

177Lu-Labeled Amidoxime-Functionalized Biodegradable Microspheres for Transarterial Radioembolization of Hepatocellular Carcinoma*

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Abstract

Transarterial radioembolization (TARE) has become an important locoregional therapy for hepatocellular carcinoma (HCC). However, the clinically used yttrium-90 (^{90}Y) microspheres are non-biodegradable and provide limited imaging capability, while biodegradable polymeric microspheres prepared through conventional radiolabeling strategies often exhibit poor radionuclide stability. Here, we report a biodegradable radiometal-chelating microsphere based on amidoxime-functionalized polylactic acid microspheres (PLA-g-PAO-Ms) engineered via electron-beam-induced graft polymerization. The resulting PLA-g-PAO-Ms exhibit a high Lu 3+ loading capacity (33.83 mg g⁻¹) and undergo controlled biodegradation in vitro after approximately 20 days. In a rat orthotopic live tumor model, intra-arterial administration of ¹⁷⁷Lu-labeled PLA-g-PAO-Ms (¹⁷⁷Lu-PLA-g-PAO-Ms) resulted in pronounced tumor suppression with sustained in vivo radionuclide retention for at least 14 days. Notably, the radiometal chelation strategy enables tunable specific radioactivity of the microspheres through modulation of coordination conditions, offering precise control over therapeutic dose deposition. These findings suggest that PLA-g-PAO-Ms provide a biodegradable microsphere system capable of stable radiometal incorporation and effective tumor suppression following intra-arterial administration, offering a promising approach for interventional radionuclide therapy of HCC.

Full Text

Preamble

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Transarterial radioembolization (TARE) has become an important locoregional therapy for hepatocellular carcinoma (HCC).

However, the clinically used yttrium-90 (⁹⁰Y) microspheres are non-biodegradable and provide limited imaging capability, while biodegradable polymeric microspheres prepared through conventional radiolabeling strategies often exhibit poor radionuclide stability.

Here, we report a biodegradable radiometal-chelating microsphere based on amidoxime-functionalized polylactic acid microspheres (PLA-g-PAO-Ms) engineered via electron-beam-induced graft polymerization. The resulting PLA-g-PAO-Ms exhibit a high Lu³⁺ loading capacity (33.83 mg g⁻¹) and undergo controlled biodegradation *in vitro* after approximately 20 days. In a rat orthotopic live tumor model, intra-arterial administration of ¹⁷⁷Lu-labeled PLA-g-PAO-Ms (¹⁷⁷Lu-PLA-g-PAO-Ms) resulted in pronounced tumor suppression with sustained *in vivo* radionuclide retention for at least 14 days. Notably, the

radiometal chelation strategy enables tunable specific radioactivity of the microspheres through modulation of coordination conditions, offering precise control over therapeutic dose deposition. These findings suggest that PLA-g-PAO-Ms provide a biodegradable microsphere system capable of stable radiometal incorporation and effective tumor suppression following intra-arterial administration, offering a promising approach for interventional radionuclide therapy of HCC.

Keywords

Transarterial radioembolization, Radiation-induced graft polymerization, Biodegradable microspheres, Radiometal chelation, Hepatocellular carcinoma

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1 1 INTRODUCTION

Hepatocellular carcinoma (HCC) remains a major cause of cancer-related mortality worldwide.[1-3] Radical hepatectomy

3 remains the primary curative treatment for HCC and offers the best chance for long-term survival[4]. For unresectable HCC, 4 clinical management relies on alternative therapeutic strategies, including local ablation, transarterial therapies, external beam 5 radiotherapy (EBRT), and systemic anticancer treatments, with the aim of improving survival and disease control.[5]

TARE is an interventional form of internal radiotherapy that has been widely applied in the treatment of hepatic

7 malignancies. TARE provides localized radionuclide retention within the tumor and enables continuous internal irradiation 8 while largely sparing surrounding normal tissues[6]. In addition, owing to the relatively long tissue penetration range of β 9 radiation, TARE can effectively deliver cytotoxic radiation to tumor cells located deep within the lesion, which may provide 10 advantages over transarterial chemoembolization (TACE) in certain hypovascular tumors[7]. Several key challenges remain

11 in the development of TARE microsphere systems. These include efficient

radionuclide loading, stable retention of the radionuclide within tumor tissue, and physicochemical properties that ensure stable suspension and smooth delivery through microcatheters. These parameters critically influence radiation dose distribution and therapeutic efficacy. The ideal microsphere platform should also possess controllable biodegradability. Permanent retention of non-degradable microspheres within the hepatic vasculature may lead to long-term complications such as vascular stenosis or chronic inflammatory responses. Controlled degradation after completion of radionuclide decay could facilitate microsphere clearance from liver tissue, potentially alleviating local hypoxia and reducing hypoxia-driven tumor aggressiveness, while theoretically enabling safer retreatment with subsequent cycles of radioembolization. However, despite increasing research interest in biodegradable radioembolization platforms, no clinically approved microsphere products with predictable and controllable degradation profiles are currently available. Achieving an optimal balance between degradability, radionuclide retention, and therapeutic safety therefore remains a major challenge in the development of next-generation TARE microspheres.[8]

Poly(lactic acid) (PLA) microspheres are widely considered promising carriers for biodegradable radioembolization because

of their favorable biocompatibility and tunable degradation properties.[9] However, radiolabeling strategies for PLA-based microspheres often suffer from limited labeling stability and radionuclide leakage in vitro and in vivo, which has hindered their clinical translation for TARE[10]. Holmium-166 (^{166}Ho)-labeled PLA microspheres represent one of the earliest biodegradable radioactive microsphere systems investigated for TARE. These microspheres are typically produced by encapsulating non-radioactive holmium acetylacetonate ($\text{Ho}(\text{acac})_3$) within poly(L-lactic acid) (PLLA) microspheres through an emulsion-solvent evaporation process, followed by neutron activation to convert ^{165}Ho into ^{166}Ho . [11, 12] The high-energy neutron irradiation required for activation can induce radiolytic degradation of the PLA matrix, leading to polymer chain scission and loss of structural integrity. Such damage to the carrier material increases the risk of radionuclide leakage and unintended systemic radiation exposure.[13]

The physical characteristics of currently used radionuclides also impose important limitations on TARE. ^{90}Y , the clinical

standard for radioembolization, is a pure β -emitter that lacks γ -emission suitable for nuclear imaging, preventing direct visualization of microsphere distribution and accurate in vivo dosimetry using SPECT. ^{166}Ho emits both β -particles and γ -rays

but has a short physical half-life of 26.8 h, which restricts the duration of intratumoral irradiation and complicates clinical logistics. Lutetium-177 (^{177}Lu) provides several advantages for interventional radionuclide therapy. With a physical half-life of 6.7 days, it allows sustained radiation delivery while main-

taining practical flexibility for radiopharmaceutical preparation 38 and administration. Its β - particles ($E_{\max} = 0.497$ MeV) have a tissue penetration range of approximately 2 mm, enabling 39 localized tumor irradiation with reduced exposure to surrounding liver tissue. In addition, the concomitant emission of 208 40 keV γ -rays permits real-time nuclear imaging and quantitative dosimetry using SPECT/CT, supporting image-guided therapy 41 and treatment monitoring. To overcome the limitations associated with conventional radiolabeling strategies, we developed a 42 surface chelation-mediated labeling approach based on amidoxime-functionalized PLA microspheres. Amidoxime (AO) 43 groups were introduced onto the microsphere surface to enable stable coordination with Lu^{3+} ions while preserving the 44 structural integrity of the polymer matrix. Compared with neutron activation-based methods, this strategy enables efficient 45 radiolabeling under mild conditions without radiation-induced damage to the carrier material. The AO ligand exhibits strong

46 affinity for trivalent metal ions and forms stable coordination complexes with Lu^{3+} . Our previous density functional theory 47 (DFT) studies demonstrated that AO groups can generate thermodynamically stable hexadentate chelation structures with 48 favorable enthalpy and Gibbs free energy characteristics.[14, 15] Unlike conventional chelators such as DOTA or EDTA, 49 AO-mediated coordination can be achieved under mild conditions at room temperature, avoiding thermal damage to the 50 microsphere matrix.[16]

In this work, monodisperse PLA microspheres were fabricated using microfluidic technology. Acrylonitrile groups were

52 introduced via electron beam-induced graft polymerization and subsequently converted to amidoxime groups through 53 amidoximation, yielding PLA-g-PAO-Ms. Physicochemical characterization confirmed successful surface functionalization 54 and high ^{177}Lu chelation capacity. The resulting ^{177}Lu -PLA-g-PAO-Ms demonstrated favorable biocompatibility, stable 55 radionuclide retention, and pronounced antitumor efficacy in hepatocellular carcinoma models (Fig. 1 [Figure 1: see original paper]). The γ -emission of 56 ^{177}Lu further enabled non-invasive SPECT imaging of microsphere distribution in vivo, providing intrinsic theranostic 57 capability.

59 Fig. 1 Schematic illustration of the synthesis and anti-tumor application of PLA-g-PAO-Ms. Monodisperse poly microspheres 60 (PLA-Ms) were first generated by via microfluidic technology. Acrylonitrile monomers was then grafted onto the PLA-Ms 61 surface through electron beam-induced graft polymerization, followed by amidoximation reaction to transform the cyano 62 groups in the grafted chains into amidoxime chelating ligands, thus obtaining the target PLA-g-PAO-Ms. The scheme also 63 outlines the workflow of in vivo administration and anti-tumor therapeutic efficacy evaluation of ^{177}Lu -radiolabeled 64 PLA-g-PAO-Ms in a rat orthotopic HCC model. Created in <https://BioRender.com> 65 2 MATERIALS AND METHODS 66 2.1 Materials

PLA (Aladdin, \$ \$99%), Acetone (Aladdin, \$ \$99%), methanol (Aladdin,

\$ \$99%), and N,N-dimethylformamide (DMF, Aladdin,

68 \$ \$99%), Polyvinyl alcohol (PVA, Macklin, \$ \$99%), Acrylonitrile (AN, Aladdin, \$ \$99%), Hydroxylamine hydrochloride 69 ($\text{NH}_2\text{OH} \cdot \text{HCl}$, Macklin, \$ \$99%) and sodium hydroxide (NaOH, Macklin, \$ \$99%) were used as received. ^{177}Lu chloride 70 ($^{177}\text{LuCl}_3$) solution (37 MBq) was used as received. Ultrapure water was used in all experiments. 71 2.2 Synthesis of PLA-g-PAO-Ms

Preparation of PLA Microspheres (PLA-Ms): PLA-Ms were fabricated via microfluidic technology combined with a phase

73 separation method, which enabled the production of PLA-Ms with uniform diameter, regular morphology, and excellent size 74 controllability by tuning the flow rates of the continuous/mobile phases and solution concentration. To prepare the continuous 75 phase, 1 mL of PLA/acetone solution (15 mg mL⁻¹) was mixed with 1.32 mL of methanol, followed by vortex mixing for 10 76 min. Meanwhile, 10 mL of 0.25% (w/v) PVA aqueous solution was prepared as the mobile phase. The as-prepared continuous

77 phase and mobile phase were loaded into syringes respectively, and the flow rate ratio of the two phases was precisely 78 adjusted via the syringe plunger to generate uniformly sized dispersed microspheres suspended in the PVA solution. The 79 resulting PLA-Ms were collected by filtration, and the filtrate was freeze-dried and stored for subsequent characterization and 80 further modification.

Preparation of PLA-g-PAO-Ms: Prior to graft polymerization, PLA-Ms were pre-irradiated in nitrogen at room temperature

82 using an electron beam accelerator (Pioneer Electric Company GJ-2-II, Institute of Applied Radiation, Shanghai University), 83 with a beam energy of 2 MeV, a maximum beam current of 10 mA, and a total irradiation dose of 20 kGy. In the graft 84 polymerization reaction, a monomer solution consisting of 3 mL of AN, 1 mL of AA, and 6 mL of DMF was added to a flask, 85 and deoxygenated for 10 min via nitrogen purging. The pre-irradiated PLA-Ms were subsequently added to the flask, and the 86 graft polymerization reaction was carried out at 57°C for 4

h. Upon completion of the reaction, the resulting grafted product

87 was washed with DMF thoroughly to remove residual monomers and homopolymers. After vacuum freeze-drying, the desired 88 product acrylonitrile-functionalized polylactic acid microspheres (PLA-g-PAN-Ms) was successfully prepared. For the 89 amidoximation reaction, 10 g of $\text{NH}_2\text{OH} \cdot \text{HCl}$ was dissolved in 100 mL of ultrapure water, and the pH of the solution was 90 adjusted to neutral with 5.7 g of NaOH. The as-prepared PLA-g-PAN-Ms were dispersed in the neutral $\text{NH}_2\text{OH} \cdot \text{HCl}$ solution, 91 and the mixture was heated to 60 ° C for 12 h to fully convert the cyano groups into AO functional groups. After the 92 amidoximation reaction was completed, the product was centrifuged at 8000 r min⁻¹, the supernatant was discarded, and the 93 solid was washed repeatedly

with ultrapure water to remove residual $\text{NH}_2\text{OH} \cdot \text{HCl}$ and NaOH . The final product 94 PLA-g-PAO-Ms was obtained after vacuum drying for 24 h followed by freeze-drying. The chemical structure, 95 microstructure, mechanical properties, functional group density and distribution of the obtained materials were characterized 96 correspondingly.

Radiolabeling of PLA-g-PAO-Ms with ^{177}Lu : 1 mL of PLA-g-PAO-Ms dispersion (30 mg mL⁻¹) was mixed with 10 μL of

98 $^{177}\text{LuCl}_3$ solution (37 MBq), and 10 μL of 0.04 M NaOH solution was added to adjust the pH of the mixture to 6 -

7. The 99 mixture was oscillated at 60°C for 30 min, followed by repeated washing and centrifugation to remove unbound ^{177}Lu .

100 2.3 Characterization

Fourier-transform infrared (FT-IR) spectra were recorded on a Fourier-transform infrared spectrometer (Bruker GmbH,

102 Germany) with the KBr pellet method to confirm the chemical structure of PLA-g-PAO-Ms, with a scan range of 4000-500 103 cm^{-1} , a resolution of 4 cm^{-1} and 32 scans per sample.

X-ray photoelectron spectroscopy (XPS) spectra were collected on an X-ray photoelectron spectrometer (Kratos Ltd., UK)

105 with a monochromatic Al $\text{K}\alpha$ X-ray source. Twenty milligrams of PLA-Ms, PLA-g-PAN-Ms, PLA-g-PAO-Ms, and 106 Lu-PLA-g-PAO-Ms were used for the measurements, with the test voltage set to 10 kV, a full-spectrum energy range of 160 107 eV and a scanning range of 0-1200 eV. The pressure of the analysis chamber was maintained at 1×10^{-9} Torr during the test, 108 and the obtained XPS data were analyzed using Thermo Avantage software.

X-ray diffraction (XRD) patterns were recorded on an X-ray diffractometer (Rigaku Corporation, Japan) with Cu $\text{K}\alpha$

110 radiation as the excitation source, a scanning range of 5-80° and a scanning rate of 5° min⁻¹ to characterize the crystalline 111 structure of PLA-Ms, PLA-g-PAN-Ms, and PLA-g-PAO-Ms.

Scanning electron microscopy (SEM) images were acquired on a field emission scanning electron microscope (JEOL Ltd.,

113 Japan) to study the morphological characteristics of PLA-Ms, PLA-g-PAN-Ms, and PLA-g-PAO-Ms. Prior to testing, the 114 microspheres were dried in a vacuum drying oven at 50°C for 6 h, then mounted on sample stubs with double-sided 115 conductive tape and subjected to gold sputtering at a current of 20 mA for 180

- s. The morphological observations were 116 conducted at an accelerating voltage of 5 kV.

Contact angles were measured on a contact angle meter (KSV Instruments Ltd., Finland) for PLA-Ms and PLA-g-PAO-Ms

118 pellets, which were prepared by tableting under 5 MPa pressure for 30

- s. A 50 μL drop of ultrapure water (18.2 M Ω cm) was 119 dispensed onto the sample surface, with video recording to capture images every 50 ms. Contact angles were determined

120 from the images captured at the moment of droplet-sample contact, and measurements were repeated five times at different 121 positions for each sample.

Thermogravimetric analysis (TGA/DTG) was performed on a TA Instruments Discovery Series TGA 550

123 Thermogravimetric Analyzer (TA, USA) for PLA-Ms, PLA-g-PAN-Ms, and PLA-g-PAO-Ms under a high-purity nitrogen 124 atmosphere with a flow rate of 50 mL min⁻¹. The measurements were carried out over a temperature range of 25–800°C at a 125 heating rate of 10°C min⁻¹, the temperature corresponding to the maximum thermal degradation rate (T_{max}) of each material 126 was determined from the DTG curves, and the thermal stability of materials during modification was analyzed by comparing 127 the TGA/DTG curves of PLA-Ms, PLA-g-PAN-Ms and PLA-g-PAO-Ms.

Laser diffraction particle size distribution was determined on a Mastersizer 3000 (Malvern Panalytical Ltd., UK) for

129 PLA-g-PAO-Ms in wet dispersion mode with ultrapure water as the dispersant. The samples were ultrasonicated for 5 min 130 before testing for uniform dispersion, the median particle diameter (D₅₀) and the proportion of particles in specific diameter 131 ranges were obtained, and the uniformity of microsphere size distribution was evaluated from the test results.

Dynamic light scattering (DLS) measurements were conducted on a Brookhaven NanoBrook Omni Nanoparticle Size and

133 Zeta Potential Analyzer (Brookhaven, USA) at a scattering angle of 90° to determine the hydrodynamic diameter of 134 PLA-g-PAO-Ms under simulated physiological conditions. A 5 mL suspension of PLA-g-PAO-Ms (10 mg mL⁻¹) was 135 dispersed in phosphate-buffered saline (PBS, pH 7.4, 0.01 mol L⁻¹) and transferred to sterilized centrifuge tubes, which were 136 incubated in a shaking incubator at 37°C with agitation at 200 rpm for 35 days. An aliquot of the suspension was collected 137 every 5 days, and the particle diameter was analyzed immediately; the average particle size was plotted against degradation 138 time to assess microsphere stability and degradation rate.

Inductively coupled plasma mass spectrometry (ICP-MS) measurements were performed on an Thermo Scientific iCAP TQ

140 ICP-MS (Thermo Scientific, USA) to determine the Lu³⁺ concentration in the supernatant of chelation reaction mixtures, with 141 three parallel replicates

for each sample and all centrifugation steps conducted at 8000 rpm for 5 min. For pH regulation 142 experiments, a 100 mL Lu³⁺ aqueous solution (1 mg L⁻¹) was prepared and its pH adjusted to 5-9, 0.2 mg of PLA-g-PAO-Ms

143 was added and incubated at 37 °C with shaking at 150 rpm, and 1 mL of supernatant was collected at 10, 20, 30, and 60 min 144 then transferred to 2% (v/v) HNO₃ for analysis. For different initial concentration experiments, 100 mL Lu³⁺ solutions (0.1, 1, 145 10 mg L⁻¹) with pH adjusted to 7 were used with the same chelation and testing procedure. For different solid-liquid ratio 146 experiments, 0.1, 0.2, and 2 mg of PLA-g-PAO-Ms were added to 100 mL Lu³⁺ solution (1 mg L⁻¹, pH 7), and the Lu³⁺ 147 concentration was measured at the same time points after chelation. 148 2.4 Cell Counting Kit-8 (CCK-8) Assay

CCK-8 assay was conducted using a PerkinElmer multimode microplate reader (PerkinElmer Inc., USA) to evaluate the

150 cytotoxicity of Lu-PLA-g-PAO-Ms on HepG2 and LO2 cells. HepG2 and LO2 cells in the logarithmic growth phase were 151 digested with trypsin to prepare single-cell suspensions, and the cell density was adjusted to 1 × 10⁴ cells mL⁻¹. The cell 152 suspension was seeded into 96-well cell culture plates at 100 μL per well, with medium-only wells set as blank controls. The

153 plates were pre-incubated in a 5% CO₂ humidified incubator at 37 ° C for 24 h to allow complete cell adhesion. After 154 pre-incubation, the original medium was carefully discarded, and 100

μ L of complete medium containing

155 Lu-PLA-g-PAO-Ms at different concentrations, 500 μg mL⁻¹ LuCl₃ control solution, or blank complete medium was added to 156 each well according to experimental groups. Each concentration was set with 6 replicates. After sample addition, the plate 157 was gently tapped to mix, and incubation was continued under the same conditions. At 48 h and 72 h post-incubation, the 158 plates were removed. CCK-8 reagent was mixed with DMEM basal medium at a 1:9 (v/v) ratio, and 100 μL of the mixture 159 was added to each well under dark conditions to avoid bubble formation. After gentle mixing, the plates were returned to the 160 incubator for 2 h of dark incubation at 37°C. Immediately after incubation, the absorbance values (OD) at 450 nm were 161 measured using the microplate reader, and the raw data were recorded. The cell viability was calculated using the following 162 formula:

Cell viability (%) =

– OD – OD

× 100

where ODb, ODc, and ODt represent the absorbance values of the blank control group, negative control group (blank

164 complete medium), and experimental group, respectively. Statistical analysis of cell viability in each group was performed 165 using GraphPad Prism 8.0 software to evaluate the effects of Lu-PLA-g-PAO-Ms at different concentrations and incubation 166 times on the proliferation of HepG2 and LO2 cells. 167 2.5 Wound Healing Assay

Wound healing assay was performed using a Thermo Scientific EVOS M5000 inverted fluorescence microscope (Thermo

169 Scientific, USA) to assess the effect of Lu-PLA-g-PAO-Ms on the migration ability of HepG2 cells. HepG2 cells in the 170 logarithmic growth phase were prepared as a single-cell suspension at a density of 2.5×10^5 cells mL⁻¹, and seeded into 6-well 171 cell culture plates at 2 mL per well. The plates were cultured under standard conditions until the cells formed a confluent 172 monolayer with a fusion rate of over 90%. Two vertical straight scratches were uniformly made on the cell monolayer in each

173 well using a sterile 200 μ L pipette tip, keeping the tip vertical to avoid uneven scratch width, and the scratch position in each 174 well was consistent. After scratching, the original medium was immediately discarded, and the wells were gently rinsed 3 175 times with sterile PBS to completely remove detached cells. Under the microscope, it was confirmed that the scratch edges 176 were clear without residual floating cells. According to experimental groups, 2 mL of serum-free DMEM medium containing 177 Lu-PLA-g-PAO-Ms at different concentrations was added to each well, with serum-free DMEM medium without 178 microspheres as the blank control group. Each concentration was set with 3 replicates. Immediately after administration, the 179 6-well plate was placed under the inverted phase-contrast microscope, and images of fixed fields of each scratch were 180 captured at 100 \times magnification to record the initial scratch width at 0

- h. The plate was then returned to the incubator for 181 continued culture, and images of the same fields at the same magnification were captured again at 24 h and 48 h 182 post-incubation to record wound healing. ImageJ software was used to measure the scratch width at each time point, and the 183 cell migration distance was calculated as follows: Migration distance = Initial scratch width - Scratch width at the

184 corresponding time point. The migration distances of cells in each group at different time points were statistically analyzed to 185 compare the effects of microspheres at different concentrations on the migration ability of HepG2 cells. 186 2.6 Calcein-AM/PI Live-Dead Cell Staining Assay

Calcein-AM/PI live-dead cell staining assay was carried out using a Thermo Scientific EVOS M5000 inverted fluorescence

188 microscope (Thermo Scientific, USA) to visually evaluate the cytotoxicity of Lu-PLA-g-PAO-Ms. HepG2 cells in the 189 logarithmic growth phase were prepared into single-cell suspensions with a cell density of 1×10^5 cells mL⁻¹ –

1, and seeded into 1906 — well plates at 1 mL per well. The plates were pre — incubated for 24 h in a 5_{2} humidified incubator at 37°C to allow cell 191 adhesion. After pre-incubation, the original medium was discarded, and 1 mL of complete medium containing 192 Lu-PLA-g-PAO-Ms at different concentrations was added to each well according to experimental groups, with complete 193 medium without microspheres as the blank control group. Each concentration was set with 3 replicates, and the plates were 194 incubated for 72 h under the same standard conditions.

At the end of incubation, the medium in each well was carefully collected into a sterile centrifuge tube. The cells were then

196 gently rinsed twice with sterile PBS, and the rinse solution was also collected into the corresponding centrifuge tube to avoid 197 losing floating cells. The collected medium and rinse solution were centrifuged at 1000 r min⁻¹ for 3 min, and the supernatant 198 was discarded. According to the instructions of the live-dead cell staining kit, 5 μ L of concentrated Calcein-AM and PI 199 staining solution were mixed thoroughly with 10 mL of PBS to prepare the staining working solution. 1 mL of the staining 200 working solution was added to each centrifuge tube to resuspend the cell pellet, and the resuspended cell suspension was 201 transferred back to the original corresponding cell culture well. The plate was shaken gently to ensure uniform contact 202 between cells and the staining solution, and the cells were incubated in the dark at 37 °C for 15 min.

After incubation, dual-channel images of the same field were collected under the fluorescence microscope to observe the

204 cell survival status, morphological changes, and death situation, so as to intuitively evaluate the cytotoxicity of the 205 microspheres.

206 2.7 Coagulation Time Determination

Coagulation time determination was performed in 96-well plates to evaluate the effect of Lu-PLA-g-PAO-Ms on blood

208 coagulation. Rabbit whole blood used in the experiment was collected in sodium citrate anticoagulant tubes. 50 μL of PBS, 209 50 μL of physiological saline (9 g L⁻¹), 50 μ L of CaCl₂ solution (0.1 mol L⁻¹), and 50 μ L of Lu-PLA-g-PAO-Ms 210 physiological saline dispersions at different concentrations (50, 100, 250, 500, 1000 μg mL⁻¹) were added to consecutive 211 wells of the 96-well plate, respectively. 50 μL of rabbit whole blood was added to each well. At 5, 10, 20, 30, and 60 min 212 post-incubation, the coagulation process was terminated by rinsing the plate with physiological saline. The plate was 213 repeatedly washed and the liquid was aspirated until the solution became clear (indicating that all soluble blood components 214 had been removed). At the end of the experiment, the coagulation time and blood clot formation of each well were recorded.

215 2.8 Hemolysis Assay

Hemolysis assay was conducted using a multimode microplate reader to evaluate the hemocompatibility of

217 Lu-PLA-g-PAO-Ms. 1 mL of rabbit whole blood was added to a centrifuge tube and centrifuged at 1500 rpm for 5 min. The 218 supernatant was discarded, and 5-10 times the volume of physiological saline was added to the precipitate. The above 219 centrifugation and washing steps were repeated 3 times to prepare red blood cell suspension (RCS). Eight 1.5 mL centrifuge 220 tubes were prepared, and 1 mL of PBS, 1 mL of physiological saline, 1 mL of ultrapure water, and 1 mL of 221 Lu-PLA-g-PAO-Ms physiological saline dispersions at different concentrations (50, 100, 250, 500, 1000 $\mu\text{g mL}^{-1}$) were added 222 to the tubes, respectively. 20 μL of RCS was added to each centrifuge tube, and the tubes were incubated in a constant 223 temperature incubator at 37 °C for 2

- h. After incubation, the supernatant was aspirated from each tube and added to six wells 224 of a 96-well plate (100 μL per well). The absorbance (OD) at 540 nm was measured using a microplate reader. The 225 hemolysis rate was calculated using the following formula:

$$\text{Hemolysis rate (\%)} = \frac{\text{OD}_q - \text{OD}_p}{\text{OD}_n - \text{OD}_p} \times 100$$

where OD_q, OD_p, and OD_n represent the absorbance values of the Lu-PLA-g-PAO-Ms dispersion group, positive control

227 group (ultrapure water group), and negative control group (physiological saline group), respectively. 228 2.9 Experimental Animals and Establishment of Orthotopic N1S1 Hepatocellular Carcinoma (HCC) Rat

229 Model

Specific pathogen-free (SPF) female Sprague-Dawley (SD) rats (100-120

g) were selected as the experimental animals and

231 housed in a clean environment of the Laboratory Animal Center. Rat hepatocellular carcinoma N1S1 cells were cultured in 232 vitro, and the cells were collected when the proliferation concentration reached approximately 1×10^6 cells mL⁻¹. The collected 233 cells were rinsed three times with PBS, and the cell concentration was adjusted to 5×10^7 cells mL⁻¹ to prepare a cell 234 suspension, which was stored on ice for subsequent use.

Rats were anesthetized by intraperitoneal injection of 100 μL of 3% sodium pentobarbital solution. After complete

236 anesthesia, the rats were fixed on the operating table, and abdominal hair was removed with depilatory cream, followed by 237 disinfection of the abdom-

inal skin with iodophor. A transverse incision was made below the xiphoid process with sterile 238 ophthalmic scissors to expose the liver, and the left outer lobe of the liver was gently pulled out of the abdominal cavity with 239 a sterile cotton swab. A 100 μL aliquot of the prepared N1S1 cell suspension was aspirated with an insulin needle and slowly 240 injected into the intrahepatic subcapsular region of the exposed liver parenchyma. After injection, the needle hole was pressed 241 with a cotton swab to prevent cell suspension leakage, and then the syringe was withdrawn. The liver was returned to the 242 abdominal cavity after confirming no bleeding at the needle hole, and the surgical wound was sutured and fully disinfected 243 before the rats were placed back into the breeding cages for routine rearing. Magnetic resonance imaging (MRI) was 244 performed on a 9.4 T small animal MRI scanner at 3 days post-modeling to monitor tumor engraftment, and successful 245 orthotopic HCC model establishment was confirmed by MRI imaging results.

246 2.10 Grouping and Hepatic Artery Administration of Therapeutic Agents

Rats with successfully engrafted orthotopic liver tumors (tumor volume approximately 15 mm³) were randomly divided

248 into four experimental groups with 6 rats per group: normal saline (NS) control group, ¹⁷⁷LuCl₃ group, blank PLA-g-PAO 249 microspheres (PLA-g-PAO-Ms) group, and ¹⁷⁷Lu-labeled PLA-g-PAO microspheres (¹⁷⁷Lu-PLA-g-PAO-Ms) group. All 250 therapeutic agents were administered via hepatic artery intervention, and the specific operational steps were as follows: rats 251 were anesthetized with 3% sodium pentobarbital as described above; the abdominal cavity was opened, and the abdominal 252 artery, hepatic artery, and gastroduodenal artery were gently separated with sterile cotton swabs and forceps; two ligators 253 were placed around the gastroduodenal artery, with the distal end ligated, and a ligator was placed around the abdominal 254 artery for temporary blood flow interruption; a self-made needle was used to puncture the gastroduodenal artery at the 255 upstream of the distal ligation site, and a catheter was inserted into the hepatic artery for agent injection.

The administration dosage for each group was strictly controlled as 100 μL per rat: the NS group was injected with 100 μL

257 of physiological saline; the ¹⁷⁷LuCl₃ group with 100 μL of ¹⁷⁷LuCl₃ solution (11.1 MBq); the blank PLA-g-PAO-Ms group 258 with 100 μL of PLA-g-PAO-Ms suspension (20 mg mL⁻¹); the ¹⁷⁷Lu-PLA-g-PAO-Ms group with 100 μL of 259 ¹⁷⁷Lu-PLA-g-PAO-Ms formulation (20 mg mL⁻¹, 11.1 MBq). After agent injection, the proximal end of the gastroduodenal 260 artery (upstream of the puncture site) was ligated, the ligator around the abdominal artery was removed to restore hepatic 261 artery blood flow, and the abdominal cavity was sutured and disinfected. 262

262 2.11 In vivo Imaging and Tumor Volume Dynamic Monitoring

Single-photon emission computed tomography/computed tomography (SPECT/CT) imaging was performed on a nanoScan

264 SPECT/CT scanner at 7 and 14 days post-administration to evaluate the in vivo biological distribution and radiostability of 265 ^{177}Lu -PLA-g-PAO-Ms. Dynamic MRI monitoring was conducted on the 9.4 T small animal MRI scanner at 7 and 14 days 266 post-administration to assess the tumor growth response to different interventions. Tumor volume was calculated using the 267 formula: $V (\text{mm}^3) = (L \times S^2)/2$, where L represents the maximum diameter of the tumor and S represents the minimum 268 diameter of the tumor, with tumor size measured from the MRI imaging results.

269 2.12 Biodistribution Analysis of Radionuclides

All experimental rats were euthanized at 14 days post-administration for biodistribution analysis. Rats from the $^{177}\text{LuCl}_3$

271 group and ^{177}Lu -PLA-g-PAO-Ms group were selected for tissue collection: heart, liver, spleen, lung, kidney, stomach, 272 intestine, bone, muscle, skin, and tumor tissues were carefully dissected, placed in sterile plastic test tubes, and weighed 273 immediately. The residual radioactive concentration of each tissue sample was detected using a γ -counter, and the radioactive 274 uptake level was calculated as the percentage of injected dose per gram of tissue (%ID g⁻¹) to quantify the in vivo distribution 275 of ^{177}Lu .

All animal experiments were performed in compliance with the standard laboratory animal care protocols, and the

277 experimental procedures were designed to ensure reproducibility with detailed operational specifications.

All experimental data were processed and plotted using Origin 2021 and Graph-Pad Prism 8.0 software. Statistical analysis

279 was performed with an unpaired two-tailed Student' s t-test for two-group comparisons of continuous variables and 280 Kruskal-Wallis ANOVA with Dunn' s multiple comparison test for multiple-group comparisons, with data expressed as mean 281 \pm standard deviation (SD) and $p < 0.05$ considered statistically significant. XPS spectral data were specifically fitted and 282 analyzed using Thermo Avantage software. 283 3 RESULTS AND DISCUSSION 284 3.1 Synthesis and Characterization of PLA-g-PAO-Ms

PLA-g-PAO-Ms were generated through three-steps involving the preparation of PLA-Ms by microfluidic technology,

286 surface functionalization via electron beam-induced graft polymerization, and amidoximation modification (Fig. 1). The 287 microfluidic process yielded microspheres with uniform size distribution and regular morphology. The relevant preparation 288 parameters have been systematically optimized in our previous studies. Electron-beam-induced graft polymerization was used 289 to introduce nitrile-containing chains onto the surface of PLA-Ms under room-temperature, additive-free conditions. 290 Subsequent amidoximation converted these nitrile groups into amidoxime ligands, yielding the target

PLA-g-PAO-Ms and 291 providing coordination sites for radiometal binding. FT-IR confirmed the stepwise surface modification (Fig. 2a [Figure 2: see original paper]). For 292 PLA-Ms, the characteristic peaks observed at 1082 cm⁻¹ and 1748 cm⁻¹ are assigned to the symmetric stretching vibration of 293 the C-O-C ether bond and the stretching vibration of the ester carbonyl (C=O) group, respectively.[17] After 294 irradiation-induced grafting, a new characteristic peak emerged at 2243 cm⁻¹ in the FT-IR spectrum of PLA-g-PAN-Ms, 295 which is attributed to the stretching vibration of the cyano (C N) group, confirming that acrylonitrile was successfully 296 grafted onto the surface of the microspheres. After the amidoximation reaction, the characteristic cyano peak at 2243 cm⁻¹ in 297 the FT-IR spectrum of PLA-g-PAO-Ms completely disappeared. Meanwhile, new absorption peaks appeared at 3401 cm⁻¹, 298 1647 cm⁻¹ and 922 cm⁻¹, which correspond to the stretching vibration of N-H bond, the stretching vibration of C=N bond, and 299 the bending vibration of N-O bond in the amidoxime group, respectively, verifying the successful introduction of the target 300 functional groups.[18] XPS analysis further confirmed the completion of grafting modification and amidoximation reaction. 301 The N 1s characteristic peak at 399.1 eV was observed in the full survey XPS spectra of both PLA-g-PAN-Ms and

302 PLA-g-PAO-Ms, while no such signal was detected in pristine PLA-Ms, indicating that nitrogen-containing groups were 303 successfully introduced onto the microsphere surface via the grafting reaction. The high-resolution N 1s spectrum of 304 PLA-g-PAO-Ms was subjected to deconvolution fitting, and three characteristic peaks were fitted with binding energies at 305 399.33 eV, 399.84 eV and 400.88 eV, which are assigned to C-NH, C-NH₂ and C=N bonds, respectively (Fig. 2b and c). The 306 high-resolution C 1s spectrum further confirmed the presence of chemical structures including O-C-O, -CN, C-H and C=C 307 (Fig. S1a, Supporting Information). XPS analysis was performed on PLA-g-PAO-Ms after Lu³⁺ chelation, and the 308 characteristic double peaks of Lu 4d_{5/2} and Lu 4d_{3/2} were observed at 197.48 eV and 206.43 eV, which confirmed the

309 coordination binding between Lu³⁺ and the amidoxime groups on the microsphere surface (Fig. 2d).

311 Fig. 2 Physicochemical and functional characterization of the microspheres. (a) FT-IR spectra of PLA-Ms, PLA-g-PAN-Ms, 312 and PLA-g-PAO-Ms;(b) XPSsurvey spectra of PLA-Ms, PLA-g-PAN-Ms, PLA-g-PAO-Ms, and Lu-loaded PLA-g-PAO-Ms 313 (Lu-PLA-g-PAO-Ms);(c) High-resolution N 1s XPS spectrum of Lu-PLA-g-PAO-Ms;(d) High-resolution Lu 4d XPS 314 spectrum of Lu-PLA-g-PAO-Ms;(e) TGA curves of PLA-Ms, PLA-g-PAN-Ms, and PLA-g-PAO-Ms;(f) Particle size

315 distribution curve of PLA-g-PAO-Ms;(g) Static water contact angle profile of PLA-Ms;(h) Static water contact angle profile 316 of PLA-g-PAO-Ms;(i) Optical photograph of the dispersibility of PLA-Ms and PLA-g-PAO-Ms in aqueous solution;(j) 317 Particle size variation curve of PLA-g-PAO-Ms during in vitro

degradation;(k) Time-dependent Lu³⁺ loading capacity of 318 PLA-g-PAO-Ms at different initial Lu³⁺ concentrations;(l) In vitro radiochemical stability of ¹⁷⁷Lu-PLA-g-PAO-Ms.

TGA was performed to characterize the thermal stability changes of the microspheres during the stepwise modification (Fig.

320 2e). For pristine PLA-Ms, the T_{max} was determined to be 460 °C. After irradiation-induced graft polymerization, a distinct 321 decrease in T_{max} to 330 °C was observed for the obtained PLA-g-PAN-Ms, corresponding to the successful grafting of 322 acrylonitrile on the microsphere surface. Following the amidoximation modification, the thermal stability of the final product 323 PLA-g-PAO-Ms further declined, with an onset decomposition temperature of approximately 180 °C. Although the stepwise 324 surface modification reduced the overall thermal stability of the microspheres, the thermal degradation behavior of the PLA

325 matrix backbone was not fundamentally altered, which still meets the thermal stability requirements for room-temperature 326 radiolabeling and subsequent interventional therapy. SEM observations show that both PLA-Ms and PLA-g-PAO-Ms exhibit 327 a regular spherical morphology and smooth surface structure (Fig. 3 [Figure 3: see original paper]). Laser particle size analysis (wet dispersion mode) 328 results show that the median particle size (D₅₀) of PLA-g-PAO-Ms is 33.73±\$0.03 μm, with more than 90% of the 329 microspheres presenting a particle size distribution in the range of 20–80 μm (Fig. 2f), which meets the particle size 330 requirements of embolic microspheres for TARE therapy.[19] In addition, the dispersion test confirms that the microspheres 331 can be stably dispersed in PBS without obvious aggregation (Fig. 2l). WCA tests showed that the WCA of PLA-g-PAO-Ms 332 significantly decreased from 119.1° for PLA-Ms to 15.9° (Fig. 2g and h). The remarkable reduction in contact angle confirms 333 that amidoximation modification can greatly improve the surface hydrophilicity of the microspheres. In vitro degradation 334 assays of PLA-g-PAO-Ms were carried out in a 10% fetal bovine serum (FBS)-containing buffer system under 335 physiological-mimicking conditions (37 °C, 100 rpm). As shown in Fig. 2(j), the as-prepared PLA-g-PAO-Ms initiated the 336 degradation process on the 20th day of incubation, and achieved a mass loss rate of 72% (corresponding to 28% residual mass) 337 at day

35. Given that the physical half-life of ¹⁷⁷Lu is 6.7 days, its radioactivity will decay to less than 5% of the initial level 338 after 5 half-lives (approximately 33.5 days). Noteworthily, PLA-g-PAO-Ms maintained excellent structural integrity during 339 the entire effective therapeutic irradiation window (the first 20 days after administration), which can effectively avoid 340 premature leakage of the loaded radionuclide and its subsequent non-specific systemic distribution, thus guaranteeing the 341 biosafety of the carrier during the whole treatment cycle. [20]

343 Fig. 3 SEM images and EDS mapping of PLA-Ms (a), PAN-g-PLA-Ms (b),

and PAO-g-PLA-Ms (c).

To optimize the Lu³⁺ chelation efficiency of PLA-g-PAO-Ms, the effects of key parameters including solution pH,

345 solid-to-liquid ratio, reaction time and initial Lu³⁺ concentration on the chelation capacity were systematically investigated. In 346 the pH range of 5 to 9, the chelation capacity increased with increasing pH, with values of 2.19, 2.28, 2.32, 3.06 and 3.60 mg 347 g⁻¹, respectively (Fig. S1b). The significantly improved chelation efficiency at pH 8-9 can be attributed to two factors: first, 348 the formation of a Lu(OH)₃ precipitate can be avoided at pH < 9; second, the deprotonation degree of amidoxime groups 349 increases under alkaline conditions, which enhances their chelating affinity for Lu³⁺ ions. Kinetic studies under different pH 350 conditions showed that the chelation capacity increased with prolonged reaction time and reached equilibrium within 30 351 minutes (Fig. S1c). The equilibrium chelation capacities at pH 5, 7 and 9 were 2.20, 2.32 and 3.60 mg g⁻¹, respectively, with 352 corresponding Lu³⁺ utilization efficiencies of 44.6%, 47.2% and 73.1%, respectively. Solid-to-liquid ratio optimization 353 experiments showed that the Lu³⁺ chelation capacity per unit mass of microspheres increased correspondingly with 354 decreasing solid-to-liquid ratio (0.1, 0.5 and 2 mg mL⁻¹) (Fig. S1d), and chelation equilibrium was reached within 30 minutes 355 for all groups. The chelation capacity of PLA-g-PAO-Ms exhibited a positive correlation with the initial Lu³⁺ concentration 356 (Fig. 2k). When the Lu³⁺ concentrations were 0.1, 1 and 10 ppm, the corresponding equilibrium chelation capacities were 357 0.12, 3.49 and 33.83 mg g⁻¹, respectively.

The reported specific activity of good manufacturing practice (GMP)-grade 177Lu varies with the production reactor,

359 ranging from approximately 740 to 2960 GBq mg⁻¹. [21] Based on the maximum chelation capacity (33.83 mg g⁻¹) measured 360 in this study and the above clinical specific activity data, the theoretical 177Lu radioloading capacity of 1 mg PLA-g-PAO-Ms 361 can reach 24.7-98.8 GBq, which fully meets the requirements for a single clinical therapeutic dose (0.5-3 GBq). In 362 comparison, the radioloading capacity of commercially available clinical-grade 90Y glass microspheres is approximately

363 0.05-0.1 GBq mg⁻¹, while that of resin microspheres is only 0.002 GBq mg⁻¹, indicating that PLA-g-PAO-Ms have significant 364 advantages in radionuclide loading capacity. In vitro stability studies of 177Lu-PLA-g-PAO-Ms in PBS and 10% FBS showed 365 that the 177Lu retention rates in both media were above 98.9% after 10 days of incubation at 37 °C (Fig. 2l). The above results 366 indicate that 177Lu-PLA-g-PAO-Ms exhibit excellent radiochemical stability and physiological stability, ensuring the 367 continuous and stable emission of radiation energy from 177Lu during TARE therapy, which lays a critical foundation for its 368 clinical translation. In summary, we successfully fabricated PLA-g-PAO-Ms with well-defined structure and controllable 369 performance, which

exhibit high Lu³⁺ chelation capacity and excellent labeling stability, providing a qualified carrier material 370 for subsequent biological evaluation. [20, 22],[23] 371 3.2 Cytocompatibility and Hemocompatibility of Lu-PLA-g-PAO-Ms

Cytocompatibility is a core metric for evaluating the biosafety of carrier materials used in TARE. [24] To systematically

373 assess the cytocompatibility of Lu-PLA-g-PAO-Ms, HCC HepG2 cells and human normal hepatic LO2 cells were selected as 374 in vitro models, and comprehensive analysis was performed using a battery of assays including plate colony formation assay, 375 CCK-8 proliferation assay, scratch wound healing assay, and live/dead cell staining assay. The results of the plate colony 376 formation assay showed no significant differences in the colony number and morphological characteristics of HepG2 cells 377 between the control group and groups treated with gradient doses of Lu-PLA-g-PAO-Ms, with no statistically significant 378 difference ($p > 0.05$, Fig. 4a [Figure 4: see original paper] and b). Colony formation ability reflects the long-term proliferation potential and self-renewal 379 capacity of cells. The above results indicated that the microspheres exerted no inhibitory effect on the colony formation 380 process of HepG2 cells within the tested dose range, suggesting that the matrix material of the microspheres did not 381 significantly interfere with cell proliferation behavior, providing an experimental basis for subsequent in vivo safety 382 assessment.

384 Fig. 4 Experimental results obtained under different concentrations of Lu-PLA-g-PAO-Ms: (a) Representative images of 385 colony formation assay; (b) Quantitative statistical analysis of colony formation assay; (c) Quantitative statistical results of 386 CCK-8 cell viability assay; (d) Quantitative analysis of cell migration rate from scratch wound healing assay; (e) 387 Representative images of scratch wound healing (cell migration) assay; (f) Representative images of live/dead cell viability 388 staining assay; (g) Results of in vitro blood coagulation assay; (h) Visual images of hemolysis assay; (i) Quantitative 389 statistical analysis of hemolysis assay.

The CCK-8 proliferation assay further confirmed the favorable cytocompatibility of the microspheres. After LO2 cells were

391 co-cultured with different concentrations of Lu-PLA-g-PAO-Ms for 72 h, the cell viability of each group exceeded 90%; the 392 viability of HepG2 cells also remained above 90% at 48 h and 72 h, with no statistically significant difference from the 393 control group ($p > 0.05$, Fig. 4c). As a control, treatment with free Lu³⁺ ($50 \text{ mg} \cdot \text{L}^{-1} \text{ LuCl}_3$) for 72 h significantly reduced the 394 viability of HepG2 cells to 51.4% ($p < 0.001$). This result was consistent with the conclusion from the in vitro release assay of 395 Lu-PLA-g-PAO-Ms that the microspheres can stably chelate Lu element, indicating that Lu-PLA-g-PAO-Ms exhibited 396 favorable in vitro cytocompatibility and providing preliminary experimental support for subsequent in vivo biosafety 397 evaluation.

The scratch wound healing assay showed no significant difference in cell migration rates between the control group and

399 groups treated with different concentrations of Lu-PLA-g-PAO-Ms at 24 h and 48 h ($p > 0.05$, Fig. 4d and e), suggesting that 400 the microspheres did not affect the intrinsic migratory capacity of HepG2 cells. The live/dead staining results further 401 supported the above conclusion. After co-culturing with different concentrations of microspheres for 48 h and 72 h, the cell 402 population was dominated by Calcein-AM-positive (green, viable) cells, while PI-positive (red, dead) cells were extremely 403 rare (Fig. 4f), with no evidence of impaired cell membrane integrity.

Multiple in vitro assays, including plate colony formation assay, CCK-8 proliferation assay, scratch wound healing assay,

405 and live/dead cell staining, consistently demonstrated that Lu-PLA-g-PAO-Ms exhibited favorable cytocompatibility within 406 the tested dose range.

Given that embolic carriers are in direct contact with blood during clinical TARE procedures, hemocompatibility is a

408 prerequisite for their translational application.[25] The hemocompatibility of Lu-PLA-g-PAO-Ms was systematically

409 evaluated by measuring the in vitro coagulation time and hemolysis rate using whole blood from New Zealand White rabbits 410 (all animal experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of our 411 institution). The coagulation assay showed that after co-incubation of microsphere suspensions at different concentrations 412 with rabbit whole blood, there was no statistically significant difference in coagulation time between each experimental group 413 and the normal saline (NS) control group ($p > 0.05$, Fig. 4g), indicating that Lu-PLA-g-PAO-Ms did not activate the 414 coagulation system. The hemolysis assay showed that after co-incubation of microspheres at different concentrations with 415 rabbit erythrocytes at 37°C for 2 h, the supernatant of each group was clear and transparent (Fig. 4h), and the hemolysis rate 416 of each group was below 5% (Fig. 4i), meeting the requirements of ISO 10993-4 for blood-contacting materials.[26] The 417 favorable hemocompatibility can effectively prevent microsphere-induced adverse reactions such as thrombosis and 418 hemolysis, ensure successful embolization of the microspheres into the tumor microvasculature, and guarantee the safety of 419 TARE procedures. 420

3.3 Radiostability and efficacy of ^{177}Lu -PLA-g-PAO microspheres

Due to the well-validated excellent in vitro performance of ^{177}Lu -PLA-g-PAO-Ms, we constructed an orthotopic N1S1

422 HCC rat model to validate the in vivo radiostability, tumor targeting capability, and anti-tumor therapeutic efficacy of 423 ^{177}Lu -PLA-g-PAO-Ms (Fig. 5a [Figure 5: see original paper]). The orthotopic HCC rat model was established via intrahepatic subcapsular injection of 424 N1S1 cells, and successful tumor engraftment was confirmed by MRI. Tumor-bearing rats were randomly divided into four 425 groups ($n = 6$ per group): NS control group, $^{177}\text{LuCl}_3$

solution group, blank PLA-g-PAO-Ms embolization group, and ^{177}Lu -PLA-g-PAO-Ms treatment group, with all interventions administered via the hepatic artery. SPECT/CT imaging was performed on day 7 and day 14 post-treatment to evaluate the in vivo distribution and radiostability of the microspheres (Fig. 428 [Figure 428: see original paper] 5d). Representative SPECT/CT images of the $^{177}\text{LuCl}_3$ group showed a significant systemic spread of radioactive signals throughout the body over time, with predominant ^{177}Lu accumulation in the skeleton. Conversely, representative SPECT/CT images of the ^{177}Lu -PLA-g-PAO-Ms group showed that radioactive signals were highly restricted to the tumor lesions, with no detectable off-target radionuclide migration observed at both day 7 and day 14 post-administration. This indicates that ^{177}Lu -PLA-g-PAO-Ms possess excellent in vivo radiostability and tumor targeting capability, which is attributed to the stable

chelation of PLA-g-PAO-Ms with ^{177}Lu , effectively hindering the off-target diffusion of radionuclides and reducing the risk of unnecessary radiation exposure to non-target organs and tissues. MRI was performed on day 7 and day 14 post-treatment to monitor the tumor response to different interventions (Fig. 5b). Quantitative MRI analysis showed that tumors in the NS control group and $^{177}\text{LuCl}_3$ group grew continuously over the observation period; although a mild inhibitory trend was observed in the $^{177}\text{LuCl}_3$ group, the difference did not reach statistical significance ($p > 0.05$). Treatment with blank PLA-g-PAO-Ms only partially inhibited tumor growth compared with the NS control and $^{177}\text{LuCl}_3$ groups, which is attributed to the embolization effect of PLA-g-PAO-Ms that blocks the tumor blood supply, thereby slowing down tumor growth. Notably, ^{177}Lu -PLA-g-PAO-Ms yielded a more remarkable anti-tumor effect, with significant tumor growth suppression and even partial tumor regression observed on day 14 post-treatment, exhibiting superior therapeutic efficacy compared with the blank embolization-only group. The tumor volumes in both the blank PLA-g-PAO-Ms group and ^{177}Lu -PLA-g-PAO-Ms

group were significantly smaller than those in the NS control group on day 14 post-treatment ($p < 0.001$).

Fig. 5 Antitumor efficacy and in vivo biological behavior evaluation after hepatic artery administration in rat N1S1 orthotopic hepatocellular carcinoma models. (a) Schematic diagram of the hepatic artery administration procedure and post-treatment evaluation workflow, outlining the establishment strategy of rat orthotopic hepatocellular carcinoma model, the experimental grouping scheme for hepatic artery administration, as well as the serial imaging evaluation and biological detection procedures after administration. Created in <https://BioRender.com> (b) MRI images of tumors in the Saline group, $^{177}\text{LuCl}_3$ group, PLA-g-PAO-Ms group, and ^{177}Lu -PLA-g-PAO-Ms group on day 0, day 7, and day 14 after administration. Red circles indicate the tumor boundaries. (c) Tumor growth curves of the above four experimental groups after administration. (d) Representative SPECT images of the

$^{177}\text{LuCl}_3$ group and $^{177}\text{Lu-PLA-g-PAO-Ms}$ group on day 5 and day 10 after 453 administration. Red circles indicate the tissue uptake sites of ^{177}Lu . (e) In vivo biodistribution profile of the $^{177}\text{LuCl}_3$ group 454 and $^{177}\text{Lu-PLA-g-PAO-Ms}$ group in rat N1S1 orthotopic hepatocellular carcinoma models at 14 days after hepatic artery 455 administration.

Biodistribution analysis performed on day 14 post-treatment further verified the in vivo SPECT/CT imaging results (Fig.

457 5e). The biodistribution results showed that tumor tissues in the $^{177}\text{Lu-PLA-g-PAO-Ms}$ group exhibited the highest 458 radioactive uptake, reaching 8.79 ± 1.74 %ID g-1, with radioactive counts significantly higher than those in normal liver 459 tissue (1 ± 0.54 %ID g-1) and other normal organs and tissues including the heart (0.15 ± 0.11 %ID g-1), spleen (0.65 ± 0.06 %ID g-1), lung (0.38 ± 0.03 %ID g-1), kidney (0.22 ± 0.002 %ID g-1), and blood (0.45 ± 0.03 %ID g-1) ($p < 0.001$). In 461 contrast, the radioactive uptake values of other non-target organs and tissues in the $^{177}\text{Lu-PLA-g-PAO-Ms}$ group remained at 462 an extremely low level, causing no severe radiation damage to normal tissues. However, the $^{177}\text{LuCl}_3$ group showed only 0.30

463 ± 0.28 %ID g-1 of radioactive uptake in tumor tissues, with systemic distribution of radionuclides and significant 464 accumulation in non-target organs, especially the bone, kidney and spleen. This was mainly attributed to the rapid clearance 465 and non-specific protein binding of

Lu, leading to non-targeted retention in normal organs. Therefore,

466 $^{177}\text{Lu-PLA-g-PAO-Ms}$ can effectively avoid off-target radiation damage to normal tissues in vivo, and greatly improve the 467 effectiveness and biosafety of in vivo TARE applications. 468 4 CONCLUSIONS

This work focuses on the critical material challenges encountered in the clinical application of TARE. Currently, clinically

470 used radioactive microspheres generally have inherent limitations including non-degradability, insufficient radionuclide 471 labeling stability, and lack of integrated theranostic capability. To address these issues, we developed PLA-g-PAO-Ms, and 472 systematically evaluated their physicochemical properties, biosafety, and in vivo anti-tumor efficacy as a ^{177}Lu -labeled carrier 473 for TARE. Our results demonstrate that the amidoxime coordination sites on the microsphere surface enable efficient and 474 stable chelation of ^{177}Lu , with a degradation behavior well matched to the physical half-life of ^{177}Lu . This system maintains 475 excellent biocompatibility while exhibiting a significant tumor growth inhibitory effect in hepatocellular carcinoma models. 476 These findings indicate that PLA-g-PAO-Ms provide a novel material design strategy and experimental basis for the 477 construction of biodegradable multifunctional radionuclide carriers for TARE therapy. In terms of preparation methodology, 478 we established a fabrication strategy combining microfluidic fabrication,

electron beam irradiation-induced grafting, and 479 amidoximation functionalization, which addresses key technical bottlenecks in the traditional preparation process of 480 radioactive microspheres to a certain extent. Microfluidic technology enables precise regulation of the particle size and 481 morphology of the microspheres. The obtained PLA-g-PAO-Ms have a median particle size of $33.73 \pm 0.03 \mu\text{m}$, with 482 approximately 90% of the microspheres distributed in the size range of 20 to $80 \mu\text{m}$, which meets the basic requirements for 483 embolic microspheres in TARE therapy, while simultaneously balancing catheter deliverability and tumor microvascular 484 embolization efficiency. Compared with traditional radionuclide labeling methods relying on reactor neutron activation, we 485 adopted electron beam-induced graft polymerization to construct functional sites on the microsphere surface. This approach 486 achieves controllable modification of the material surface under relatively mild conditions and avoids significant damage to

487 the polymer chain structure. This method does not require the additional introduction of chemical initiators, which reduces 488 the risk of impurity introduction to a certain extent, while preserving the inherent physicochemical properties and degradation 489 characteristics of the polylactic acid matrix. Material characterization results further confirm that amidoxime groups can be 490 stably anchored on the microsphere surface and provide effective coordination sites, offering a structural foundation for the 491 stable chelation of radionuclides and thereby improving the stability of radionuclide labeling. Biodegradability is a key 492 feature that distinguishes this carrier from clinically available non-degradable 90Y microspheres, with its degradation process 493 rationally designed to balance both therapeutic efficacy and biosafety. In vitro degradation experiments show that 494 PLA-g-PAO-Ms initiate obvious degradation on approximately day 20 in a simulated physiological environment, with a mass 495 loss rate reaching 72% by day

35. This degradation profile is well matched to the physical half-life of ^{177}Lu . After 496 approximately 3 half-lives of ^{177}Lu (around day 20), its radioactivity has dropped to below 12.5% of the initial value, with the 497 treatment-related radiation energy essentially fully released, at which point the microspheres undergo gradual degradation.

498 This characteristic maintains the structural stability of the microspheres during the radiotherapy window period, thereby 499 reducing the risk of systemic off-target toxicity caused by premature radionuclide release. Meanwhile, it avoids long-term 500 retention of the microspheres in liver tissue, reduces the incidence of potential complications such as long-term vascular 501 stenosis and chronic inflammation, and reserves a certain safety margin for subsequent repeated TARE treatments that may be 502 required. The amidoxime groups on the surface of PLA-g-PAO-Ms endow the material with strong radionuclide chelating 503 capacity and lay a foundation for functional expansion. Experimental results show that the maximum chelating capacity of 504 the microspheres for Lu^{3+} can reach 33.83 mg g^{-1} . Estimated based on the specific ac-

tivity of clinical-grade ^{177}Lu , its 505 theoretical radioloading capacity can meet the dose requirements of a single TARE treatment, and exhibits high drug loading 506 potential. Stability evaluation results demonstrate that the radionuclide retention rate of ^{177}Lu -labeled microspheres remains 507 above 98.9% after 10 days of incubation in physiological media. In vivo SPECT/CT imaging and 14-day biodistribution study 508 both confirm that the radionuclide can be stably retained in the tumor lesion area, with no obvious off-target migration 509 observed, which verifies the stability of the labeling system at both in vitro and in vivo levels. Compared with the limitation 510 of mainstream clinical ^{90}Y microspheres that lack real-time imaging capability, the gamma rays emitted by ^{177}Lu enable 511 non-invasive visualized monitoring during the treatment process via SPECT/CT. Meanwhile, the strong coordination ability 512 of amidoxime groups towards a variety of transition metal radionuclides endows the microspheres with the potential to 513 further construct a co-labeling system of therapeutic and imaging radionuclides, providing new possibilities for the 514 optimization of TARE treatment strategies in different clinical scenarios.

From the perspective of clinical translation, the ^{177}Lu -labeled PLA-g-PAO-*Ms* developed in this work provide a promising

516 candidate carrier for transarterial radioembolization of unresectable hepatocellular carcinoma. There remains a significant 517 unmet therapeutic need for patients with intermediate and advanced hepatocellular carcinoma at present. Clinically used 518 radioactive microspheres have certain limitations in their safety and applicable scope to a certain extent due to their 519 non-degradability and potential risk of radionuclide leakage. On the basis of preserving the synergistic effect of embolization 520 and internal irradiation of TARE technology, the biodegradable microspheres prepared in this study overcome several 521 limitations of existing carrier materials. The material exhibits excellent biocompatibility, stable radionuclide retention

522 capacity, and definite in vivo tumor inhibitory effect, which lays an experimental foundation for subsequent preclinical 523 evaluation in large animal models and systematic toxicological studies, and also provides a new material option for further 524 optimization of treatment strategies for unresectable hepatocellular carcinoma. This study still has certain limitations that 525 need to be further addressed in subsequent work. First, this study only completed the short-term efficacy and safety 526 evaluation in a rat N1S1 orthotopic hepatocellular carcinoma model, and has not yet been validated in large animal models 527 that more closely mimic the clinical application scenario. The rabbit VX2 hepatocellular carcinoma model or canine normal 528 liver model can better simulate the conditions of clinical interventional procedures, and have important reference value for 529 evaluating catheter deliverability, dosimetry distribution, and long-term safety. Second, this study has not performed a direct 530 comparison with widely used clinical commercial ^{90}Y microspheres, thus lacking a systematic assessment of the differences 531 in efficacy and safety between the two. Third, this study mainly verified the chelation and therapeutic efficacy

of ^{177}Lu , while 532 the labeling efficiency and in vivo performance of the amidoxime coordination structure for other common TARE 533 radionuclides such as ^{166}Ho or ^{90}Y still need further investigation. Finally, the in vivo observation period of this study was 14 534 days, and systematic data on the histological response, chronic inflammation, and long-term systemic toxicity that may occur 535 during the complete in vivo degradation cycle of the microspheres are still lacking. This study still has certain limitations that 536 need to be further addressed in subsequent work. First, this study only completed the short-term efficacy and safety 537 evaluation in a rat N1S1 orthotopic hepatocellular carcinoma model, and has not yet been validated in large animal models 538 that more closely mimic the clinical application scenario. The rabbit VX2 hepatocellular carcinoma model or canine normal 539 liver model can better simulate the conditions of clinical interventional procedures, and have important reference value for 540 evaluating catheter deliverability, dosimetry distribution, and long-term safety. Second, this study has not performed a direct 541 comparison with widely used clinical commercial ^{90}Y microspheres, thus lacking a systematic assessment of the differences 542 in efficacy and safety between the two. Third, this study mainly verified the chelation and therapeutic efficacy of ^{177}Lu , while 543 the labeling efficiency and in vivo performance of the amidoxime coordination structure for other common TARE 544 radionuclides such as ^{166}Ho or ^{90}Y still need further investigation. Finally, the in vivo observation period of this study was 14 545 days, and systematic data on the histological response, chronic inflammation, and long-term systemic toxicity that may occur 546 during the complete in vivo degradation cycle of the microspheres are still lacking.

547 Author contributions

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553 Competing interests

554 The authors declare no competing interests.

555 Reference

556 [1] 559 [2] 561 [3] 563 [4] 565 [5] 567 [6] 569 [7] 571 [8] 573 [9] 576 [10] 579
[11] 582 [12] 585 [13] 588 [14] 590 [15] 592 [16] 595 [17]

F. Bray,

M. Laversanne,

H. Sung et al., Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA. Cancer*

J. Clin. 74, 229-263, (2024). <https://doi.org/10.3322/caac.21834>.

R.

L. Siegel, T.

B. Kratzer, A.

N. Giaquinto et al., *Cancer statistics,*

2025. *CA. Cancer*

J. Clin. 75, 10-45, (2025). <https://doi.org/10.3322/caac.21871>.

R.

L. Siegel, A.

N. Giaquinto and

A. Jemal, *Cancer statistics,*

2024. *CA. Cancer*

J. Clin. 74, 12-49, (2024). <https://doi.org/10.3322/caac.21820>.

M. Lian,

Y. Zhang,

J. Huang et al., Lasiodin sensitizes hepatocellular carcinoma cancer to sorafenib via SNARE complex disruption. *Phytomedicine.* 149, 157603, (2025).

M.

E. Abdelsalam,

K. Ahrar, R.

A. Sheth et al., Interventional oncology: a primer for clinicians on the role of ablation and embolization for solid tumors. *CA. Cancer*

J. Clin. 76, e70051, (2026). <https://doi.org/10.3322/caac.70051>.

B. Sangro,

M. Iñarrairaegui and J.

I. Bilbao, Radioembolization for hepatocellular carcinoma. *Journal of Hepatology.* 56, 464-473, (2012). <https://doi.org/10.1016/j.jhep.2011.07.012>.

J.-L. Raoul,

E. Boucher,

Y. Rolland et al., Treatment of hepatocellular carcinoma with intra-arterial injection of radionuclides. *Nat Rev Gastroenterol Hepatol.* 7, 41-49, (2010). <https://doi.org/10.1038/nrgastro.2009.202>.

M.

M. Welling,

N. Duszenko, M.

P. van Meerbeek et al., Microspheres as a carrier system for therapeutic embolization procedures: achievements and advances.

J. Clin. Med. 12, 918, (2023). <https://doi.org/10.3390/jcm12030918>.

R.

J. Mumper, U.

Y. Ryo and

M. Jay, Neutron-activated holmium-166-poly (L-lactic acid) microspheres: a potential agent for the internal radiation therapy of hepatic tumors.

J. Nucl. Med.: Off. Publ. Soc. Nucl. Med. 32, 2139-2143, (1991).

J.

F. Nijsen, A.

D. van Het Schip, M.

J. van Steenberg et al., Influence of neutron irradiation on holmium acetylacetonate loaded poly(L-lactic acid) microspheres. *Biomaterials.* 23, 1831-1839, (2002). [https://doi.org/10.1016/s0142-9612\(01\)00309-x](https://doi.org/10.1016/s0142-9612(01)00309-x).

J.

F. Nijsen, B.

A. Zonnenberg, J.

R. Woittiez et al., Holmium-166 poly lactic acid microspheres applicable for intra-arterial radionuclide therapy of hepatic malignancies: effects of preparation and neutron activation techniques.

Eur.

J. Nucl. Med. 26, 699-704, (1999). <https://doi.org/10.1007/s002590050440>.

A.

G. Arranja, W.

E. Hennink,

C. Chassagne et al., Preparation and characterization of inorganic radioactive holmium-166 microspheres for internal radionuclide therapy. Mater. Sci. Eng.,

C. 106, 110244, (2020). <https://doi.org/10.1016/j.msec.2019.110244>.

S.

W. Zielhuis, J.F.

W. Nijsen,

R. de Roos et al., Production of GMP-grade radioactive holmium loaded poly(L-lactic acid) microspheres for clinical application. Int J Pharm. 311, 69-74, (2006). <https://doi.org/10.1016/j.ijpharm.2005.12.034>.

X. Xu,

H. Chen,

P. He et al., 3D Hollow Porous Radio-Granular Hydrogels for SPECT Imaging-Guided Cancer Intravascular Brachytherapy. Adv Funct Materials. 33, 2215110, (2023). <https://doi.org/10.1002/adfm.202215110>.

X. Xu,

H. Zhang,

J. Ao et al., 3D hierarchical porous amidoxime fibers speed up uranium extraction from seawater.

Energy & Environmental Science.

2019.

X. Xu,

H. Chen,

Z. Zhao et al., Engineering radioactive microspheres for intra-arterial brachytherapy using radiation-induced graft polymerization. *Adv. Funct. Mater.* 33, 2306215, (2023). <https://doi.org/10.1002/adfm.202306215>.

R.

N. Darie-Niță,

A. Irimia,

F. Doroftei et al., Bioactive and physico-chemical assessment of innovative poly(lactic acid)-based biocomposites containing sage, coconut oil, and modified nanoclay. *Int.*

J. Mol. Sci. 24, 3646, (2023).

598 [18] 601 [19] 603 [20] 605 [21] 608 [22] 610 [23] 613 [24] 615 [25] 618 [26]

<https://doi.org/10.3390/ijms24043646>.

X. Xu,

L. Xu,

J. Ao et al., Ultrahigh and economical uranium extraction from seawater via interconnected open-pore architecture poly(amidoxime) fiber.

J. Mater. Chem.

A. 8, 22032-22044, (2020). <https://doi.org/10.1039/D0TA07180C>.

J.

F. Nijsen, A.

D. Schip,

W. Hennink et al., Advances in nuclear oncology: microspheres for internal radionuclide therapy of liver tumours. *Curr. Med. Chem.* 9, 73-82, (2002). <https://doi.org/10.2174/0929867023371454>.

A. Vogel, S.

L. Chan, L.

A. Dawson et al., Hepatocellular carcinoma: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann. Oncol.* 36, 491-506, (2025). <https://doi.org/10.1016/j.annonc.2025.02.006>.

C. Kratochwil, W.

P. Fendler,

M. Eiber et al., EANM procedure guidelines for radionuclide therapy with ¹⁷⁷Lu-labelled PSMA-ligands (¹⁷⁷Lu-PSMA-RLT). *Eur.*

J. Nucl. Med. Mol. Imaging. 46, 2536-2544, (2019). <https://doi.org/10.1007/s00259-019-04485-3>.

H. Cai HW (Cai,

F. Pang FW (Pang,

W. Zhang WJ (Zhang et al., Preparation of ^{131}I -collagen-chitosan microspheres for interventional radionuclide therapy.

J. Nucl. Med. (2016).

J.

J. Zaknun,

L. Bodei,

J. Mueller-Brand et al., The joint IAEA, EANM, and SNMMI practical guidance on peptide receptor radionuclide therapy (PRRNT) in neuroendocrine tumours. Eur.

J. Nucl. Med. Mol. Imaging. 40, 800-816, (2013). <https://doi.org/10.1007/s00259-012-2330-6>.

X. Jiang,

L. Chen and

X. Xu, Advancements in the investigation of radioactive microspheres for brachytherapy. Front.

Bioeng. Biotechnol. 13, 1621418, (2025). <https://doi.org/10.3389/fbioe.2025.1621418>.

Z. Zhao,

Y. Chen,

H. Liu et al., Amidoxime-based radio-microspheres for internal irradiation combined with a checkpoint-blocking nanobody boost antitumor immunity. Nano Today. 57, 102383, (2024). <https://doi.org/10.1016/j.nantod.2024.102383>.

L. Xiao,

Y. Li,

R. Geng et al., Polymer composite microspheres loading ^{177}Lu radionuclide for interventional radioembolization therapy and real-time SPECT imaging of hepatic cancer. Biomater. Res. 27, 110, (2023). <https://doi.org/10.1186/s40824-023-00455-x>.

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