Biodegradable Samarium-153–labelled microspheres for hepatic radioembolization: preparation, characterization and radiolabelling evaluation after neutron activation

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Biodegradable Samarium-153–labelled microspheres for hepatic radioembolization: preparation, characterization and radiolabelling evaluation after neutron activation

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Abstract. A biocompatible and biodegradable radioactive samarium-153 (^{153}Sm)-labelled Poly-(L-lactic acid) (PLLA) microspheres was developed for hepatic radioembolization. Samarium acetylacetonate was encapsulated in PLLA microspheres using oil-in-water solvent evaporation method. Physicochemical characterization of the microspheres were analysed using Field Emission Scanning Electron Microscopy, Energy Dispersive X-ray spectroscopy and particle size analyser. The prepared microspheres were irradiated in a nuclear reactor with a neutron flux of 1.49×10^{12} n.cm-2.s^{-1}, converting ^{152}Sm to ^{153}Sm (E_{max} = 807.6 keV, half-life = 46.3 h). Gamma spectroscopy was carried out to determine the presence of radionuclide impurities while the in-vitro radiolabelling efficiency was performed to analyse the retention of ^{153}Sm on the microspheres. The Sm-labelled PLLA microspheres was found spherical with the diameters within 20–60 μm, as indicated by the scanning electron microscopy and particle size distribution results. Gamma spectrometry suggested that no long half-life radioimpurities present after neutron activation. The ^{153}Sm-labelled PLLA microspheres has achieved a nominal activity of 5.9 GBq.g^{-1} after 6 h neutron activation. The formulation showed more than 97% radiolabelling efficiency in saline and human blood plasma over 550 h. The ^{153}Sm-labelled PLLA microspheres are potentially useful for hepatic radioembolization due to their biodegradability, favourable radiation characteristics and excellent radiolabelling efficiency. The preparation of the formulation does not involve ionizing radiation and hence reduces the costs of production.

1. Introduction

Hepatocellular carcinoma (HCC) is a complex primary liver malignancy, which has accounted for 90% of primary liver cancers. It is the third leading cause of all cancer-related deaths globally and responsible for approximately 7% of all cancers, representing the fifth most common cancer in men and eighth for women (1-3). HCC is often diagnosed at later stages as the symptoms of HCC often do not appear until the cancer has progressed to later stages. Hence, curative approaches such as liver transplantation and surgical resection are no longer feasible (2).

Selective internal radiation therapy or radioembolization is a useful alternative therapy for HCC where radioactive microspheres with sufficient size to lodge in the end-arterioles are intra-arterially injected. The radioembolization treatment is based on the differentiated blood supply to the normal liver and liver tumours. In contrast to the normal liver that receives most of the blood supply from the portal vein, liver tumours are almost solely dependent on arterial blood supply (4, 5). Intra-arterial injection of radioactive microspheres within the size range of 20 and 60 μm via a catheter will lodge in the vascular bed of the liver and deliver all the radiation in situ (6). Currently, there are two commercially available radioembolic agents for radioembolization; glass-based TheraSphere® (Nordion, Canada) and resin based SIR-Spheres® (SIRTex, Australia) microspheres. Both the TheraSphere® and SIR-Spheres® are labelled with Yttrium-90 (^{90}Y), which is produced by high-purity separation from Strontium-90 in a ^{90}Y generator. This has contributes to a higher cost of ^{90}Y microspheres. In addition, the ^{90}Y is a pure beta emitter and hence the distribution of ^{90}Y microspheres after each procedure is difficult to be verified.

An ideal radionuclide for therapeutic purpose should has an optimum physical half-life, suitable linear energy transfer (LET) and range in tissue, high ratio of non-penetrating to penetrating radiation, short-lived or stable daughter, good and selective concentration with prolonged retention in tumour and minimum uptake by normal tissue (7). In addition, radionuclides with both beta and a diagnostic range of gamma energies would be ideal for theranostic purposes. Besides, due to relatively simpler production process and widely availability of nuclear reactors, neutron activation is preferred in radionuclide production. Samarium-153 (^{153}Sm) has been well-known as a therapeutic beta agent in palliative treatment of bone metastasis. It is administered in the form of Samarium Lexidronam through a single intravenous injection of about 18 to 37 MBq per kg. The ^{153}Sm has also been used in several pharmacoscintigraphy studies and it has potential to be used as an imaging agent for gastrointestinal gamma imaging (6, 8). In view of the above, ^{153}Sm could be a potentially suitable as alternative to ^{90}Y.

Therefore, this study was taken to develop a microsphere with ^{153}Sm encapsulated as alternative to ^{90}Y microspheres for hepatic radioembolization. The use of poly lactic acid (PLA) microspheres to encapsulate the ^{153}Sm could combine the biocompatible, biodegradable and near plasma density characteristics of PLA with the neutron-activated and theranostic properties of ^{153}Sm for hepatic radioembolization.

2. Materials and Methods

2.1. Materials

Poly (L-lactic acid) (PLLA), samarium acetylacetonate, polyvinyl alcohol (PVA) and chloroform were procured from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade purity. Deionized water was used in all the experiment unless otherwise stated.

2.2. Synthesis of PLLA and Sm-labelled PLLA Microspheres

The Sm-labelled PLLA microspheres were synthesized using oil-in-water solvent-evaporation method. Briefly, 1.0 g of PLLA and 1.75 g of samarium acetylacetonate were dissolved in 50 mL of chloroform. The solution mixture was then added dropwise into 600 mL of 3% (w/v) PVA solution under stirring at 950 rpm with an overhead stirrer. The stirring process was continued for at least 12 h at room temperature for the evaporation of the chloroform. The Sm-labelled PLLA microspheres was collected through vacuum filtration. The collected microspheres was then washed three times with distilled water and 0.1 M HCl to dissolve the free samarium acetylacetonate. The PLLA microspheres was prepared following the same procedure without the samarium acetylacetonate. The microspheres were then dried in vacuum oven at 35°C for 48 h and kept at –20°C for further analysis.

2.3. Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-ray (EDX) Spectroscopy

Structural observations and validation of chemical compositions of the PLLA and Sm-labelled PLLA microspheres were made using Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-ray (EDX) spectroscopy, respectively, on a FESEM system (Quanta FEG 450, FEI, Oregon, USA).

2.4. Particle Size Distribution

The mean particle size and particle size distribution were measured by a laser scattering particle size analyzer (Microtrac X100, Honeywell, USA). Aliquots of PLLA and Sm-labelled PLLA microspheres were dispersed in distilled water by ultrasonication, which was then loaded into the particle size analyzer.

2.5. Neutron Activation

Neutron activation of the Sm-labelled PLLA microspheres was performed at TRIGA PUSPATI Reactor (RTP) (Triga Mark II, General Atomics, USA) at the Malaysian Nuclear Agency (MNA), which regularly operated at 750 kW power level. Prior to the sample neutron activation, the Sm-labelled PLLA microspheres was transferred into a polyethylene vial (3.15 cm height and 1.4 cm diameter) and the top of the vial was heat sealed to ensure that the sample is safely confined during neutron activation. The heat sealed polyethylene vials were further put into separate polyethylene ampoule of 10 cm height and 3 cm diameter before the neutron activation procedure. The samples were neutron activated using either the pneumatic transfer system (PTS) or rotary specimen rack (RR) (6). The samples were left for 48 h after neutron activation to allow for complete decay of the short-lived contaminate radionuclides. The activity of the activated ^{153}Sm-labelled PLLA were determined using a dose calibrator (CRC 25R, Capintec, USA).

2.6. Gamma Spectrometry

Gamma spectrometry was made on the samples after 24 and 48 h after neutron activation on a coaxial, p-type hyper-pure germanium detector (Canberra, Meriden, USA). Each sample was counted for 5 min live time at a calibrated distance. The presence of radionuclide impurities, especially the long-lived radionuclides were determined using gamma spectrum analysis software (GenieTM 2000 Ver. 3.2, Canberra, USA). Each sample was counted at 18 cm distance from the detector so that the detection yield was less than 20% for minimal dead time effect.

2.7. In Vitro Stability of the ^{153}Sm-labelled Microspheres

The Sm-labelled PLLA microspheres were activated via PTS for 5 min. The activated ^{153}Sm-labelled PLLA microspheres were transferred into the glass tubes containing 10 mL of saline at a concentration of 2.5% (w/v). The tubes containing the activated microspheres were mixed at 50 rpm for 1 h. The samples were then centrifuged at 2000 rpm for 10 min and 1 mL of the supernatant was transferred into a separate gamma assay tubes. The procedure was continued until a total of 8 mL of supernatant obtained over a period of 550 h. The activity of the supernatant was assayed using a gamma scintillation counter (2470 Wizard2, PerkinElmer, USA). The experiment was repeated in human blood plasma, which was obtained from the Department of Transfusion Medicine, University Malaya Medical Centre (UMMC), Malaysia. Medical ethics approval was not required with reference to Scope 2.1.3, Standard Operating Procedure (SOP) of UMMC Medical Ethics Committee, since no personal identity information of the donor was acquired for this research. Labelling efficiency of each formulation was calculated using the following equation:

$$\textbf{Retained activity}\ (\%) = \frac{A_{sus} - A_{sup}}{A_{sus}} \times 100\%$$

where;

A_{sus} = Activity of microspheres suspension before each extraction of 1 ml supernatant

A_{sup} = Activity of 1 ml supernatant

3. Results

The oil-in-water solvent evaporation method was employed to synthesize the Sm-labelled PLLA microspheres with chloroform as solvent and samarium acetylacetonate as the source of ^{152}Sm. The morphology of the PLLA microspheres and Sm-labelled PLLA microspheres were analysed using FESEM. **Figure 1** shows the SEM images of the PLLA microspheres and Sm-labelled PLLA microspheres. As can be seen from **Figure 1**, the PLLA microspheres without the addition of samarium acetylacetonate was found to be spherical. Addition of samarium acetylacetonate to the PLLA microspheres does not seems to affect the particles shape, in which, the microspheres still remained spherical even at high samarium acetylacetonate loading (175% samarium acetylacetonate). The average size and size distribution of the PLLA microspheres and Sm-labelled PLLA microspheres were determined using particle size analyser and the results are given in **Figure 2**. The PLLA microspheres and Sm-labelled PLLA microspheres were within 20 to 60 µm with the average size of the microspheres at 34.35 ± 10.22 µm and 37.61 ± 9.32 µm for PLLA microspheres and Sm-labelled PLLA microspheres, respectively.

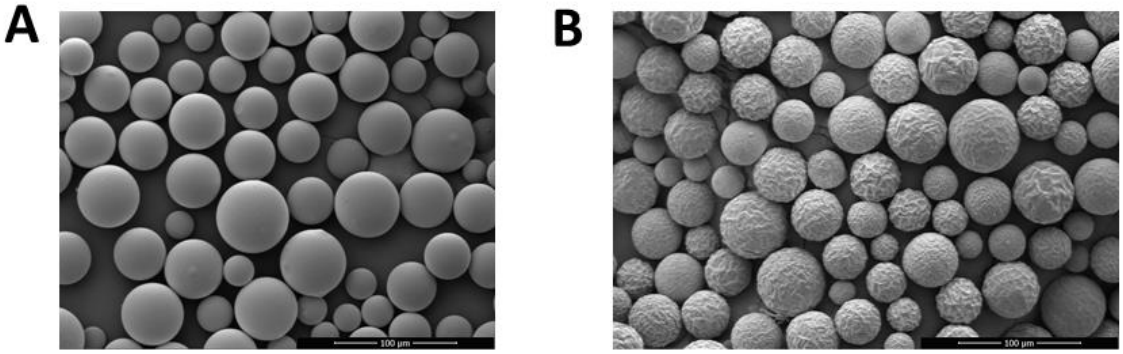

Figure 1. Scanning electron microscope images of (A) PLLA microspheres and (B) Sm-labelled PLLA microspheres.

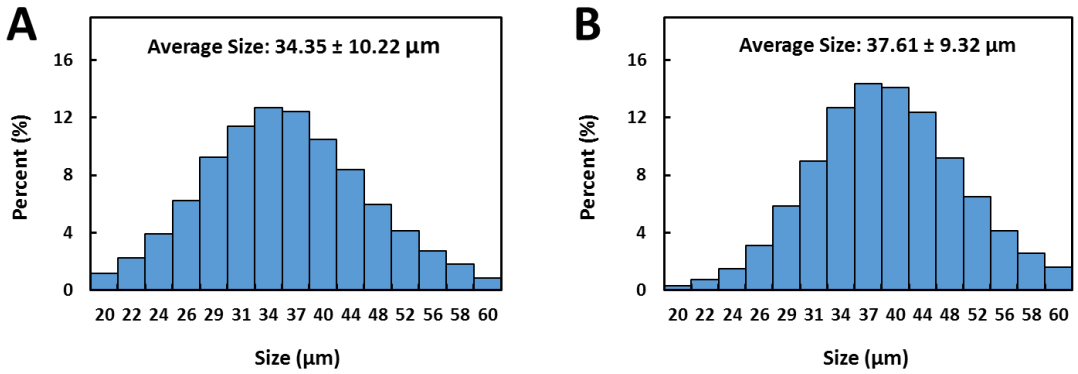

Figure 2. Particle size distribution of (A) PLLA microspheres and (B) Sm-labelled PLLA microspheres.

The elemental composition of the PLLA microspheres and Sm-labelled PLLA microspheres was determined using EDX spectroscopy. The PLLA microspheres has been shown to contains elements carbon (C), oxygen (O) while the hydrogen (H) could not be detected as the EDX spectroscopy is only able to detect chemical element with atomic number higher than 6. On the other hand, the samarium (Sm) was detected in the Sm-labelled PLLA microspheres, suggesting the presence of samarium

acetylacetonate in the Sm-labelled PLLA microspheres. The percentage of the samarium content present in the Sm-labelled PLLA microspheres was found to be 9.4%.

Gamma spectrometry was carried out on the Sm-labelled PLLA microspheres at 24 and 48 h after neutron activation to determine the presence of radionuclide impurities. Gamma spectrum of the samples shown a few photopeaks at 103.1 ± 0.2 keV, 69.4 ± 0.2 keV, 40.7 ± 0.2 and 46.5 ± 0.2 keV. Among these different peaks, the most dominant peak at 103 keV and smaller peak at 69.4 keV were the principal gamma energy emitted by ^{153}Sm. On the other hand, the photopeaks at 40.7 and 46.5 keV were resulted from the K-shell characteristic X-rays following radioactive decay. Overall, no radionuclide impurity was observed in the Sm-labelled PLLA microspheres at 24 and 48 h after neutron activation. A specific activity per gram of 5.91 ± 0.04 GBq.g^{-1} was achieved with the Sm-labelled PLLA microspheres after 6 h neutron activation with RR method. This is corresponded to 2.88 ± 0.03 GBq.g^{-1} after storage for one half-life of the ^{153}Sm (~46 h). The specific activity was found approximate to the initial target of 3 GBq for liver radioembolization. Radiolabelling efficiency of the Sm-labelled PLLA microspheres in saline and human blood plasma are given in **Figure 3**. The radiolabelling efficiency of Sm-labelled PLLA microspheres were excellent in both saline and blood plasma (99.7 and 99.5%, respectively) over 550 h.

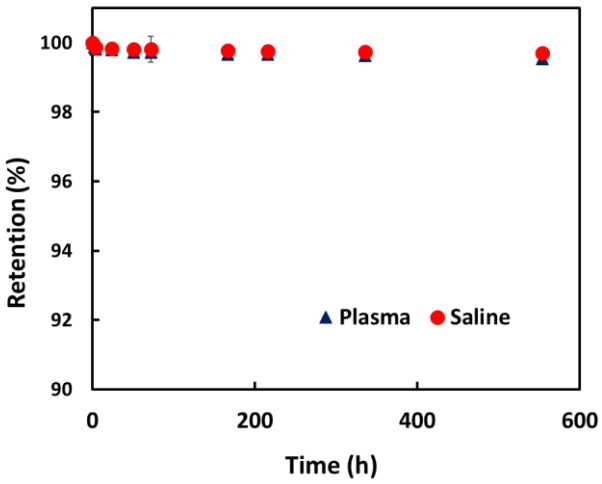

Figure 3. Percentage retention of ^{153}Sm on Sm-labelled PLLA microsphere in saline and human blood plasma over 550 h.

4. Discussion

The ^{152}Sm with a neutron capture cross section of 210 barns has been encapsulated into PLLA microspheres at high concentration under non-hazardous conditions. The ^{153}Sm is produced by neutron activation of a rare earth lanthanide, ^{152}Sm. In spite of the complex decay scheme, which includes various beta particles (E_{max} = 0.632, 0.702, 0.805 MeV), this reactor-produced radiolanthanide also emits low energy gamma radiation (E = 0.103 MeV, 28% abundance), which is well-suited for gamma scintigraphic imaging (8). The ^{153}Sm has a physical half-life of 46.3 h and its average and maximum beta particle ranges in water are 0.5 mm and 3.0 mm, respectively. These characteristic made the ^{153}Sm a suitable alternative to the ^{90}Y.

Unlike the permanent implant of glass-based TheraSphere® and resin-based SIR-Spheres®, the PLLA microspheres is biodegradable. Ideally, the microspheres labelled with the radionuclide in radioembolization should be degraded after the particular radionuclide fully decayed, leaving no permanent embolization in the liver. Thus, the use biodegradable PLLA microspheres as an agent for radioembolization could prevent the formation of anoxic foci for future radiotherapy or chemotherapy and reduces the possibility for neo-vascularization in the resistant tumour tissues (9). The rate of

biodegradation of PLLA microspheres does not appear to be rapid where it shows a high retention of ^{153}Sm on the PLLA microspheres until the isotope's complete decay at approximate 10 half-life of the ^{153}Sm (equivalent to 460 h). A similar in-vivo degradation rate of the PLA microsphere has been reported earlier (10, 11).

The ^{153}Sm-labelled PLLA microspheres developed in this study are potentially useful for hepatic radioembolization due to its biodegradability, favourable radiation characteristics and excellent radiolabelling efficiency. The preparation of the formulation does not involve ionizing radiation and hence reduces the costs of production.

5. Conclusion

The Sm-labelled PLLA microspheres with an average diameter of 37 μm has been successfully developed. The preparation method of the microsphere is relatively easy and does not involve any hazardous procedures. It also does not involve any unnecessary radiation exposure during the sample preparations. Owning to the biodegradability of the PLLA, the microsphere could be fully degraded after the ^{153}Sm fully decayed. The Sm-labelled PLLA microspheres have an excellent retention of ^{153}Sm over 550 h and this lowers the chance of radionuclide leaking to the surrounding organs. In addition, no radioactive impurity was produced from neutron activation of the microspheres. In view of the above, the developed Sm-labelled PLLA microspheres has the potential as an alternative to ^{90}Y microspheres, with added advantage of gamma radiation for imaging.

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Acknowledgments

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