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Development of ^{166}Ho Poly Lactic Acid Microspheres for Radiosynovectomy

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Abstract: Microsphere and particle technology with selective transport of radiation represents a new generation of therapeutics in radiosynovectomy. ^{166}Ho-PLLA-MS (Poly L-lactic acid microspheres loaded with holmium-166 acetylacetonate) are novel microdevices for intra-cavital radiosynovectomy in this work. ^{166}Ho- PLLA agent was developed and quality controls of the compound were described. ^{165}HoAcAc-PLLA microparticles were prepared by dissolving holmium acetylacetonate and incorporating into PLLA spheres by the solvent evaporation technique. Microspheres were irradiated at TRR (tehran research reactor). The diameter and surface morphology were characterized by particle sizer and SEM (scanning electron microscopy) before and after irradiation. The complex stability, radiochemical purity and *in vivo* biodistribiotion were checked in the final solution up to 3 days. In this study, ^{166}Ho-PLLA spherical particles with a smooth surface and diameter of 5-10 μm were obtained, which were stable *in vitro* and *in vivo* studies. Neutron irradiation did not damage the particles, and heightened activity stimulated radiosynovectomy. No significant leakage of dose from injection site and its distribution in organs was observed up to 3 days for ^{166}Ho-PLLA. The ease with which the PLLA spheres could be made in the optimal size range for later irradiation and their ability to retain the ^{166}Ho made them attractive agents for radionuclide synovectomy.

Key words: Holmium-166, poly L-lactic acid, radiosynovectomy, artrit romatoid, drug delivery.

1. Introduction

Developing new radiosynovectomy agents is of great importance due to the aging of human populations throughout the world and increasing outbreak of inflammatory diseases [1, 2].

In the treatment of rheumatoid arthritis, a surgical, chemical or radiation synovectomy may be done. Surgical synovectomy suffers from the risks of surgery and anesthesia, the need for hospitalization and a prolonged period of rehabilitation, albeit to some extent [2, 3]. In chemical synovectomy highly toxic agents such as osmic acid, alkylating substances such as nitrogen mustards, methotrexate and cobra venom were used initially but were then abandoned because of possible joint tissue damage [4, 5].

A well designed controlled drug delivery system can be regarded as a solution to conventional therapy problems and enhance the therapeutic efficacy of active pharmaceutical compounds. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion; and suchlike approach is utilizing microspheres as carriers for drugs [6, 7].

A promising local radionuclide therapy employs radioactive microspheres for nonoperable group of patients suffering from rheumatoid arthritis. Two ^{90}Y microspheres products are currently commercially available in clinical use: glass-based TheraSpheres and resin-based microspheres [5-8].

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Radionuclide synovectomy consists of the intra-articular administration of a therapeutic radionuclide such as beta emitter radionuclide in colloidal or particulate form to a diseased joint to reduce the inflamed synovium [8, 9]. This procedure was first reported by Fellinger et al. [10] in 1952 and has been used extensively in Europe [11-14].

The selection criteria for radionuclide synovectomy have to include the physical and chemical characteristics of radionuclide and microspheres. The ideal properties for radio labeled micro-spheres or particles for intra-cavital therapy are as: high mechanical stability to resist breakdown, high chemical stability to resist elution of radioactive label, macrophage removal or radiolysis, uniform size, unit density to prevent settling or streaming, relative ease of labeling with high-energy beta particle, low photofraction and intermediate (days) half-life [15].

The particulate material used as vehicle for radionuclides may be based on synthetic or natural biodegradable polymers, glass, resins, plastics, etc. [16]. The first plastic microspheres were labeled with ^{90}Y and showed an unpredictable and catastrophic leaching of yttrium, which brought them into question [17]. This problem was solved later by the use of glass and resin-based microspheres. Although the glass spheres have several advantages, their high density [18] and their non biodegradability are major drawbacks [18-22]. Different resins such as Bio-Rex 70, Cellex-P, Chelex 100, Sephadex SP and AG 50W-X8 have been investigated revealing encouraging results [23]. However, the obvious disadvantages of these systems are their non biodegradability, limited surface adsorption and low labeling capacity [17, 23-25].

Polymer-based microspheres have many advantages over other materials, in particular their near-plasma density, biodegradability and biocompatibility [26, 27]. Polylactic acid which can be irradiated is regarded as a vector. Polylactic acid, a synthetic polymer, is a biodegradable and biocompatible polymer with interesting semi-crystalline properties [28]. Poly lactic acid has been recently chosen as a matrix of microparticles, incorporating either (before their preparation) the neutron-activatable ^{165}holmium-complexes or metallic 185-187 rhenium or being labeled (after their preparation) by the radioactive 90 yttrium or a 188 rhenium salt for intra-cavital therapy [29-37]. In addition to microspheres, the type of radionuclide plays a significant role on the success of the therapeutic strategy. Yttrium- 90, rhenium-188 and holmium-166 radioisotopes have characteristics, which make them potentially suitable for the treatment of inflamed arthritis [38-41]. However, Yttrium-90 has a major disadvantage as a radioisotope for therapy. The biodistribution of microspheres loaded with ^{90}Y cannot be directly determined in clinical trials, since ^{90}Y is a pure β- emitter and does not produce imageable γ-rays [16-18, 29, 30].

Natural rhenium is composed of two isotopes (185Re and 187Re) that form β-emitting ^{186}Re and ^{188}Re radioisotopes respectively, upon neutron activation. The nuclear and dosimetric properties of the ^{186}Re and ^{188}Re radioisotopes are comparable to those of ^{90}Y, but they have imageable γ-photons [38-41]. Holmium-166 (^{166}Ho) is a very attractive candidate, since this radionuclide emits gamma rays in addition to high-energy beta particles, allowing both nuclear imaging and radioablation, respectively. Moreover, holmium can be visualized by CT and MRI due to its high attenuation coefficient and paramagnetic properties [37, 41-44]. Also, favorable its half-life of 26.9 hr is long enough to eliminate logistic problems encountered with the short-lived radionuclides is sufficient to provide a high radiation dose rate. Its cross section is comparable with rhenium, but ^{165}Ho has a natural abundance of 100% and thus only one radioisotope, ^{166}Ho, is formed by neutron bombardment. Taking these characteristics into consideration, ^{166}Ho is therefore an attractive candidate for use in future treatments [38-44].

In this research, we have reported on the potential

use of biodegradable neutron activated poly-L-lactic acid microspheres (5-10 μm) containing ^{166}Ho for the radionuclide synovectomy. In this novel application, PLLA microspheres containing sufficient amounts of stable ^{165}Ho, in the form of ^{165}Ho-acetylacetona (^{165}Ho-AcAcc), prepared in the optimal size range and their physiochemical properties fully characterized before and after becoming radioactive by neutron bombardment.

2. Material and Methods

2.1. Preparation of the Ho-acetylacetonate Complex

The complex of holmium with acetylacetone (^{165}HoAcAc) was prepared and characterized as described by Nijsen et al. [44]. In short, acetylacetone (180 mL) was dissolved in 1,000 mL water. The pH of this solution was brought to 8 with an aqueous solution of NH4 (28.4% v/v). Holmium chloride (10 g in 30 mL water) was added to this solution, and HoAcAc crystals were formed at room temperature in 24 h. The crystals were collected by filtration, washed with water and dried off by nitrogen.

2.2 Preparation of HoAcAc-loaded Microspheres

^{165}HoAcAc-loaded poly (L-lactic acid) microspheres with different ^{165}HoAcAc amounts were prepared by solvent evaporation as described by Nijsen [44]. In this study ^{165}HoAcAc (1 g) and PLLA (0.6 g) were dissolved in 18.6 mL chloroform. The resulting homogeneous solution was added to an aqueous solution of PVA (100 mL water, 3% (w/w)). The mixture was stirred at 1200 rpm for 8 h, then the precipitated spheres were centrifuged at 2500 rpm for 10 min to remove the viscous continuous phase and later on, they were filtered using a 3.0 μm filter. The filtered spheres were resuspended in 800 mL 0.1N HCl for 2 min to remove the unincorporated ^{165}Ho-AcAc, refiltered and washed with 100 mL of deionized H_2O. Three subsequent filtrations using fresh 3.0 μm filter paper assured optimal particle size. The particles were dried off by nitrogen.

2.3 Size Distribution and Surface Characteristics

The size distribution of the microspheres was determined by a laser particle sizer from Fritsch GmbH (Analysette 22 Nanotec model). Surface morphology of HoAcAc-loaded PLLA microspheres was evaluated by scanning electron microscopy using a Philips XL30 FEGSEM. Samples of PLLA microspheres were mounted on aluminium stubs and sputter-coated with a Pt/Pd layer of about 10 nm.

2.4 Irradiation of Microparitícles

In order to stimulate heightened ^{166}Ho activity, the prepared microsphere samples were irradiated in Tehran Research Reactor installed in IAERI (Iran Atomic Energy Research Institute). Samples consisting of 10 mg of microspheres in polyethylene tube (diameter range 5-10 microns) were irradiated in the reactor for 1h in a thermal neutron flux of 4×10^{13} n/cm^{2}s.

2.5 Quality Control of the ^{166}Ho-PLLA Microsphers

Radionuclide Purity

As for evaluating any radionuclide impurity of ^{166}Ho-PLLA products, ^{166}Ho-PPLA complex was checked by γ and β spectrotometry by means of HPGe and beta scintillation detection systems respectively.

2.6 Radiochemical Purity

The labeling efficiency was evaluated by instant thin-layer chromatography (ITLC, Gelman Science Inc.) utilizing whatman nº 1 strips, eluted with 1 mM EDTA: (v/v) as solvent.

2.7 Stability of ^{166}Ho-PLLA

Samples of 1 mL fresh human blood serum were prepared. An aliquot of 25 μL of the ^{166}Ho-PLLA complex (100 μCi; 100 μg microspheres) was added to each sample, then the samples were incubated at 37 °C. Later on, they were analyzed by ITLC after 0, 24 h, 48 h and 72 h of incubation. The same procedure was repeated for incubating the complex at 4 °C, room

temperature and phosphate buffer conditions.

2.8 Biodistribution of ^{166}Ho-PLLA in Wild Type Rats after Intra-articular Administration

To determine the accumulation of ^{166}Ho-PLLA microspheres in the intra-articular cavity, radiolabeled PLLA solution was carefully administered to wild-type rats. A volume (50 μL) of final radiolabeled PLLA solution containing 60 ± 5 μCi radioactivities was injected intra-articularly into rats. The animals were sacrificed 2 h, 24 h, 48 h and 72h post injection. The specific activity of different organs was calculated and expressed as %ID/g tissue.

3. Result and Discusion

3.1 Size Distribution and Surface Characteristics

The size distribution and surface morphology of the microspheres were characterized using a laser particle sizer and SEM, both before and after neutron activation. The particle size distribution was narrow, from 5 μm to 10 μm, with an average microsphere size of 6.7 μm when the microspheres were prepared at 1,200 rpm. The average particle size could be tailored by altering the stirring rate and %PVA during emulsifying. The size distribution was narrow to control the biodistribution of the microspheres and to reduce possible shunting of small microspheres to non-target organs after administration. A sieving step resulted in more confined size distribution. More than 94% of the microspheres had a size between 5-10 μm after sieving. The particle size analyses of the ^{166}Ho-PLLA-MS showed that the required size distribution was guaranteed and small fragments due to radiation damage were not seen. SEM analysis showed that spherical particles with a smooth surface were formed (Figs. 1a and 1b). Particle surface damage, agglomeration or fragments was not observed after neutron activation of the microspheres for 1h. The size distribution and morphology findings indicated that the microspheres were resistant to neutron irradiation and there was no difference in suspending behavior of Ho-PLLA-MS before and after neutron irradiation, which indicated that the chemical composition of the surface had not changed.

Formation of size and shape of microsphere depends on several parameters. In this study some of these parameters including PVA concentration, pH of reaction, stirring rate, and stirring time were investigated. Increasing concentrations of PVA (from 1% to 3%) in continuous phase resulted in the formation of smaller spheres. Concentrations of 2%-3% PVA led to smooth microspheres. This could be attributed to higher viscosity in the continuous phase, leading to smaller droplet size in the dispersed phase. An increased stirring rate and time led to the smaller particle formation. Stirring rates with 300 rpm, 600 rpm, 900 rpm, 1,200 rpm and 1,600 rpm resulted in particles 30-40 μm, 20-30 μm, 10-20 μm, 5-10 μm and 0.5-4 μm diameters respectively. Maximum extraction of ^{165}Ho-PLLA was observed with a pH of above 7.5. The parameters examined resulted in a standard method under the following conditions: 3% PVA, 10 mL ^{165}HoAcAc in 180 mL chloroform, 25 °C and 1200 rpm for 8 h, yielding 4-5g of microspheres with a 5-10 μm diameter. These results were comparable with those of the studies of Mumper et al. [45].

In order to set up reactor conditions as comparable as possible and then define necessary irradiation conditions to produce therapeutic dosages of ^{166}Ho-PLLA-MS while maintaining their integrity and suitability for therapy, some irradiation parameters were investigated including: (1) the facility; (2) irradiation time; (3) water content of the microspheres; (4) the amount of microspheres per vial and (5) material of the vial.

Non-irradiated ^{165}Ho-PLLA-MS showed a smooth and spherical appearance (Fig. 2a). After irradiation in the reactor facility (neutron flux 4×10^{13} $cm^{-2}.s^{-1}$) for 1 h in polyethylene vials, surface changes were not seen with SEM (Fig. 2b). Overall structural integrity was maintained in terms of form and size and the microspheres did not showed tendency towards

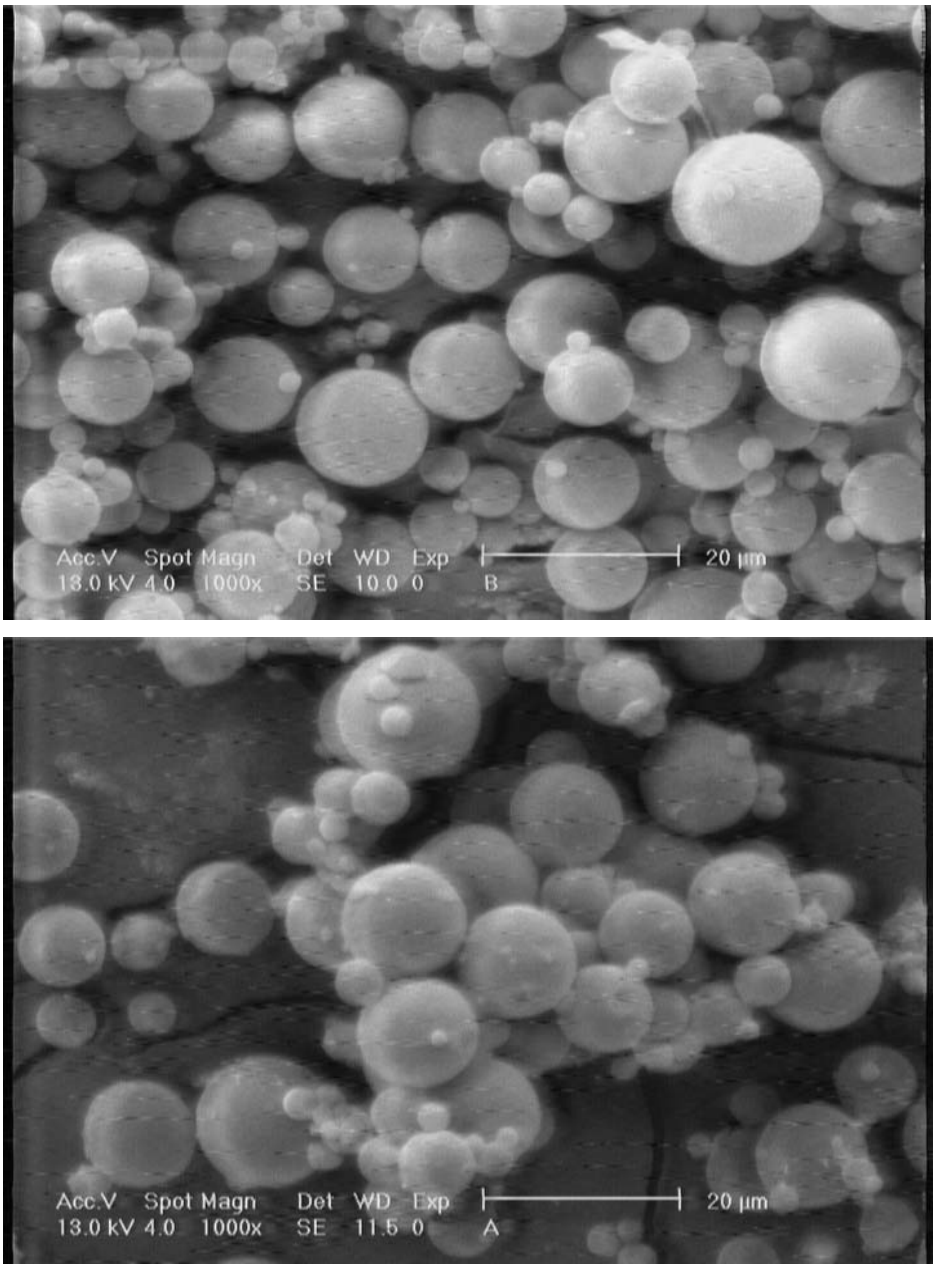
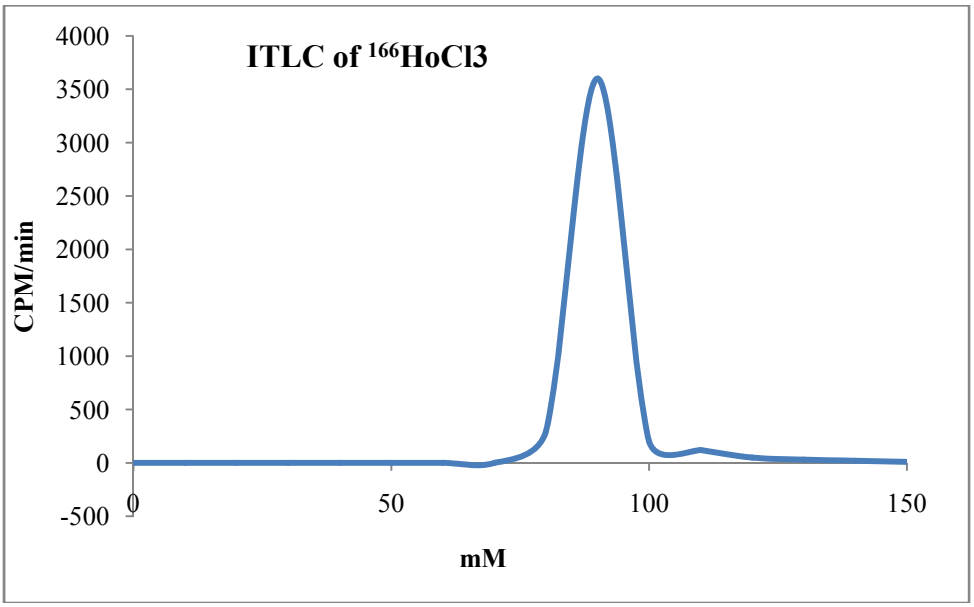
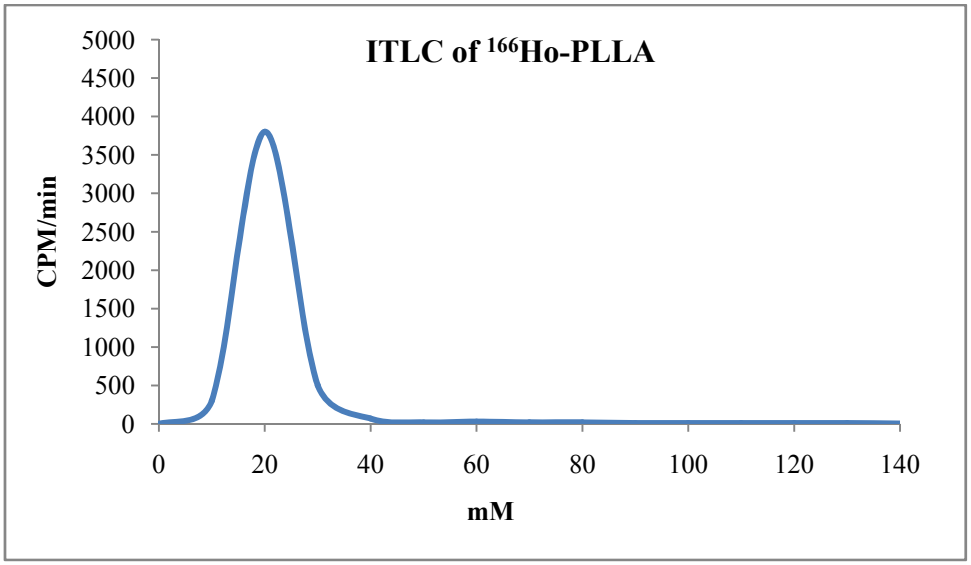


Fig. 1 Scanning electron microscopy of (a) ^{165}Ho-PLLA before neutron activation and (b) microspheres irradiated for 1 h.

aggregation, they were easily suspended in PBS and were suitable for intra-articular therapy. The results obtained from using quartz instead of polyethylene vials were similar, although some aggregation of the microspheres was observed. The presence of water had a more destructive effect. Consequently, melt appeared. Irradiation of a higher concentration of the microspheres in the reactor facility for a long time instead of 1 h had a disastrous effect on the integrity of the microspheres in both polyethylene and quartz vials. The obtained particles had a mixture of small and very large sharp edges (data are not shown), while irradiation of only 10 mg of derided microspheres in polyethylene vials for 1 h resulted in intact microspheres. This result demonstrated that microspheres must be irradiated in dehydrated conditions as well as in high neutron flux but irradiation time of lower than 1 h.



(a)



(b)

Fig. 2 ITLC of (a) $^{166}HoCl_3$ and (b) ^{166}Ho-PLLA.

These finding had minor discrepancies with studies of Mumper et al. [18, 22, 26, 45]. Mumper et al. could produce up to 1,295 MBq of ^{166}Ho within 3 h by irradiation (neutron flux 8.88 $\times 10^{12}$ cm^{-2} s^{-1}) of 50 mg microspheres with 150 mg inositol as an additive. In contrast, in this work irradiation conditions were defined such that 1,110 MBq of ^{166}Ho was obtained within 1 h by neutron activation (neutron flux 4 $\times$ 10^{13} cm^{-2} s^{-1}) of 10 mg microspheres without any additives. These discrepancies could be attributed to our work condition (short time, neutron flux 4 $\times$ 10^{13} cm^{-2}·s^{-1} and low concentration of ^{166}Ho-PLLA). This would greatly facilitate the routine microsphere production and transport to the therapy centers.

3.2 Radiochemical Purity

The relative counts recorded from the different segments of the chromatographic papers against the distance from the bottom of the papers are shown in Fig. 2. The highest activity is seen in the first segments

showing very good radiochemical purity. The Rf (retention factors) values of the $^{166}HoCl_3$ and ^{166}Ho-PLLA were 0.9-1 and 0-0.1, respectively. As can be seen, the radiochemical purity of the ^{166}Ho-PLLA was more than 99% at 1 h and pH of 5.5 (Figs. 2a and 2b). This study ensured that in the ^{166}Ho-PLLA complex free ^{166}Ho did not exist.

3.3 Stability of Radiolabeled PLLA in vitro

The stability of labeled PLLA was evaluated by instant thin layer chromatography at 4 °C, room temperature, phosphate buffer and in the incubation at 37 °C in human serum at different times. Fig. 3 shows ^{166}Ho-PLLA remained stable under these conditions and the radiochemical purity was higher than 95% for more than 72 h of storage. Also the complex showed great stability up to 72 h in human plasma (Fig. 3). These experiments showed excellent radiochemical purity results, which might indicate that no free radiometal was detected and these conditions were immaculate for radioparticle storage and applications.

3.4 Biodistribution Studies

Radiation synovectomy involves intra-articular injection into the synovial joint of colloids or particles labelled with beta emitting radionuclides. The retention of the preparation within the joint cavity is a considerable concern for this procedure. Leakage of radioactivity from the joint can result in unwanted radiation doses to non-target organs, which usually results from either the very small size of the particles or the instability of the preparation leading to dissociation of the activity from the particles. The size distribution of the particles is of importance in this regard and particles between 5mm and 10 mm in diameter are believed to suit perfectly [46- 48].

Fig. 4a presents the distribution of the injected dose in the rat organs up to 72 h after intra-articular injection of $^{166}HoCl_3$ (60 μCi/50μl) solution determined for control studies. Based on these results, it was concluded that the highest level of injected activity of $^{166}HoCl_3$ was introduced into blood circulation and distributed in the rat organs which was consistent with free Ho^{3+} distribution [49, 50] while administered intravenously (Fig. 4a).

Fig. 4b presents the distribution of the injected dose in the rat organs at various intervals after intra-articular injection of 60 μCi/50μl of ^{166}Ho-PLLA complex as a percentage of the injected dose. In case of any leak from the joint, the complex would accumulate in RE (reticuloendothelial) system due to high molecular weight of the complex, unless the complex would dissociate at serum pH, resulting in the formation of Ho^{3+}cation. In this study, almost no detectable activity was observed in spleen and lung, the two important RE organs, showing the lake of complex leak. Also, the absence of ^{166}Ho in the bone and femur after 72h is indicative of the intact PLLA particles which do not

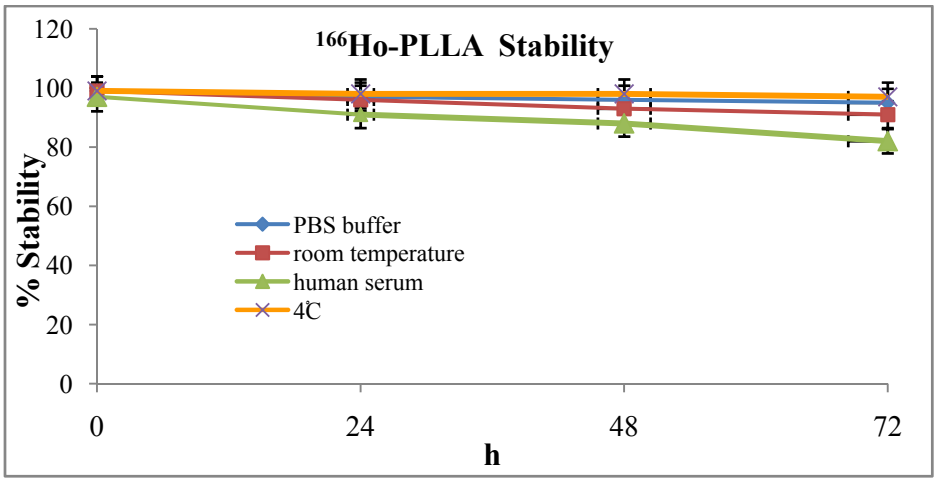
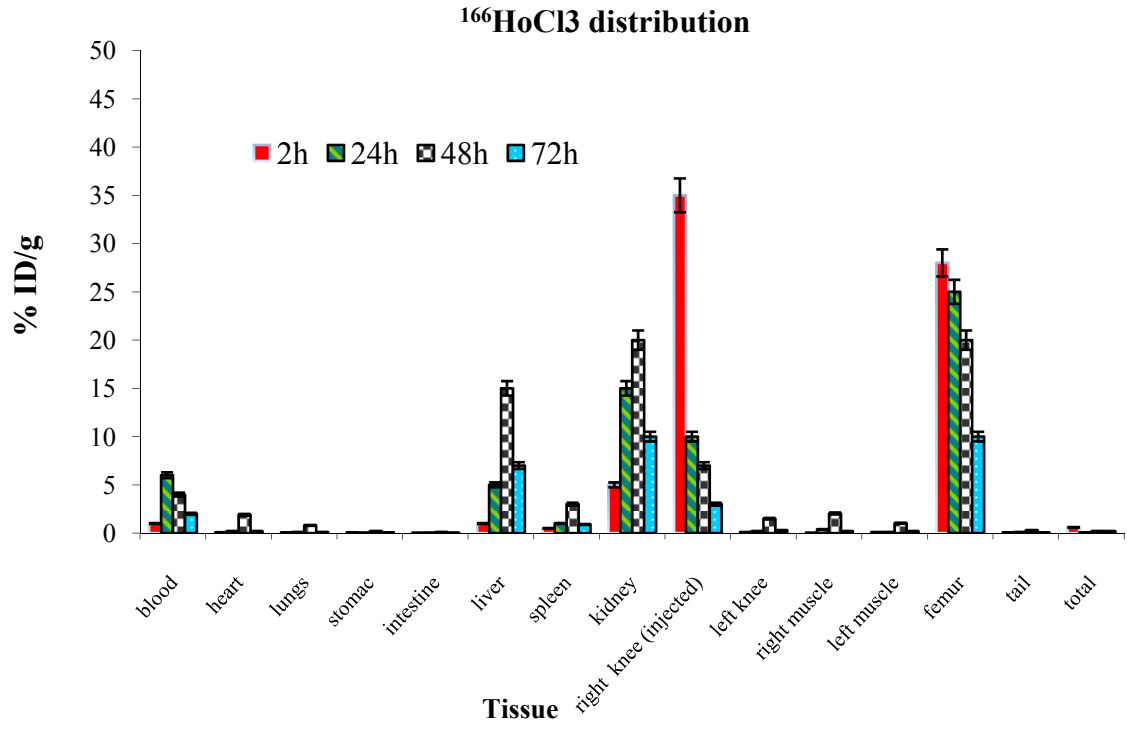
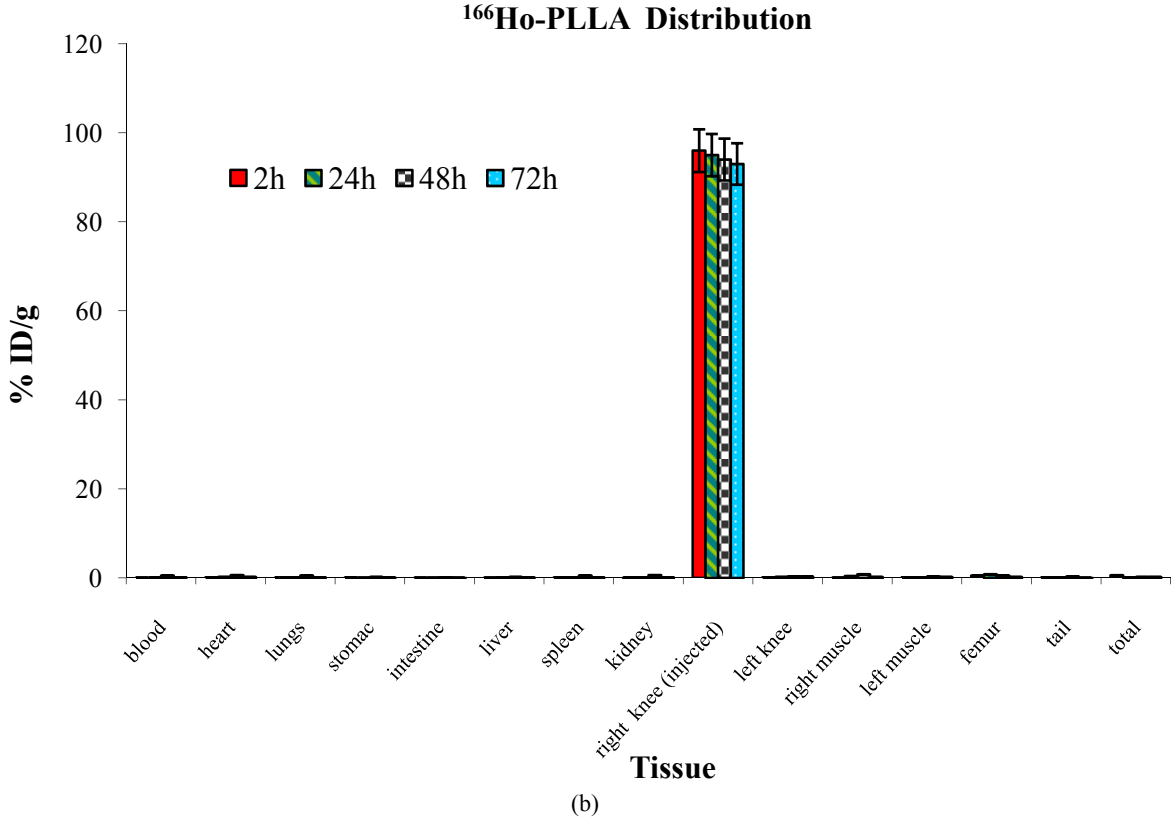


Fig. 3 In vitro stability of ^{166}Ho-PLLA in different conditions.



(a)



(b)

Fig. 4 Distribution of (a) ^{166}HoCl3 and (b) ^{166}Ho-PLLA in wild-type male rats, 2 h, 24 h, 48 h and 72 h after intra-articular injection of 60 µCi of compound. %ID—percentage of injected dose. Each bar presents mean ± SD (*n* =3).

escape the joint space. Free ^{166}Ho, known as a bone-seeking element [49, 50], could leave the joint space and deposit in the skeleton system, however, the femur and bone marrow uptake of ^{166}Ho accounted for only 0.03%-0.1% of the injected dose and only 2.7% of the total leached activity.

4. Conclusions

In summary, ^{166}Ho-PLLA-MS containing sufficient masses of neutron activatable ^{165}Ho were prepared by means of the solvent evaporation technique with high radiochemical yield (>99 %) under non-hazardous conditions. Spherical particles with a smooth surface and diameter of 5-10 μm were obtained and irradiated later to produce therapeutic amounts of ^{166}Ho-PLLA. Neutron irradiation did not damage the particles, and adequate activity was stimulated for nuclear biodistribution and radioablation of inflammations through intral-cavital injections. The irradiated particles were stable in the final solution at room temperature, 37 ºC and in the presence of human serum, and could be used even 24 h after preparation. Also, the prepared complex showed a remarkable ability to retain the encapsulated ^{166}Ho that could deliver a high radiation dose to the diseased synovium, albeit low doses to other organs. Intra-articular injection of ^{166}Ho-PLLA complex to the wild male rats and investigation of leakage of activity in the body showed that most of the injected dose has remained in injection site 72 h after injection. The high labeling yield (99%), radiochemical purity and excellent *in vitro* and *in vivo* stability of the complex, lead to the conclusion that ^{166}Ho-PLLA particles would be useful as a therapeutic agent in the arthritis treatment. Also the potential clinical use of this agent is very promising since its preparation does not require the handling of heightened activity and the half-life of ^{166}Ho removes the necessity of being close to a reactor. Finally, a kit formulation was developed for the in situ preparation of the radiopharmaceutical in clinical centers.

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