitro methods. ISO-10993-5 (EN 30993-5); 1992.