Holmium-Loaded Poly(L-lactic Acid) Microspheres: In Vitro Degradation Study

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The clinical application of holmium-loaded poly(L-lactic acid) (PLLA) microspheres for the radionuclide treatment of liver malignancies requires in depth understanding of the degradation characteristics of the microspheres. To this end, an in-vitro degradation study was conducted. PLLA-microspheres with and without HoAcAc loading, and before and after neutron or gamma irradiation, were incubated in a phosphate buffer at 37 °C for 12 months. In contrast with the other microsphere formulations, only the neutron-irradiated Ho-PLLA-MS disintegrated. At the end of the experiment (52 weeks) highly crystalline fragments, as evidenced from Differential Scanning Calorimetry, were present. Infrared spectroscopy showed that these fragments consisted of holmium lactate. In conclusion, this study demonstrates that the degradation of neutron-irradiated Ho-PLLA-MS was substantially accelerated by the HoAcAc incorporation and subsequent neutron irradiation. The degradation of these microspheres in aqueous solution resulted in the formation of insoluble holmium lactate microcrystals without release of Ho^{3+}.

1. Introduction

Radioembolization is a minimally invasive treatment for liver malignancies that can be used when curative surgery is not possible.[1−3] This treatment makes use of radionuclide-loaded microparticles with a size of 20−50 μm that are delivered into the blood vessels in and around the tumor after administration via a catheter. When these microspheres are administered into the hepatic artery of patients suffering from liver malignancies, they will lodge in and around the tumor and directly irradiate the tumor.[1,2] Radioactive microspheres can be obtained by neutron irradiation in a nuclear reactor. Therefore, the microspheres should be loaded with an element that can be easily neutron-activated. Holmium-165 is a nonradioactive element with a high cross section of 64 barn and a natural abundance of 100%,[1,4] allowing a fast and simple neutron activation procedure. Neutron irradiation of holmium-165 results in the formation of radioactive holmium-166 (physical half-life of 26.8 h), a β-emitter (E_{max} = 1.84 MeV) suitable for radionuclide therapy that also emits photons (80.6 keV, 6.2%) usable for imaging with a gamma camera. Holmium-165 can be incorporated into poly(L-lactic acid) (PLLA) microspheres as its acetylacetonate complex (HoAcAc) using a solvent evaporation technique.[5]

It is important that therapeutic radiopharmaceuticals have a high radiochemical stability. The release of radioactive holmium from the microspheres will result in distribution over other organs than the liver, which can lead to serious adverse events. Previous papers report that, although the molecular weight of the PLLA decreased due to the neutron irradiation, the microspheres retained their integrity.[5] Moreover, 2 days after administration, the amount of holmium-166 in urine and feces of treated rabbits was less than 0.1% of the administered dose,[6] indicating that holmium remained stably incorporated in the microspheres. The toxicity of lanthanides such as holmium was studied in rodents and LD50 values of intravenously or intraperitoneally lanthanide salts varied from about 50−500 mg/kg bodyweight which shows that these lanthanides are not very toxic.[7] However, the long-term effects concerning the chemical toxicity and biocompatibility of Ho-PLLA-MS are unknown and need further examination. Consequently, a clear insight into the degradation characteristics of the microspheres and the nature of the formed degradation products is essential.

Gamma irradiation is a well-established sterilization method for PLLA and might therefore be a useful option for the sterilization of Ho-PLLA-MS.[8] However, it is well-known that gamma irradiation also changes the properties of polymeric microspheres[9] and therefore the effect of gamma irradiation on the degradation characteristics of Ho-PLLA-MS was also studied.

Hydrolysis of ester bonds in poly(lactic acid) results in the formation of soluble lactic acid oligomers which are ultimately converted into lactic acid.[10−12] By this hydrolytic process, PLLA microspheres disintegrate and finally "dissolve" in time. The degradation of (drug-loaded) PLLA microspheres is dependent on many factors, such as the size, morphology, molecular weight of PLLA, and the type of drug and drug loading.[13,14] In view of this, there are two factors that can have a large influence on the degradation of Ho-PLLA-MS. The first factor is the neutron irradiation,[5] which results in substantial decrease in molecular weight of PLLA and a loss of its crystallinity.[15] The second factor is the high drug loading because 50% of the microsphere matrix consists of HoAcAc. It was demonstrated that the Ho-ion interacts with the carbonyl groups of PLLA[16] which in turn might affect the hydrolytic degradation of PLLA.

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To understand the consequences of these factors, an in vitro degradation study was conducted. Knowledge of the degradation pattern of Ho-PLLA-MS is of great importance for evaluation of the biocompatibility of these microspheres in animals and their application in patients.

2. Materials and Methods

2.1. Materials. All chemicals were commercially available and used as obtained. Acetylacetone, 2,4-pentanedione (AcAc; >99%), chloroform ($CHCl_3$; HPLC-grade), poly(vinyl alcohol) (PVA; average MW 30000−70000, 88% hydrolyzed), and ammonium hydroxide (NH_4OH; 29.3% in water) were supplied by Sigma Aldrich (Steinheim, Germany). Sodium hydroxide (NaOH; 99.9%) was purchased from Riedel-de Haën (Seelze, Germany). Holmium(III) chloride hexahydrate ($HoCl_3 \cdot 6H_2O$; 99.9%) was obtained from Phase Separations BV (Waddinxveen, The Netherlands). L-Lactic acid (90% solution; USP-grade) and poly(L-lactic acid) (PLLA; inherent viscosity 1.14 dL/g in chloroform at 25 °C) were purchased from Purac Biochem (Gorinchem, The Netherlands). Hydrochloric acid (HCl; 37%), sodium azide (NaN_3; 99%), di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$; 99%), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$; 99%), and lithium chloride (LiCl; >99%) were purchased from Merck (Darmstadt, Germany). Dichloromethane (DCM, HPLC-grade), dimethylformamide (DMF, HPLC-grade), and tetrahydrofuran (THF, HPLC-grade) were obtained from Biosolve (Valkenswaard, The Netherlands).

2.2. Preparation of HoAcAc and Microspheres. HoAcAc was prepared as described previously.[5] In brief, acetylacetone (180 g) was dissolved in water (1080 g). The pH of this solution was brought to 8.50 with an aqueous solution of ammonium hydroxide. Holmium chloride (10 g dissolved in 30 mL of water) was added to this solution. After 15 h of incubation at room temperature, the formed HoAcAc crystals were collected by centrifugation and washed with water.

HoAcAc (10 g) and PLLA (6 g) were dissolved in 186 g of chloroform. The resulting homogeneous solution was added to 1 L of an aqueous solution of PVA (2%). The mixture was stirred (500 rpm) for 40 h at room temperature and the formed microspheres were collected by centrifugation. The microspheres were washed three times with water, three times with 0.1 M HCl, and finally three times with water. The washed microspheres were size-fractionated with a wet sieving system consisting of an Electromagnetic Sieve Vibrator (EMS 755) combined with an Ultrasonic Processor (UDS 751) (both from Topas GmbH, Dresden, Germany). The collected microspheres (about 4 g, size between 20 and 50 μm) were dried at 70 °C under vacuum for 5 h using a rotating Glass Oven (B-580 GKR, Buchi). The holmium content in microspheres was determined by a complexometric titration as described previously.[17] Placebo PLLA microspheres without HoAcAc loading were prepared in the same way. After drying, microspheres (~250 mg) were packed in polyethylene vials.

2.3. Neutron and Gamma Irradiation. Neutron irradiations were performed in the pneumatic rabbit system (PRS) in the reactor facility in Delft (Department of Radiation, Radionuclides and Reactor, Delft University of Technology, The Netherlands). The polyethylene vials with Ho-PLLA-MS (~250 mg) were neutron-irradiated with a thermal neutron flux of 5×10^{12} $cm^{-2} \cdot s^{-1}$ for 6 h. The neutron-irradiated microspheres were stored in closed vials at room temperature and analyzed after 1 month. Gamma sterilization of the samples with a dose of 25.0 kGy was performed using a cobalt-60 source (Isotron, Ede, The Netherlands).

2.4. Degradation of Microspheres. Microsphere samples (100 mg) were incubated in test tubes containing 5 mL of an isotonic phosphate buffer (116 mM, pH 7.4) and continuously shaken at 37 °C. The buffer was made by dissolving 39 mmol of $NaH_2PO_4 \cdot 2H_2O$ and 77 mmol of $Na_2HPO_4 \cdot 2H_2O$ in 1 L of water and the isotonicity was checked with a freezing point osmometer. Sodium azide (0.05%) was added to the buffer to prevent bacterial growth. At predetermined time points (1, 4, 8, 12, 16, 24, 32, 40, and 52 weeks), samples were centrifuged for 5 min at 2000 rpm and the supernatant was collected. The microspheres were washed two times with water, dried for 3 days at room temperature (without further treatment), and weighed. The microspheres and supernatant were used for further analysis.

2.5. Release of Holmium. The release of holmium from neutron-irradiated microspheres was determined by measurement of metastable radioactive holmium (Ho-166m) in the supernatant and in the degraded microspheres using a low-background γ-counter (Tobor, Nuclear Chicago, USA). Ho-166m is a metastable isotope formed in a small fraction (7 ppm of the total amount of newly formed isotopes) during activation of Ho-165. The concentration of Ho-166m in the samples was low but detectable. After the decay of Ho-166 (half-life of 26.8 h) during 1 month of storage, Ho-166m (half-life ~1200 year) is the only persisting radioactive isotope in the microspheres and can therefore be reliably and accurately detected as demonstrated previously.[18]

2.6. Holmium Lactate. Holmium lactate was prepared as follows. (L)-Lactic acid (1.0 g) was dissolved in 10 mL of distilled water and the pH was adjusted to 6.0 with a sodium hydroxide solution (1 M). Holmium chloride (4.2 g) was dissolved in 20 mL of water and subsequently added to the sodium lactate solution, and a precipitate was formed. The reaction was performed at pH 6.0 since it is known that at higher pH values holmium hydroxide can be formed.[19] The precipitated holmium lactate (1.2 g) was washed three times with water and dried for 48 h at 50 °C.

2.7. Determination of Particle Size Distribution and Evaluation of the Surface Morphology of the Microspheres. The particle size distribution of microspheres was determined using a Coulter counter (Multisizer 3, Beckman Coulter, The Netherlands) equipped with a 100-μm orifice.

The surface morphology of the Ho-PLLA-microspheres was investigated by scanning electron microscopy (SEM) using a Philips XL30 FEGSEM. A voltage of 5 or 10 kV was applied. Samples of the different microsphere batches were mounted on aluminum stubs and sputter-coated with a Pt/Pd layer of about 10 nm.

2.8. Gel Permeation Chromatography (GPC). The weight-average molecular weight (M_w) and number-average molecular weight (M_n) of PLLA of nonirradiated and gamma-irradiated PLLA- and Ho-PLLA-MS were determined by GPC with two thermostated (35 °C) columns in series (PL gel Mixed-D, Polymer Laboratories, Amherst, USA). A refractive index detector (type 410, Waters, Milford, USA) was used. Samples of approximately 10 mg were dissolved in 5 mL of chloroform and filtered through 0.45 μm HPLC filters (Waters). Elution was performed with chloroform and the flow rate was 1 mL/min.

The M_w and M_n of PLLA of the neutron-irradiated PLLA-MS and Ho-PLLA-MS were determined by another GPC method using two thermostated (40 °C) columns in series (Mesopore, Polymer Laboratories). Samples of approximately 10 mg were dissolved in 5 mL of THF and filtered through 0.45 μm HPLC filters (Waters). Elution was performed with THF and the flow rate was 1 mL/min. The mesopore column was used since low molecular weights of PLLA were expected due to the neutron irradiation[15,20] and THF is a suitable solvent for (neutron-irradiated) PLLA.[20]

The columns were calibrated using polystyrene standards of known molecular weights (Shodex and Tosoh, Polymer Laboratories). Analyses were performed in duplicate.

2.9. Differential Scanning Calorimetry (DSC). Modulated DSC (MDSC) analyses were performed with a DSC Q1000 (TA Instruments, USA). Samples of approximately 5 mg were transferred into aluminum pans. Scans were recorded under "heating only" conditions, with a heating rate of 1 °C/min and cooling rate of 2 °C/min. The settings were periods of 30 s and a temperature modulation amplitude of 0.5 °C was applied. Samples were heated from 20 to 230 °C. The Universal Analysis 2000 software (version 3.9A) was used for evaluation. The melting enthalpy of holmium-containing samples was corrected for their holmium content. Analyses were performed in duplicate.

2.10. Infrared Spectroscopy. Infrared spectra were recorded on a Bio-Rad FTS 6000 spectrometer (Cambridge, USA), in the range of

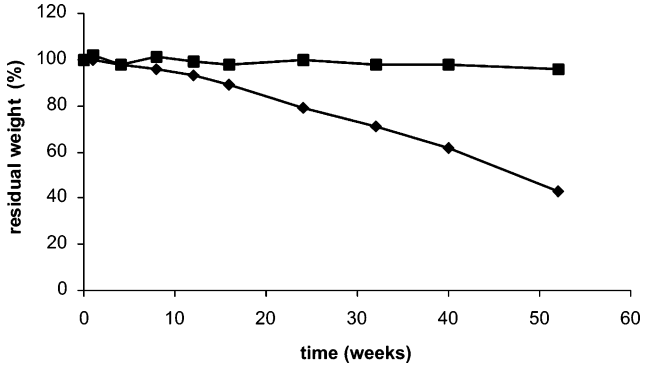

Figure 1. Residual weight of neutron-irradiated PLLA-MS (■) and neutron-irradiated Ho-PLLA-MS (◆) incubated in 116 mM phosphate buffer in time. The residual weight of neutron-irradiated PLLA-MS is representative of the other microsphere formulations (except neutron-irradiated Ho-PLLA-MS) and is shown here as a typical example.

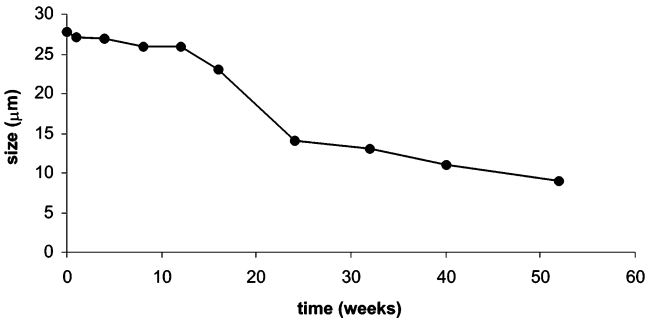

Figure 2. Mean particle size of neutron-irradiated Ho-PLLA-MS incubated in 116 mM phosphate buffer in time.

400−4000 cm^{-1} (scan number 64, resolution 2 cm^{-1}). Samples of microspheres and of holmium lactate were mixed with spectroscopy grade KBr (Merck, Darmstadt, Germany) and pressed into a tablet using a pressure of $8 \cdot 10^3$ kg/cm^2.

3. Results and Discussion

3.1. Release of Holmium and Weight Loss. The characteristics of the prepared Ho-PLLA-MS were in compliance with previous results.[5,15,17] In brief, the holmium loading was 17.0 ± 0.5%, corresponding with a loading of HoAcAc of ∼50% (w/w). More than 97% of the microspheres (with and without HoAcAc loading) had a size between 20 and 50 μm after sieving. No differences in size distributions were observed after gamma irradiation (25 kGy), whereas after neutron irradiation still more than 95% of the microspheres had a size between 20 and 50 μm.

In a previous paper it was shown that hardly any release of holmium occurred during 270 h of incubation of Ho-PLLA-MS in a phosphate buffer (0.5 ± 0.2%).[21] In the present study the release was followed for longer times and it was shown that during a time span of 52 weeks only 0.7 ± 0.2% had released.

The weight loss of neutron-irradiated Ho-PLLA-MS incubated in buffer in time is given in Figure 1. This figure shows that the weight of these microspheres decreased in time; the total weight loss was ∼60% at the end of the degradation experiment (52 weeks). In contradistinction, the other microsphere formulations showed no significant weight loss during 52 weeks, which is in line with data reported on the in vitro degradation of PLLA fibers.[22]

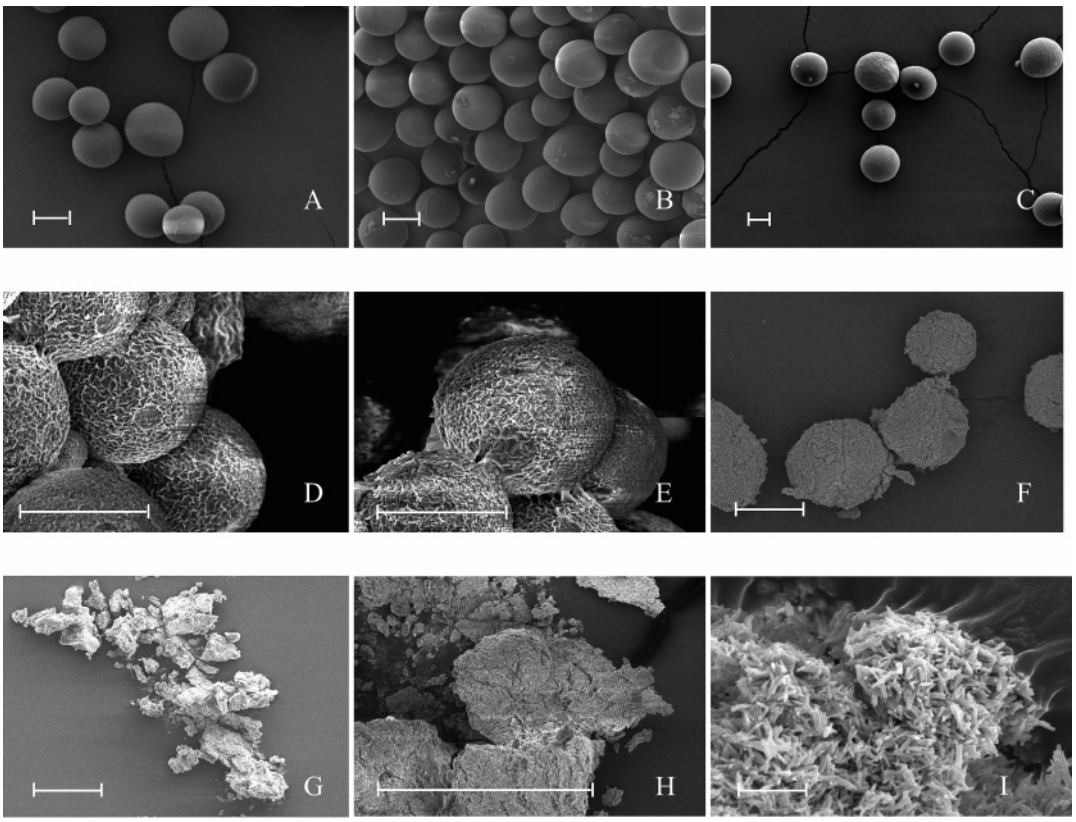

Figure 3. SEM pictures of PLLA-MS (A), gamma-irradiated PLLA-MS (B), neutron-irradiated PLLA-MS (C), Ho-PLLA-MS (D), and gamma-irradiated Ho-PLLA-MS (E) after 52 weeks of incubation in buffer. (F), (G), and (H) are neutron-irradiated Ho-PLLA-MS after 12, 24, and 52 weeks of incubation in buffer, respectively. (I) is a detailed picture of (H) and shows the presence of needle-shaped crystals. Bars represent 20 μm in (A−H) and the bar in (I) represents 1 μm.

Table 1. M_w and M_n of PLLA in Nonirradiated ("non"), Gamma-Irradiated ("gamma"), and Neutron-Irradiated ("neutron") PLLA-MS

sample	irradiation	M_w	M_n
PLLA-MS	non	114 000	65 000
PLLA-MS	gamma	66 000	48 000
PLLA-MS	neutron	2200	1900
Ho-PLLA-MS	non	63 000	46 000
Ho-PLLA-MS	gamma	52 000	30 000
Ho-PLLA-MS	neutron	1900	1500

In a recent long-term biocompatibility study, we investigated the degradation characteristics of Ho-PLLA-MS after implantation into the liver of rats. No clinical and biochemical toxic effects were observed. Also, no release or transfer of holmium from the site of implantation of the holmium-loaded microspheres to other parts of the liver or organs was measured over a period of 14 months. This is consistent with the findings of the present in vitro study.

3.2. SEM and Particle Size Distribution. Particle size analysis showed that the size of neutron-irradiated Ho-PLLA-MS remained more or less constant during the first 16 weeks of incubation (Figure 2). Thereafter, the particle size changed considerably and remained almost constant again from week 24 until week 52. No significant particle size changes were observed for the other microsphere formulations.

SEM analysis showed that PLLA-MS without HoAcAc loading, including the neutron-irradiated ones, retained their spherical shape and smooth surface until the end of the degradation experiment (52 weeks) (Figure 3A−C). In contradistinction, SEM showed that Ho-PLLA-MS (Figure 3D) and gamma-irradiated Ho-PLLA-MS (Figure 3E) had a wrinkled surface, but retained their spherical shape. SEM analysis of neutron-irradiated Ho-PLLA-MS showed that these microspheres disintegrated after 24 weeks of incubation in buffer (Figure 3G) and that fragments were present (Figure 3H), consisting of small needle-shaped crystals (Figure 3I).

3.3. GPC. The initial molecular weights of PLLA in the different microsphere formulations before incubation in buffer

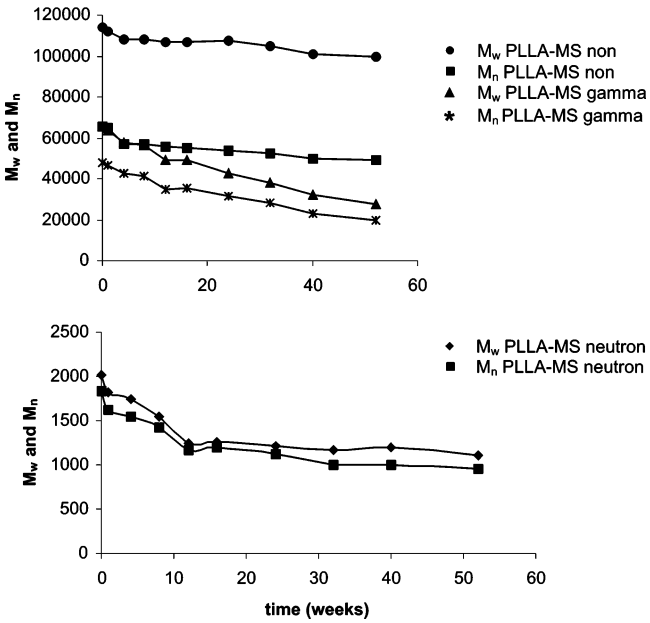

Figure 4. M_w and M_n of PLLA in nonirradiated ("non"), gamma-irradiated ("gamma"), and neutron-irradiated ("neutron") PLLA-MS as a function of degradation time.

Table 2. T_g, T_m, and Melting Enthalpy of PLLA-MS and Ho-PLLA-MS

sample	irradiation	T_g (°C)	T_m max (°C)	melting enthalpy (J/g)[a]
PLLA-MS	non	70	177	55
PLLA-MS	gamma	68	174	48
PLLA-MS	neutron	nd	116	15
Ho-PLLA-MS	non	60	152	30[a]
Ho-PLLA-MS	gamma	60	150	30[a]
Ho-PLLA-MS	neutron	50	nd	nd

[a] Melting enthalpy was corrected for the HoAcAc loading; nd: nondetectable.

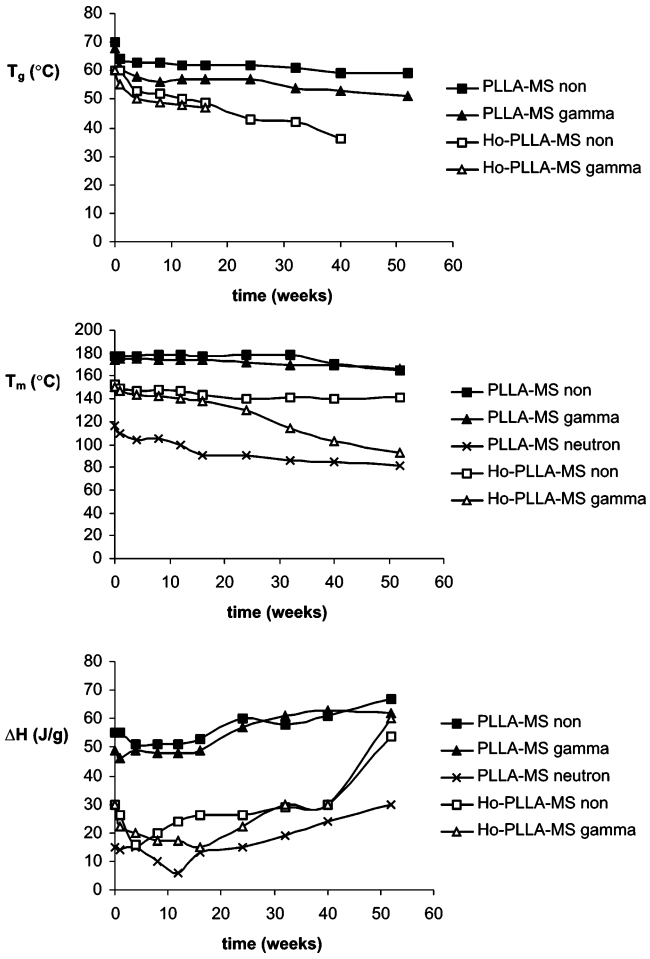

Figure 5. T_g, T_m, and melting enthalpy of nonirradiated ("non"), gamma-irradiated ("gamma"), and neutron-irradiated ("neutron") microspheres as a function of degradation time.

are given in Table 1 and were in compliance with previous studies.[15,16] In summary, the M_w and M_n of PLLA in Ho-PLLA-MS were lower than the molecular weights in PLLA-MS. This decrease in molecular weight is not caused by holmium-induced degradation of PLLA,[15,16] but is due to interactions between PLLA and Ho-AcAc which results in a decrease in the hydrodynamic volume of PLLA and thus in an apparently lower molecular weight. After gamma irradiation (25 kGy) the M_w and M_n of PLLA in PLLA-MS decreased with ∼45% and ∼25%, respectively, and the M_w and M_n of PLLA in Ho-PLLA-MS decreased with ∼20% and ∼35%, respectively. Neutron irradiation of (Ho)-PLLA-MS caused a substantial decrease (∼95%) of the M_w and M_n of PLLA, likely due to chain scission.[15]

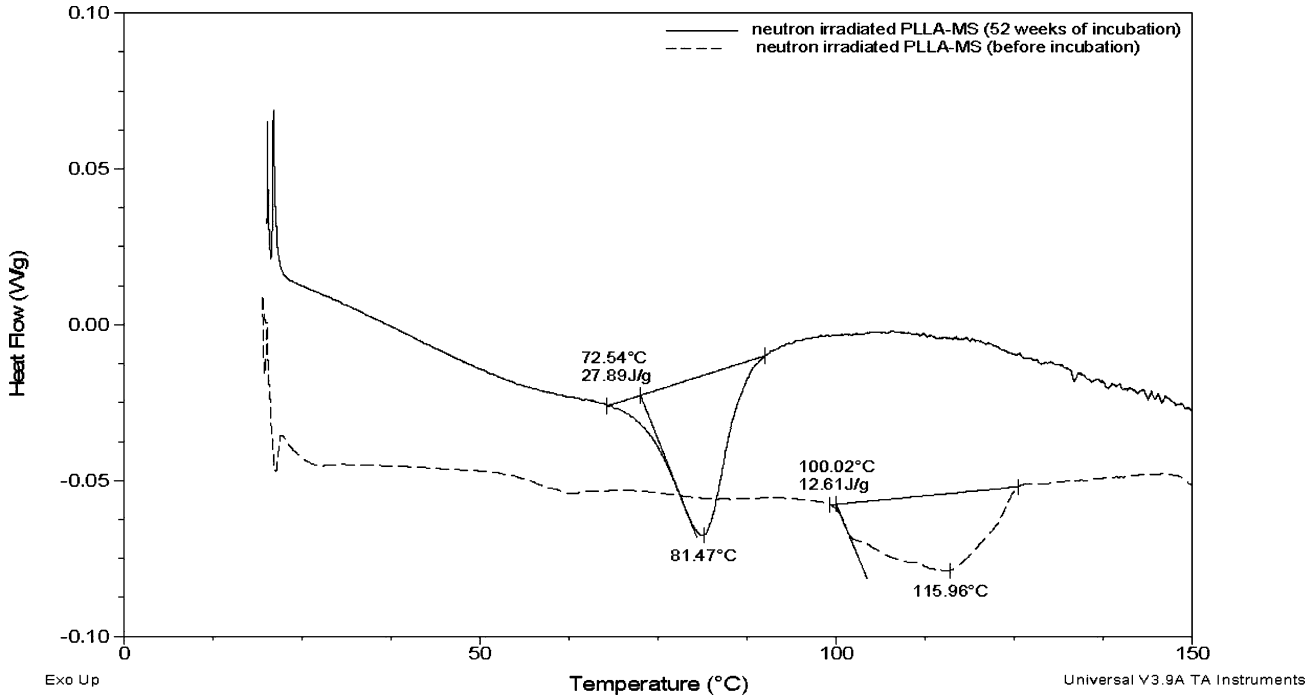

Figure 6. DSC thermogram of neutron-irradiated PLLA-MS before and after 52 weeks incubation in 116 mM phosphate buffer.

The M_w and M_n of PLLA in PLLA-MS as a function of degradation time are shown in Figure 4. A slight decrease in both M_w and M_n was observed for nonirradiated PLLA-MS in time. The M_w and M_n of PLLA in gamma-irradiated PLLA-MS decreased faster in time, ending up with a M_w and M_n of ~27 000 and ~20 000 g/mol, respectively. It has indeed been reported that gamma irradiation accelerates the hydrolytic degradation of PLA,[23] which is ascribed to the higher number of hydrophilic end groups (COOH and OH) which accelerates PLLA decomposition autocatalytically.[23]

The M_w of PLLA in neutron-irradiated PLLA-MS decreased from ~2000 to ~1100 g/mol in 12 weeks of incubation in buffer and remained more or less constant hereafter, which explains that these microspheres stayed intact during the degradation experiment (Figure 3C, SEM analysis). These GPC results are in agreement with the paper of Suuronen et al.,[24] who followed the degradation of PLLA plates for a period of 5 years and showed that the molecular weight of PLLA decreased from ~50 000 to ~3000 g/mol after 2 years of incubation in buffer and remained at ~3000 g/mol during the following 3 years.

Remarkably, PLLA in degraded nonirradiated as well as gamma- and neutron-irradiated Ho-PLLA-MS could not be analyzed with GPC since it was not possible to dissolve Ho-PLLA-MS in suitable solvents such as chloroform, DCM, THF, and DMF with and without 10 mM of LiCl, even after heating of the solvents to 50 °C. As shown before, Ho^{3+} interacts with the carbonyl groups of PLLA,[16] and obviously these interactions increase in time, yielding an insoluble matrix.

3.4. DSC. Table 2 summarizes the results of the DSC analysis of the different microspheres before incubation in buffer. The results were in compliance with previous studies.[15,16] In summary, compared to nonirradiated PLLA-MS, gamma irradiation of PLLA-MS did not result in major changes in the T_g, T_m, and melting enthalpy. However, after neutron irradiation both the melting temperature (T_m) and melting enthalpy of PLLA decreased tremendously, whereas no glass transition temperature (T_g) was detected. Nonirradiated Ho-PLLA-MS had a lower T_g, T_m, and melting enthalpy than nonloaded PLLA-MS (Table 2),

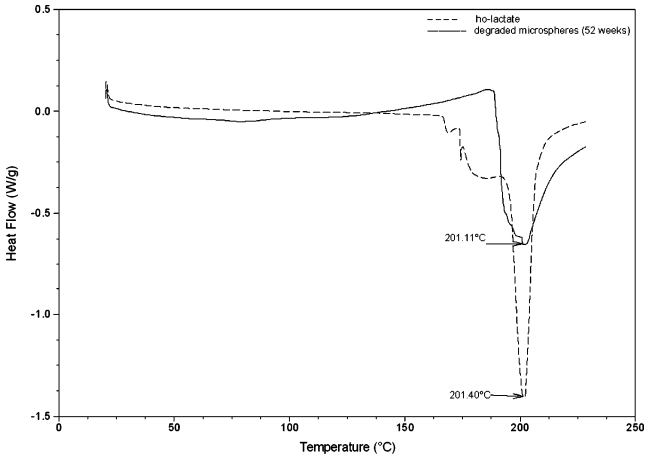

Figure 7. DSC thermogram of holmium lactate and the insoluble residue of neutron-irradiated Ho-PLLA-MS after 52 weeks incubation in 116 mM phosphate buffer.

indicating that the PLLA phase in Ho-PLLA-MS had a low degree of crystallinity.[16] Gamma irradiation of Ho-PLLA-MS did not result in changes of the T_g, T_m, and melting enthalpy. In contrast, after neutron irradiation of Ho-PLLA-MS lowering of the T_g was observed, whereas no T_m was detected due to the loss of crystallinity.[15]

The T_g, T_m, and melting enthalpy of PLLA-MS and Ho-PLLA-MS after incubation in buffer are shown in Figure 5.

In time, a slight decrease in T_g and T_m was observed for nonirradiated PLLA-MS and gamma-irradiated PLLA-MS, whereas the melting enthalpy first slightly decreased and later increased. A decrease in T_m and an increase in the melting enthalpy was also observed for neutron-irradiated PLLA-MS (Figure 6). The increase in crystalline fraction in time can be explained by the observation that degradation of PLLA mainly occurred in its amorphous phase.[22,25] Moreover, it has been reported that crystallization of the amorphous phase can occur during the degradation process,[22,26] which is likely also the case in this study.

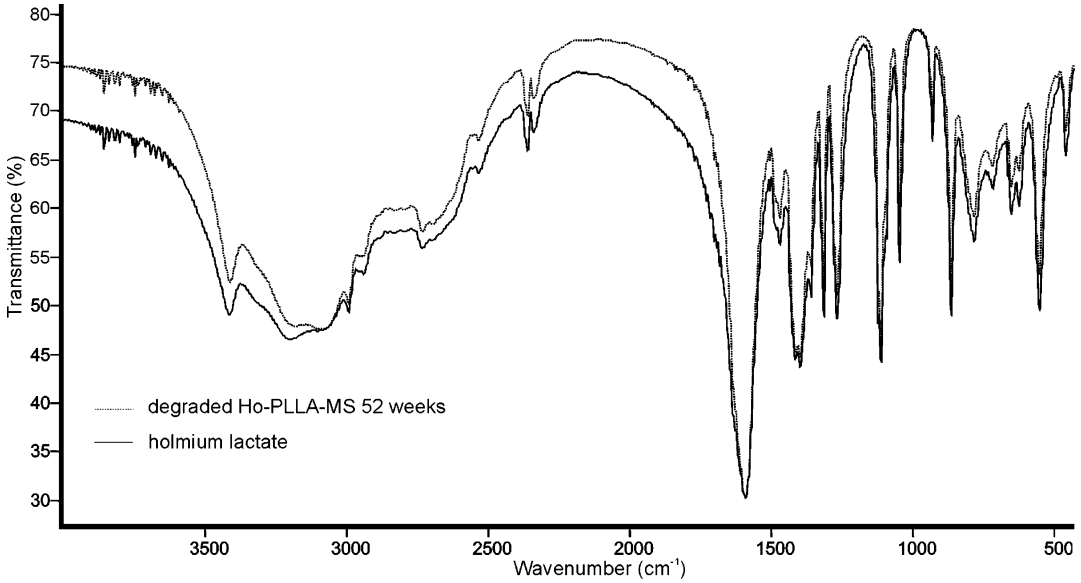

Figure 8. IR spectra of holmium lactate and the insoluble residue of neutron-irradiated Ho-PLLA-MS after 52 weeks incubation in 116 mM phosphate buffer.

The T_g and T_m of both non- and gamma-irradiated Ho-PLLA-MS decreased somewhat faster than the T_g and T_m of PLLA-MS formulations as a function of the degradation time. No T_g of nonirradiated and gamma-irradiated Ho-PLLA-MS could be observed after 40 and 16 weeks of incubation in buffer, respectively, probably due to crystallization of the amorphous phase during the degradation process.[22,26]

For neutron-irradiated Ho-PLLA-MS neither a T_g nor a T_m could be detected in the degraded microspheres. The absence of a T_g might be caused by the interaction of Ho^{3+} with the carbonyl groups of PLLA,[16] which results in a rigid structure. The absence of crystallinity in neutron-irradiated Ho-PLLA-MS likely caused their relatively fast degradation. Generally, with decreasing crystallinity in microspheres the degradation rate increases.[14] The reason for the observation that neutron-irradiated Ho-PLLA-MS did degrade while neutron-irradiated PLLA-MS did not degrade in the time frame studied might be because of the HoAcAc loading. Previous research has shown that HoAcAc is responsible for a low degree of crystallinity of the PLLA phase.[16] Remarkably, neutron-irradiated Ho-PLLA-MS, which was incubated in buffer for 52 weeks, showed a sharp T_m at 201 °C (Figure 7), which corresponds with that of holmium lactate (Figure 7; see also section 3.5. Infrared Spectroscopy).

3.5. Infrared Spectroscopy. The IR spectra of neutron-irradiated Ho-PLLA-MS, which were degraded for 52 weeks, and holmium lactate are given in Figure 8. The IR spectra were in compliance with each other, which demonstrates, and is supported by the DSC results (Figure 7), that the degradation of neutron-irradiated Ho-PLLA-MS results in the formation of insoluble holmium lactate microcrystals.

4. Conclusions

The degradation of Ho-PLLA-MS is strongly influenced by its HoAcAc loading and neutron irradiation. In contradistinction to neutron-irradiated PLLA-MS and nonirradiated Ho-PLLA-MS, neutron-irradiated Ho-PLLA-MS disintegrated after a period of 24 weeks of incubation in buffer, with the result of the formation of insoluble holmium lactate microcrystals. Hardly any holmium was released into the buffer (<1%), and the total weight after degradation, namely, 40% of the initial weight of the microspheres, corresponds with the calculated weight of holmium lactate, assuming that Ho^{3+} is complexed by three lactate groups.

In conclusion, this study demonstrates that in contradistinction to neutron-irradiated PLLA-MS and nonirradiated Ho-PLLA-MS, neutron-irradiated Ho-PLLA-MS undergoes hydrolytic degradation and as a result disintegrates. This process resulted in the formation of insoluble holmium lactate. An in vivo study in which the consequences of the degradation characteristics and formation of holmium lactate for the biocompatibility of neutron-irradiated Ho-PLLA-MS was evaluated in rats has been submitted for publication.

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