Synthesis and Preliminary Biological Evaluation of $^{177}\text{Lu}$-Labeled Polyhydroxamic Acid Microparticles Toward Therapy of Hepatocellular Carcinoma

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Abstract

Background: Transarterial radioembolization (TARE) represents an effective targeted therapeutic option for hepatocellular carcinoma (HCC), a cancer with high mortality and poor prognosis. The aim of this study was the preparation and preliminary biological evaluation of $^{177}\text{Lu}$-labeled polyhydroxamic acid (PHA) microparticles toward possible use in the therapy of HCC.

Materials and Methods: PHA microparticles were synthesized starting from polyacrylamide. They were characterized by Fourier-transform infrared spectroscopy (FT-IR), visual color test, and laser diffraction particle size analysis. Experimental variables such as reaction pH, amount of PHA microparticles, carrier Lu content, and incubation time were optimized for maximum uptake of $^{177}\text{Lu}$ on PHA microparticles. Stability of $^{177}\text{Lu}$–PHA microparticles was tested in the presence of competing Fe(III) ions in solution. In vitro stability of $^{177}\text{Lu}$–PHA microparticles was evaluated in 0.05 M sodium phosphate solution (pH 7.5), saline, and serum. Bioevaluation studies were performed in normal Wistar rats by intrahepatic artery injection of the $^{177}\text{Lu}$–PHA microparticles.

Results: Successful synthesis of PHA microparticles could be confirmed from the results of FT-IR analysis and visual color test. Laser diffraction-based particle size analysis confirmed median particle size to be 54 $\mu$m, suitable for TARE. Under the optimized conditions, >99% loading of $^{177}\text{Lu}$ on PHA microparticles could be achieved. Even in the presence of high concentration of Fe(III) ions, $^{177}\text{Lu}$ binding to PHA microparticles was stable. $^{177}\text{Lu}$–PHA microparticles exhibited excellent in vitro stability in sodium phosphate solution, saline, and serum up to 5 d at 37°C. In the bioevaluation studies performed in normal Wistar rats, 92.8% ± 3.1% of $^{177}\text{Lu}$–PHA microparticles were retained in the liver at 96 h postinjection without any significant leakage to other organs.

Conclusion: This preliminary study demonstrates the potential of synthesized PHA microparticles as carriers of therapeutic radioisotopes such as $^{177}\text{Lu}$ for treatment of HCC.

Keywords: hepatocellular carcinoma, transarterial radioembolization, polyhydroxamic acid microparticles, $^{177}\text{Lu}$, $^{177}\text{Lu}$–PHA

Introduction

Hepatocellular carcinoma (HCC) has emerged as one of the most common causes of cancer-related mortality globally, and its incidence is expected to increase in the coming decades.1,2 Delayed manifestation of clinical symptoms and absence of dedicated screening programs for specific markers in underprivileged countries mean that majority of the patients are at advanced stages of the disease at the time of diagnosis, rendering them unsuitable for resective...
surgery. Several treatment options have been explored, among which transarterial chemoembolization (TACE) and systemic therapy with sorafenib are considered as the first-line treatments for patients with intermediate and advanced stage disease. Although significant survival benefits could be achieved using these treatment modalities, contraindications and treatment-related toxicities pose serious challenges.

In such a scenario, transarterial radioembolization (TARE) employing microspheres bearing β-emitting radioisotopes is advantageous as it delivers localized radiation dose to the tumor through the hepatic artery. Both TACE and TARE take advantage of the fact that neoangiogenic vasculature arising from tumor growth is primarily supplied by the hepatic artery while the normal liver is served by portal vein. Compared with TACE, TARE can be performed as an outpatient procedure and requires fewer treatment sessions. In terms of quality of life also, TARE is superior to TACE.

The aforementioned benefits of TARE ushered the development of radiopharmaceuticals such as lipiodol labeled with 131I, 90Y, and 188Re, glass microspheres radiolabeled with 32P and 186/188Re, 166Ho-labeled polyactic acid microspheres and 90Y microspheres, among which 90Y microspheres have received prominence in the clinic. Sirtex Medical Limited, Australia, launched SIR-Spheres (90Y-labeled resin microspheres) of 20–60 μm diameter. Alkaline medium at ambient temperature. The reaction was allowed to proceed for 16 h, after which the solution was washed several times with deionized water and air dried.

Fourier-transform infrared spectroscopy (FT-IR) spectra were recorded on an ALPHA-P ATR-FTIR Fourier Transformed Infrared Spectrometer. Particle size measurements were performed on a Cilas 1090 Particle Size Analyzer (Cilas, France). Energy dispersive X-ray fluorescence (ED-XRF) analysis was performed using EX 3600-MXenemetrix EDXRF spectrometer (Jordan Valley AR Ltd., Israel). A well-type Na(Tl) scintillation counter (ECIL, India) was used for radioactivity counting during the radiolabeling experiments.

Normal adult Wistar rats (female, 150–200 g) from the institutional animal house were used for bioevaluation experiments. Six millimeters 31G needles (Becton-Dickinson) were employed to administer the radiolabeled particles during the animal experiments. Ethicon polyglactin surgical sutures (Johnson & Johnson, India) were utilized for postsurgery suturing. Counting of radioactivity retained in animal organs/tissues was performed on a flat-bed Na(Tl)I detector (Harshaw). Animal experiments were carried out after obtaining the approval of the institutional animal ethics committee.

Synthesis and characterization of PHA microspheres

Crosslinked micron-sized polyacrylamide (PAM) was synthesized by free radical polymerization. In the first step, acrylamide monomer and N,N'-methylenebisacrylamide (crosslinking agent) were dissolved in 25 mL of 2-methoxyethanol with continuous stirring. Subsequently, ammonium persulfate initiator was added to the clear solution to initiate polymerization. The reaction mixture (RM) was heated in a controlled manner as its temperature was gradually raised from 25°C to 70°C in 30 min. The polymer powder obtained was further warmed to completely evaporate the solvent. Then it was washed several times with deionized water and air dried. In the next step, the PAM powder was functionalized by reacting with hydroxylamine hydrochloride solution in alkaline medium at ambient temperature. The reaction was allowed to proceed for 16 h, after which the solution was neutralized using 3N HCl. PHA solid thus formed was washed several times with deionized water to ensure complete removal of excess reagents. It was then air dried.

Experimental

Materials and methods

All the chemicals used were analytical reagent grade. Acrylamide, N,N'-methylenebisacrylamide, anhydrous sodium acetate (>99.999% pure on trace metal basis), ferric chloride, lutetium chloride, sodium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from Sigma–Aldrich (Steinheim, Switzerland). Ammonium persulfate, hydroxylamine hydrochloride, sodium hydroxide, and hydrochloric acid were obtained from S.D. Fine Chemicals (Mumbai, India). LuCl3 (24.9 ± 0.6 mCi/μg at end of irradiation) was produced by thermal neutron irradiation of enriched lutetium oxide target (84.6% enriched in 176Lu, >99.99% pure) in the Dhruva reactor, Bhabha Atomic Research Centre (BARC) at a thermal neutron flux of ~1.2 × 1014 n/cm²·s for 21 d.

In such a scenario, transarterial radioembolization (TARE) employing microspheres bearing β-emitting radioisotopes is advantageous as it delivers localized radiation dose to the tumor through the hepatic artery. Both TACE and TARE take advantage of the fact that neoangiogenic vasculature arising from tumor growth is primarily supplied by the hepatic artery while the normal liver is served by portal vein. Compared with TACE, TARE can be performed as an outpatient procedure and requires fewer treatment sessions. In terms of quality of life also, TARE is superior to TACE.

The authors considered polyhydroxamic acid (PHA) microspheres as possible carriers of radiolanthanides owing to their favorable physicochemical characteristics, biocompatibility, and ability to form stable complexes with 177Lu and other radiolanthanides. The authors recently demonstrated the utility of PHA–cellulose-based radioactive skin patches for the treatment of superficial skin tumors. This article describes a synthesis procedure for the preparation of 177Lu–PHA microspheres and their preliminary biological evaluation in Wistar rats to assess their suitability for TARE. To the best of the authors’ knowledge, this is the first report to explore the applications of radiolabeled PHA microspheres for treatment of hepatic malignancies.
Finally, the particles were segregated by repeated grinding and sieving.

The synthesized PHA microparticles were characterized by FT-IR analysis in comparison with the starting material PAM. Furthermore, they were treated with Fe(III) solution for the visual color test. To determine the particle size, ~50 mg of the synthesized PHA microparticles was dispersed in deionized water, the suspension was ultrasonicated, and the particle size was determined using a laser diffraction-based particle size analyzer.

Optimization of radiolabeling with \(^{177}\text{Lu}\)

PHA microparticles were radiolabeled with \(^{177}\text{Lu}\) by incubation in 1 mL of 0.05 M sodium acetate solution in the presence of \(^{177}\text{LuCl}_3\) (37–925 MBq) and the required amount of nonradioactive Lu carrier. The RM was incubated with shaking on a mechanical shaker at ambient temperature. After incubation, it was centrifuged at 2000 rpm for 10 min to separate the supernatant and the particles. Aliquots of the RM and supernatant were measured for radioactivity. The radiolabeled particles were finally suspended in 1 mL of saline.

To obtain maximum uptake of \(^{177}\text{Lu}\) on the PHA microparticles, the following expression was calculated:

\[
\% \text{ Uptake} = \left( \frac{\text{RM counts} - \text{counts in supernatant}}{\text{counts in RM}} \right) \times 100
\]

The \(^{177}\text{Lu}\) bound PHA microparticles were washed several times with normal saline to remove loosely bound \(^{177}\text{Lu}\) radioactivity. The radiolabeled particles were finally suspended in 1 mL of saline.

To obtain maximum uptake of \(^{177}\text{Lu}\) on the PHA microparticles, experimental variables such as reaction pH (pH 3–8 adjusted with 0.1 N HCl or 0.1 N NaOH solutions), amount of \(^{176}\text{Lu}\) carrier (0–1 mg), amount of PHA microparticles (1–20 mg), and incubation time (15 min–2 h) were optimized. Each reaction was performed in triplicates to ensure reproducibility and accuracy of the results.

In vitro stability studies

To determine the stability of \(^{177}\text{Lu}\)-PHA in the presence of competing metal ions, \(^{177}\text{Lu}\)-PHA microparticles were incubated in 1 mL of saline containing Fe(III) ions in concentrations ranging from 50 μg to 500 μg/mL for 5 d at ambient temperature. At regular time intervals, the leaching of \(^{177}\text{Lu}\) out of the radiolabeled PHA microparticles was determined by separating the supernatant and counting the radioactivity associated with it in comparison with the radiolabeled preparation.

In vitro stability studies were also carried out in 0.05 M sodium phosphate solution (pH 7.5), saline, and serum for 5 d at 37°C to estimate the release of \(^{177}\text{Lu}\) from \(^{177}\text{Lu}\)-PHA microparticles containing ~925 MBq of \(^{177}\text{Lu}\). In brief, \(^{177}\text{Lu}\)-PHA microparticles were prepared under the optimized reaction conditions. At periodic intervals, percentage radioactivity released into the sodium phosphate solution, saline, and serum was determined by centrifuging the suspension and counting the radioactivity associated with the supernatant, as described previously.

**ED-XRF analysis of nonradioactive Lu–PHA microparticles**

Nonradioactive Lu–PHA microparticles were synthesized according to the protocol standardized for \(^{177}\text{Lu}\)-PHA. They were subjected to ED-XRF analysis to confirm the presence of lutetium.

**In vivo evaluation in normal Wistar rats**

The localization of \(^{177}\text{Lu}\)-labeled PHA microparticles in the liver of normal Wistar rats and their stability in vivo were assessed. Normal adult female Wistar rats weighing 150–200 g were used for the study. The rats were fasted overnight before the procedure. Viable surgery was performed in aseptic conditions under xylazine–ketamine (10:1)-induced anesthesia, and \(^{177}\text{Lu}\)-PHA microparticles suspended in saline were administered as per the previously reported protocol.

In brief, \(^{177}\text{Lu}\)-PHA microparticles in 50 μL volume (1.85 MBq) were injected slowly into the hepatic blood supply using a 30G 0.5 inch needle. The injection site was gently pressed with a gauze pad to stop any bleeding and the incision was closed with surgical suture. After surgery, the animals were allowed to recover from anesthesia and were kept in normal conditions for the specified periods of incubation (24 and 96 h postinjection [p.i., 4 animals per time point]). The animals were provided with food and water ad libitum and were closely observed during the experimental period for any signs of discomfort or disability. At the end of the respective time points, the animals were euthanized in saturated carbon dioxide atmosphere. Blood was collected through cardiac puncture, the relevant organs/tissues were excised and counted to assess the radioactivity retained. The radioactivity resident in individual organs/tissues was expressed as percentage of the total injected activity (%ID/organ) at the respective time point. Radioactivity resident in the blood, bone, and muscle was calculated by considering that they constitute 7%, 10%, and 40% of the body weight of the animal, respectively.

**Results**

**Synthesis and characterization of PHA microparticles**

PHA microparticles were synthesized as described in the Experimental section. The first step involved the synthesis of PAM from acrylamide monomer, and in the second step, PAM was reacted with hydroxylamine hydrochloride to get the final product. Figure 1 schematically represents the reactions involved in the synthesis. Results of the IR analysis confirmed the synthesis of PHA wherein the IR spectrum of

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**FIG. 1.** Reaction scheme for synthesis of PHA microparticles. PHA, polyhydroxamic acid.
PHA microparticles was compared with that of PAM, as shown in Figure 2. It is evident from the spectra that PAM and PHA have similar backbones. The oxime (-N-OH) functional group in PHA was confirmed by FT-IR analysis, wherein the absorption peak at 960–930 cm\(^{-1}\) in PHA was mainly due to N-O stretching vibration of -C=\(\text{N-OH}\) group (oxime functional group, highlighted in Fig. 2), which was absent in PAM. Absorption peaks at 3345 and 3190 cm\(^{-1}\) correspond to N-H asymmetric and symmetric stretching vibrations, respectively, of amide group. The -NH bending vibration peak appeared at 1122 cm\(^{-1}\). Peaks at 2922 and 1426 cm\(^{-1}\) are due to the stretching and bending vibrations of -CH\(_2\) group (R-CH\(_2\)-NHR). Peak at 1652 cm\(^{-1}\) corresponding to the stretching vibration of C=O (carbonyl group) that usually appears as a doublet in solid state (which is a characteristic of amide) is observed in both PAM and PHA. The IR spectra thus confirm the conversion of amide group of PAM to hydroxamic acid group in PHA.

Furthermore, the synthesis of PHA microparticles was independently confirmed by a visual color test in which the PHA microparticles turned dark brown due to the uptake of Fe(III) ions. This is evident from Figure 3a and b. Figure 3a shows the PHA microparticles while Figure 3b represents Fe(III)-treated PHA microparticles. This color change with Fe(III) ions was not exhibited by the starting material PAM.

Particle size distribution of PHA microparticles, as analyzed on a laser diffraction particle size analyzer, is shown in Figure 4. Results of the analysis revealed median particle size as 54 \(\mu\)m with \(>90\%\) of the particles distributed in the 20–80 \(\mu\)m diameter range.

**Optimization of radiolabeling microparticles with \(^{177}\text{Lu}\)**

To obtain maximum uptake of \(^{177}\text{Lu}\) on the PHA microparticles, experimental variables such as pH of the reaction medium, amount of PHA microparticles, binding capacity of the PHA microparticles for Lu, and incubation time were optimized. Figure 5 depicts the influence of reaction pH on the uptake of \(^{177}\text{Lu}\) on PHA microparticles. It is evident from Figure 5 that maximum uptake of \(^{177}\text{Lu}\) on the PHA microparticles could be obtained in the 4–5 pH range. With further increase in pH, uptake of \(^{177}\text{Lu}\) on the microparticles was found to decrease due to its tendency to form hydroxides under alkaline conditions. In the experiments performed to determine the amount of PHA microparticles required for maximum uptake of Lu, it could be seen that as low as 5 mg of PHA microparticles was sufficient to achieve \(>99\%\) uptake of Lu.

Experiments performed to evaluate the binding capacity of the PHA microparticles for Lu (using nonradioactive Lu carrier and trace \(^{177}\text{Lu}\) radioactivity) showed that up to 100 \(\mu\)g of Lu could bind with 5 mg of the microparticles with near quantitative efficiency. Addition of Lu beyond 100 \(\mu\)g resulted in decreased uptake. Results of these studies are graphically represented in Figure 6. Fifteen minutes of incubation at ambient temperature was adequate to achieve \(>99\%\) uptake of \(^{177}\text{Lu}\) on the PHA microparticles. In the optimized radiolabeling protocol, to 1 mL of 0.05 M sodium acetate solution, 10 \(\mu\)L of nonradioactive carrier \(^{176}\text{Lu}\)Cl\(_3\) solution (1 mg/mL) along with 0.1 mL of \(^{177}\text{Lu}\)Cl\(_3\) solution (37–925 MBq) and mixed well. The solution pH was adjusted to 4–5 with 0.1 N HCl after which 5 mg of PHA microparticles were added to it. The RM was kept shaking for 15 min at ambient temperature. Subsequently, the particles were separated from the supernatant by centrifugation followed by counting, and percentage uptake was calculated as described in the Experimental section.

**In vitro stability studies**

Studies performed to evaluate the stability of binding of \(^{177}\text{Lu}\) to PHA microparticles showed that even in the
presence of high concentrations of Fe(III) ions (50–500 µg), no displacement of Lu(III) ions from radiolabeled microparticles was observed. Results of the stability studies in the presence of 500 µg of Fe(III) ions are given in Table 1.

The $^{177}$Lu–PHA microparticles exhibited excellent in vitro stability in 0.05 M sodium phosphate solution, saline, and serum as shown in Table 1. It is evident from the results obtained that the binding of $^{177}$Lu with the PHA microparticles was strong and irreversible, resulting in minimal release of radioactivity when incubated in sodium phosphate solution, saline, and serum. In sodium phosphate solution, 97.5% ± 0.3% of the $^{177}$Lu radioactivity was associated with the microparticles at 120 h postincubation. At 24 h postincubation in saline, the stability of $^{177}$Lu–PHA was

FIG. 4. Particle size profile of PHA microparticles by laser diffraction analysis.

FIG. 5. Influence of pH of reaction on the yield of $^{177}$Lu–PHA microparticles.

FIG. 6. Influence of carrier Lu on the yield of $^{177}$Lu–PHA microparticles.
99.3% ± 0.2%. At 120 h postincubation also, the stability was not significantly affected at 98.5% ± 0.4%. Transchelation of 177Lu from 177Lu–PHA to serum proteins was also not observed as can be seen readily from the results of serum stability studies. At the end of 5 d of incubation in serum at 37°C, 177Lu–PHA exhibited excellent stability, the value being 97.3% ± 0.2%.

**ED-XRF analysis of nonradioactive Lu–PHA microparticles**

Uptake of Lu on PHA microparticles was independently confirmed by ED-XRF analysis using a Rh target (20.22 keV). The data obtained is depicted in Figure 7. Figure 7a represents the ED-XRF spectrum of PHA microspheres that contains a broad peak because of Rh source. Figure 7b represents the ED-XRF spectrum of Lu-loaded PHA microspheres. Figure 7b contains the broad peak from Rh source along with sharp peaks at 7.64 and 8.70 keV corresponding to Lα and Lβ of Lu, respectively, which corroborates its presence on the PHA microparticles. Since both the characteristic X-rays of Lu are seen in equal intensities, the presence of Lu is established.

**In vivo evaluation in normal Wistar rats**

The 177Lu-labeled PHA microparticles could be administered to all the animals without causing significant bleeding or other complications. All the rats recovered from the surgical procedure within few hours. The animals did not show any signs of distress or impaired mobility till the time of euthanasia. No internal abnormalities attributable to the surgical protocol were noted in the course of animal experiments.

The pattern of in vivo distribution/retention of 177Lu–PHA microparticles in normal Wistar rats is shown in Figure 8, wherein the %ID/organ values for all the major organs/tissues and blood are given for the biodistributions performed at 24 and 96 h p.i. At 24 h p.i., 93.0% ± 2.6% of the radioactivity was localized in liver with minimal accumulation in other organs such as lungs (0.3% ± 0.2%) and bone (0.01% ± 0.01%). Liver remained the major organ of retention (92.80% ± 3.1%) at 96 h p.i. (the maximum period of study), indicating good localization of the 177Lu–PHA microparticles in the liver, which also corroborated their in vivo stability. Any degradation of the radiolabeled microspheres in vivo would have resulted in radioactivity uptake in nontarget organs. Absence of notable amount of radioactivity in lungs (0.20% ± 0.17%), bone (0.02% ± 0.01%), and kidney (0.25% ± 0.20%) even at 96 h injection confirmed the in vivo stability of the preparation. This is crucial from the perspective of nonspecific organ dose and radiation safety of the preparation.

**Table 1. In Vitro Stability of 177Lu–PHA-Microparticles**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>In presence of Fe(III) ions (500 μg) Saline Serum</th>
<th>% Stability of 177Lu–PHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 M sodium phosphate</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>99.6 ± 0.2</td>
<td>99.2 ± 0.2</td>
</tr>
<tr>
<td>48</td>
<td>99.4 ± 0.2</td>
<td>98.9 ± 0.3</td>
</tr>
<tr>
<td>72</td>
<td>99.0 ± 0.3</td>
<td>98.5 ± 0.1</td>
</tr>
<tr>
<td>96</td>
<td>98.7 ± 0.2</td>
<td>98.1 ± 0.4</td>
</tr>
<tr>
<td>120</td>
<td>98.4 ± 0.4</td>
<td>97.5 ± 0.3</td>
</tr>
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PHA, polyhydroxamic acid.

**Discussion**

Over the years, TARE, a form of selective internal radiation therapy employing radiolabeled microspheres, has emerged as a viable therapeutic option for inoperable HCC. Commercially available 90Y microspheres have dominated the field thus far and newer developments on the radioactive microsphere technology are expected to have a far-reaching clinical impact. Although the utility of TheraSphere and

**FIG. 7.** ED-XRF pattern of (a) PHA microparticles (b) Lu-loaded PHA microparticles. ED-XRF, energy dispersive X-ray fluorescence.
There are several reports on the synthesis of chelating polymers containing hydroxamic acid groups employing PAM, polyethyl acrylate, polymethyl acrylate, etc. as starting materials.51,52 In this study, the PAM route was chosen to synthesize PHA microspheres as PAM finds use in various pharmaceutical and biomedical applications.53 The authors aimed to synthesize PHA microspheres for incorporating 177Lu and other radioisotopes such as 166Ho based on the authors’ recent experience on the preparation of 177Lu-loaded PHA-grafted cellulose films as radioactive skin patches for the treatment of superficial skin cancers.40

In this study, synthesis protocol for PHA was optimized to avail PHA microspheres that were characterized by FT-IR analysis and visual color test. Their median particle size was 54 μm. For maximum loading of 177Lu on PHA microspheres, influence of pH of the solution, amount of PHA, capacity of the microspheres for Lu, and the time of incubation were thoroughly investigated. Under the optimized conditions, >99% uptake of the 177Lu radioactivity in solution could be achieved. It was seen that in the pH range of 3–6, >90% of 177Lu radioactivity could be bound to the PHA microspheres. Even at pH 8, ~80% uptake of the 177Lu on the PHA microspheres could be obtained, which translated to a broad pH range for their use. The PHA microspheres also exhibited very high binding capacity for Lu. About 100 μg of Lu could be readily loaded on to 5 mg of the PHA microspheres, which is equivalent to ~2.3–2.5 Ci of 177Lu (considering its specific activity in the range of 23–25 mCi/μg). In this study, in vitro stability of 177Lu–PHA microparticles containing up to 925 MBq of 177Lu could be demonstrated. Radiolytic degradation of the polymeric material in the presence of higher radioactivity levels is planned to be evaluated as a part of future investigations.

The results of this study indicate that facile preparation of therapeutic doses of 177Lu–PHA microparticles is possible using 5 mg of PHA microspheres. The Lu loading capacity of the PHA microspheres would depend on their surface area and chelate density. The percentage conversion of amide group to hydroxamic acid was determined by C, H, N analyses as reported in the authors’ previous publication.54 More than 95% conversion of acid amide to hydroxamic group could be achieved under the optimized reaction conditions.54 Robust and irreversible binding of 177Lu to the PHA microparticles was evident from the results of in vitro stability studies. Even in the presence of high concentrations of Fe(III) ions, there was no displacement of 177Lu ions bound to the PHA microparticles by the Fe(III) ions, confirming strong binding of the 177Lu(III) ions to the PHA microspheres.

In the bioevaluation studies, 177Lu–PHA microparticles showed high retention in liver (92.8 ± 0.3%ID at 96 h p.i.) without any shunting to the lungs. Absence of bone uptake also confirmed the in vivo stability of the preparation as Lu3+ ions released are expected to localize in bone. These results give early indications of the usefulness of 177Lu–PHA microparticles and warrant further investigations of their in vivo behavior in animal models of HCC.

177Lu-labeled microparticles have been proposed as theranostic agents for use in dual isotope preparations with 90Y for radioembolization.55,56 To achieve therapeutic effect akin to the 90Y-based products, higher 177Lu radioactivity may be required as the medium energy β− particles of 177Lu
have a maximum range of 1.5 mm. Reportedly, tumor dose equivalent to 1.82 GBq of $^{90}$Y can be achieved with 4.44 GBq of $^{177}$Lu. It has been also shown that use of $^{177}$Lu-based preparations results in lower dose to the normal liver cells than $^{90}$Y, which is advantageous for sparing the normal liver tissues from radiation. However, owing to the gamma emissions of $^{177}$Lu, radiation dose to the organs in the proximity of the liver throughout the duration of the treatment needs to be ascertained. A Monte Carlo simulation study using Geant4 for radioembolization with a $^{177}$Lu-based product reported that the absorbed doses to the other organs were found to be far below the maximum tolerance limit.

An important advantage of the PHA microparticles reported in this communication is that owing to their similar chemistry, the studies performed with $^{177}$Lu can serve as a template for preparation of similar $^{90}$Y- or $^{166}$Ho-labeled PHA microparticles as their behavior is expected to be similar. Having successfully completed the preliminary evaluation of $^{177}$Lu–PHA microparticles for use in TARE, further studies are envisaged, which include evaluation of stability with higher $^{177}$Lu radioactivity, bioevaluation studies in animal model of liver cancer to assess tumor regression, assessment of toxicity in animals, and dosimetry studies.

**Conclusions**

The objective of preparation of $^{177}$Lu–PHA microparticles and their preliminary bioevaluation studies for possible utility in HCC therapy could be successfully accomplished. The PHA microparticles reported herein can be synthesized by a facile and scalable synthesis protocol using inexpensive starting chemicals and exhibit very high binding capacity for $^{177}$Lu. The $^{177}$Lu-labeled PHA microparticles exhibited excellent *in vitro* stability and favorable *in vivo* behavior. This preliminary study highlights the potential of $^{177}$Lu–PHA microparticles for HCC therapy. However, detailed investigations are warranted to establish their prospects for treatment of hepatic malignancies.

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**Authors’ Contributions**

U.P. was involved in conceptualization of study, experiment design, radiolabeling, and *in vitro* experiments and analysis of data, article preparation, and responsibility of corresponding author. S.S. carried out experimental design, planning, and execution of bioevaluation study as well as analysis of the corresponding data, and article preparation. S.S. performed synthesis and characterization of PHA microspheres. N.G. carried out radiolabeling experiments and assisted in bioevaluation experiments. S.K. was involved in synthesis and characterization of PHA microspheres and article writing related to their synthesis. A.D. was involved in conceptualization of study, article writing, and editing. It is hereby confirmed that all the coauthors have reviewed and approved the article before submission.

**Disclosure Statement**

There are no existing financial conflicts.

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