Radiolabelling of poly(histidine) derivatized biodegradable microspheres with the $^{188}$Re tricarbonyl complex

$[^{188}\text{Re} (\text{CO})_3 (\text{H}_2\text{O})_3]^+$

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Objectives Many radiopharmaceuticals have been studied as radiation synovectomy agents. In this study, we developed a new potential agent for radiation synovectomy: poly(lactic acid)–histidine (PLA–his) microspheres radiolabelled with $[^{188}\text{Re} (\text{CO})_3 (\text{H}_2\text{O})_3]^+$. 

Methods The reaction conditions for the chelation of $[^{188}\text{Re} (\text{CO})_3 (\text{H}_2\text{O})_3]^+$ and the radiolabelling of PLA microspheres were optimized and the stabilities for both steps tested in vitro.

Results The chelation efficiency of $[^{188}\text{Re} (\text{CO})_3 (\text{H}_2\text{O})_3]^+$ reached 93.12 ± 1.82% with > 95% radiochemical purity once the colloidal and free $^{188}$Re were removed by a small Sep-Pak column (Plus QMA). More than 90% of radioactivity stayed in the $[^{188}\text{Re} (\text{CO})_3 (\text{H}_2\text{O})_3]^+$ form over 5 h. The radiolabelling efficiency of PLA–his microspheres with $[^{188}\text{Re} (\text{CO})_3 (\text{H}_2\text{O})_3]^+$ was above 92%. After 3 days incubation at 37°C in calf serum, more than 80% of the radioactivity was still bound to the microspheres.

Conclusion Such microspheres are potentially useful as a radiation synovectomy agent for the treatment of chronically inflamed arthritic joints. Furthermore, they might be valuable in cancer brachytherapy. Nucl Med Commun 26:453–458 © 2005 Lippincott Williams & Wilkins.

Keywords: microsphere, radiolabelling, $^{188}$Re, tricarbonyl, histidine, radiation synovectomy

Introduction Radiation synovectomy is a non-invasive therapeutic alternative to surgical synovectomy. It consists of an intra-articular injection of a radionuclide in colloidal or particulate form to ablate the inflamed synovium. Many radiopharmaceuticals have been developed for use as synovectomy agents, such as $^{90}$Y, $^{166}$Dy, $^{153}$Sm, $^{198}$Au, $^{169}$Ho and $^{186/188}$Re [1–7]. $^{188}$Re is an excellent candidate for radiotherapy with a half-life of 17.0 h. Beta emissions with energies of 2.12 MeV (71.6%) and 1.96 MeV (25.1%) are suitable for therapy and the gamma emission of 155 keV (15%) allows imaging and calculating dosimetry during therapy. Another good property is that it can be conveniently acquired as a ‘no carrier added’ radioisotope from a $^{188}$W/$^{188}$Re generator [8]. Many materials, such as hydroxyapatite, microspheres, sulfur colloid, sulfur suspension and tin colloid, have been labelled with $^{188}$Re to be evaluated for radiation synovectomy [4,6,9–12]. A suitable agent for radiation synovectomy should minimize leakage of radioisotopes from the treated joint. In addition, it would be ideal if the radioactive particles biodegrade after treatment.

The technetium tricarbonyl complex $[^{99m}\text{Tc} (\text{CO})_3 (\text{H}_2\text{O})_3]^+$ has been widely studied for radiopharmaceutical application because of the high substitution stability of its three CO ligands and the substitution lability of the coordinated water molecules [13,14]. The rhenium tricarbonyl complex $[^{188}\text{Re} (\text{CO})_3 (\text{H}_2\text{O})_3]^+$ has also been studied. It is more difficult to prepare than the technetium carbonyl complex because rhenium requires harsher conditions to be reduced from its original oxidation state, + VII, to lower oxidation states and it has a higher tendency to reoxidize [15].

In this study, we used biodegradable polyester microspheres made from poly(lactic acid) (PLA) [16] with a mean particle diameter of 2 μm. A particle size of 2–5 μm seems to be ideal to reduce leakage from the treated joint [10]. In order to radiolabel microspheres with a rapid and mild method, the surface of PLA microspheres was covalently derivatized with histidine to be labelled with the organometallic aqua ion $[^{188}\text{Re} (\text{CO})_3 (\text{H}_2\text{O})_3]^+$. Histidine is an essential amino acid and forms a stable organometallic complex with the $[\text{M} (\text{CO})_3 (\text{OH}_2)_3]^+$ (where $\text{M} = \text{Tc}$ or Re) core through its imidazolyl group.
Methods
Production of 188Re
Carrier-free [188Re(perrhenate was freshly eluted with saline from an alumina based 188W/188Re generator (Shanghai Kexing Pharm. Co., Ltd, P. R. China; 188W was supplied by Oak Ridge National Laboratory, Tennesse, USA). The radionuclide purity of 188Re was greater than 99%, as analysed by gamma spectroscopy with a high-purity germanium (HPGe) detector (GEM-15190, EG & ORTEC, USA) and the radiochemical purity of Na188ReO4 was more than 95% by paper chromatography developed with 0.9% NaCl [17].

Preparation of [188Re(CO)3(H2O)3]+

The method of Schibli et al. was adapted for the preparation of the [188Re(CO)3(H2O)3]+ core [15]. Two to ten milligrams of BH3-NH3 (from Fluka Co.) was placed in a 10 ml glass vial. The vial was sealed with an aluminium capped rubber stopper and flushed with CO gas for 5–30 min. One millilitre of [188Re]perrhenate containing, typically, 74–370 MBq with 6 μl of H3PO4 (85%) was added to the vial and incubated in a water bath at 50–90°C for 10–30 min. A 20 ml syringe was used to keep the balance of H2 gas. Both colloidal and free 188Re could be removed using a small Sep-Pak column (Plus QMA; Waters Co.).

Quality control
The chelation efficiency of [188Re(CO)3(H2O)3]+ was determined by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The TLC system consisted of a GF254 silica gel glass plate as stationary phase and CH3OH/hydrochloric acid (36%) (99:1) as mobile phase. The plates were scanned with a Bioscan AR-2000 radioanalyser. HPLC analyses were performed on a Dionex P680 system equipped with a PDA-100 photodiode array detector and a Bioscan AR-2000 radioanalyser. HPLC solvents consisted of methanol (solvent A) and 0.05 M TEAP (triethylammonium phosphate solution) buffer, pH 2.25 (solvent B). HPLC was performed using a C18 reversed phase column (10 μm, 300 × 3.9 mm, Waters Co.) with the following parameters: 0–3 min, 100% B; 3–9 min, 75% B and 25% A; 9–20 min, 66% B and 34% A; 20–22 min, 100% A; 22–25 min, 75% B and 25% A; 25–30 min, 100% B.

Radiolabelling of PLA microspheres with [188Re(CO)3(H2O)3]+

Biodegradable PLA microspheres were prepared by a solvent evaporation method [16] and yielded a mean particle size of 2 μm, as determined on a multichannel particle size analyser COULTER Multisizer II (Beckman Coulter, Fullerton, California, USA). In summary, 1 g of poly(1-lactic acid) with a molecular weight of 2000 (Resomer 104, Boehringer Ingelheim, Germany) was dissolved in 4 ml of chloroform and added to 320 ml of a heavily stirred solution of 1% polyvinyl alcohol in water. After 45 min of stirring, the microspheres were washed several times with distilled water. Three types of microsphere were then radiolabelled with [188Re(CO)3(H2O)3]+. The first type consisted of the native PLA microspheres with free –COOH groups (control 1). A second type of microspheres had been further derivatized with diethylamine using dicyclohexylcarbodiimide to yield free –NH2 groups on its surface (control 2). And a third type of microspheres was derivatized with the polypeptide his–his–his–his–his–his (his6) (Molecular Biotechnology Core Laboratory of the Cleveland Clinic Foundation, USA) by carbodiimide activation of the carboxyl end groups [16].

After the preparation of 188Re(CO)3(H2O)3]+, 50–500 μl of the 188Re tricarbonyl complex was added to 2–8 mg of microspheres in 50–500 μl of water or 0.5 M of 2-N-morpholinoethanesulfonic acid (MES) and incubated at 37–90°C for 15–60 min in an Eppendorf thermomixer vibrating at 1000 rpm.

Measurement of labelling efficiency and stability in vitro
After radiolabelling the microspheres with [188Re(CO)3(H2O)3]+, the supernatant was separated by centrifugation. The labelling efficiency was calculated as

\[
1 - \frac{A_s}{A_r}
\]

where \(A_s\) is the radioactivity of the supernatant, and \(A_r\) is the total radioactivity before separation.

One millilitre of new-born calf serum or phosphate buffered saline (PBS, pH 7.4) was added to the labelled microspheres and incubated at 37°C in a shaking water bath. At time points of 1 h, and 1, 2 and 3 days, the supernatant was removed and the labelling efficiency determined.

Results and discussion
Analysis of chelation efficiency
The TLC system for the analysis of the chelation efficiency of [188Re(CO)3(H2O)3]+ always showed four peaks (Fig. 1(b)). The 188Re carbonyl complex had two peaks: [188Re(CO)3(H2O)3]+ at an \(R_f\) of 0.4 and an unknown species at an \(R_f\) of 0.7. These same two peaks had already been described by Alberto et al. [18]. The unknown peak may be [188Re(CO)3(H2O)3–(NH3)n] where \(n = 1–3\), which converts into [188Re(CO)3(H2O)3]+ when labelled with biomolecules [19]. The NH3 group comes from BH3-NH3. The other two peaks were colloidal 188Re (\(R_f = 0\)) and free 188Re[perrhenate (\(R_f = 0.8–1\) ) (Fig. 1(b)). Both colloidal and free 188Re...
could be removed using a small Sep-Pak column (Fig. 1(c)). The chelation efficiency was calculated using the sum radioactivity of the two middle peaks divided by the total radioactivity in TLC (Fig. 1).

The chelation efficiencies determined by TLC were confirmed by HPLC using the characteristic wavelength of the standard $\text{fac-}[\text{Re(CO)}_3(\text{H}_2\text{O})_3]^+$ at 262 nm for detection [20]. The HPLC gamma trace revealed the peak of the $\text{fac-}[\text{188Re(CO)}_3(\text{H}_2\text{O})_3]^+$ (80 ± 5%, retention time (RT) = 5 min) and $\text{188ReO}_4^-$ (20 ± 5%, RT = 9–10 min), which were the same as described by Schibli et al. [15] (Fig. 2).

**Investigation of the optimum reaction conditions for the preparation of $[\text{188Re(CO)}_3(\text{H}_2\text{O})_3]^+$**

Since it was not possible to reach the desired chelation efficiency of $\text{188Re(CO)}_3(\text{H}_2\text{O})_3^+$ by the reported method (Schibli and Schubiger) [13], we further investigated the reaction conditions. Figures 3–5 show the effect of temperature, reaction time, and the amount of BH$_3$/C$_3$NH$_3$ on the preparation of $[\text{188Re(CO)}_3(\text{H}_2\text{O})_3]^+$. The optimum conditions for its preparation were 15 min of heating at 75°C and using 5 mg of BH$_3$/NH$_3$. The amount of BH$_3$/NH$_3$ is a key reaction condition. When less BH$_3$/NH$_3$ is used, then less $[\text{188Re}]$ perrhenate is converted into $[\text{188Re(CO)}_3(\text{H}_2\text{O})_3]^+$. Using more BH$_3$/NH$_3$, however, leads to a higher $\text{188Re}$ colloid production. In addition, it was necessary to keep the pH below 2 to achieve significant amounts of the $\text{188Re}$ carbonyl complex (Figs 3–5).

In order to determine the length of time that the $[\text{188Re(CO)}_3(\text{H}_2\text{O})_3]^+$ can be stored at room temperature for the radiolabelling of microspheres or other compounds, we also analysed the chelation efficiency at different time points after preparation (i.e., 1, 3, 5 and 24 h after its preparation) (Fig. 6). After 5 h, there was still more than 90% of the radioactivity present in the form of $[\text{188Re(CO)}_3(\text{H}_2\text{O})_3]^+$. This value then slowly decreased to about 75% of the radioactivity in the form of $[\text{188Re(CO)}_3(\text{H}_2\text{O})_3]^+$ after 1 day (Fig. 6). $[\text{188Re(CO)}_3(\text{H}_2\text{O})_3]^+$ is thus a good precursor for developing $\text{188Re}$ radiopharmaceuticals because it binds readily to histidine groups which are often present in peptides and proteins.

The final chelation efficiency of higher than 95% with radioactivity concentrations greater than 74 MBq·mL$^{-1}$ under the optimal reaction conditions was appropriate for radiolabelling PLA microspheres.

**Labelling efficiency of PLA–his microspheres**

Figures 7 and 8 show the effect of reaction temperature and time on radiolabelling PLA–his microspheres with $[\text{188Re(CO)}_3(\text{H}_2\text{O})_3]^+$. Heating for 30 min at 70°C was sufficient to reach a high radiolabelling efficiency. We also investigated the effect of the reaction solvent (water or MES buffer), its total volume and the amount of PLA on radiolabelling PLA–his microspheres. Although MES buffer was the best for radiolabelling PLA–his microspheres with $[\text{99mTc(CO)}_3(\text{H}_2\text{O})_3]^+$ [16], it was
not very useful for radiolabelling with the \(^{188}\text{Re}\) carbonyl complex. The labelling efficiencies of 2 mg of PLA–his microspheres in 0.1, 0.5 and 1 ml of total volume were 92.66 ± 0.1%, 86.18 ± 4.04% and 70.48 ± 11.01%, respectively. With 4, 6 and 8 mg of microspheres, the radiolabelling efficiencies were 87.47 ± 0.04%, 87.92 ± 0.89% and 82.52 ± 7.04% in 1 ml of solvent. From these results, we chose radiolabelling of 4 mg of PLA microspheres in 1 ml as our procedure for further studies (Figs 7 and 8).

### In-vitro stability of PLA–his microspheres

The serum stability of PLA–his microspheres is slightly lower than the stability measured in PBS, with about 80% of the radioactivity still bound to the microspheres after 3 days (Fig. 9).

Using the optimized reaction conditions, the radiolabelling efficiency of PLA–his microspheres with \([^{188}\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+\) exceeds 92%. In contrast, radiolabelling of the control microspheres containing –COOH and
–NH₂ groups on the surface yielded a radiolabelling efficiency of 76.47 ± 0.59% and 68.99 ± 2.39%, respectively. The chelation is relatively unspecific, as can be seen by the low stability measured in serum at 37°C. After 24 h, only 40.65 ± 10.59% and 41.45 ± 0.71%, respectively, was still bound to microspheres, as compared to above 90% for the PLA microspheres with histidine groups. It is thought that the [¹⁸⁸Re(CO)₃]⁺ core binds to the imidazoles of two histidines which are separated by one histidine, as has been speculated for [⁹⁹mTc(CO)₃]⁺ [13].

**Conclusion**
Poly(histidine)-derivatized biodegradable microspheres were successfully radiolabelled with [¹⁸⁸Re(CO)₃]⁺.
(H2O)3]+ using an effective method with high radiolabelling efficiency and serum stability. When made into a kit (which has still to be perfected) that contains one vial of the histidine-derivatized microspheres and a second vial of the eluents for the [188Re(CO)3(H2O)3]+ core preparation, it is thought that such microspheres will be useful as a radiation synovectomy agent for the treatment of chronically inflamed arthritic joints. Being biodegradable will allow them to disappear after having delivered their radiation dose without inducing long-term irritation and chronic effects. A rabbit experiment to test the 188Re labelled microspheres is under way. But there are also other possible applications of such radioactive microsphere preparations, such as the treatment of cancer after direct injection into the tumour or by injection of larger particles (1–30 μm) microspheres made from the same material which then radioembolize the arterial supply of the tumour [21]. Furthermore, magnetic targeting is also possible with smaller particles (1 μm or less) that contain magnetic nanoparticles [22]. In this way, liver, lung, and head and neck tumours, for example, could be targeted by placing a magnet above the tumour, and then injecting the radioactive particles into a patient’s blood supply.

References