The Preparation of PLLA Microspheres containing the Dysprosium ion

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1. Introduction

Microspheres are defined as small spherical microscopic particles with diameters in the micrometer range (typically 1-1000 μm). The development of microspheres is an ongoing to continue for biomedical applications. [1,2] The microspheres have several advantages such as large surface area, uniform size and shape, which can improve delivery of sphere with drugs and bioactive molecules to the specific target site. [1-3] Particularly, polymer-based microspheres have received considerable attention in recent years due to their potential controlled drug release characteristics.[3] Among many synthetic polymers, Poly–L-lactic acid(PLLA) is one of the most common biodegradable polymer owing to their processibility, less energy dependence, excellent degradation and biocompatibility. [4-7]

Radioactive Holmium-166(^{166}Ho) loaded PLLA microspheres are promising radiopharmaceuticals to treat of liver malignancies [8, 9] ^{166}Ho is the ideal radionuclide to treatment for liver malignancies and have been utilized in bone marrow ablation and radiation synovectomy owing to its high-β radiation energy [$T_{1/2}$=26.6 h, E^{β}_{max}=1855 keV(51%), E^{β}_{av}=666 keV].[10] ^{166}Ho can be produced by two approach using (n,γ) reaction and (2n,γ)β reaction.

(n,γ) reaction : $^{165}Ho(n, \gamma)^{166}Ho$
(2n,γ)β reaction: $^{164}Dy(n, \gamma)^{165}Dy(n, \gamma)^{166}Dy \rightarrow {}^{166}Ho$

As mentioned, ^{166}Ho can be produced by β decay of ^{166}Dy [$T_{1/2}$=81.5 h, E^{β}_{max}=486.8 keV, E^{β}_{av}=130 keV] and also produced as a carrier free state by separation processing. ^{166}Dy as parent nuclide of ^{166}Ho can be produced by double neutron capture reaction of ^{164}Dy. [10] ^{166}Ho/^{166}Dy pair is valuable as *in vivo* generator system.

There are main two ways to manufacture microspheres containing drug. One is to mix drugs together when manufacturing the microspheres in solution state, the other is to encapsulate drugs after manufacturing the microspheres.[1]

In this research, we will present here to prepare the Dy contained microsphere in cold state for application of ^{166}Dy/^{166}Ho *in vivo* generator system. The PLLA microsphere can be synthesized by O/W suspension polymerization. The addition of Dy acting as drug to the microspheres was carried out by the two methods mentioned above.

2. Experiments

2.1 Materials and Instruments

All chemical reagents and solvents used for experiments were purchased from Sigma-Aldrich and used without further purification. The Dysprosium ion identification was performed by Atomic Adsorption spectroscopy (AA-7000, Shimadzu). The shape and morphology of microspheres were characterized by Microscopy (LEICA INVERTED) and Jeol JSM-7100 F field-emission scanning electron microscope (FE-SEM; JEOL) at an acceleration voltage of 5.0 kV.

2.2 The preparation of Dy-PLLA-microsphere by adding Dy-ACAC during O/W suspension polymerization (Dy-PLLA-MS ①)

To reduce the ionic character of Dy^{3+}, Dy-AcAc complexes were synthetized by reaction of $DyCl_3 \cdot 6H_2O$ and acetylacetonate(AcAc). The organic mixture of Dy-AcAc(0.05 g) and PLLA(1.5 g) dissolved in chloroform(30 ml) was added to 1.3% poly vinyl alcohol (PVA) aqueous solution(300 ml). The O/W emulsion mixtures were continuously stirred for 40 hours with difference of stirring speeds (500, 400 and 300 rpm). The obtained final microspheres were washed with D.W-0.1 M HCl-D.W respectively and dry at 50 ℃.

2.3 The preparation of Dy-PLLA-microsphere through absorption of Dy ion after production of microsphere (Dy-PLLA-MS ②)

PLLA-microsphere was obtained by O/W suspension polymerization. The PLLA (1 g) was dissolved in chloroform (35 ml) as solvent. Then, the solution was added to 1.3% PVA aqueous solution (400 ml) and stirred with 300 rpm for 24 hours. The obtained PLLA microspheres were washed with D.W-0.1 M HCl-D.W respectively and dry at 50 ℃.

Each Dy-AcAc complex and $DyCl_3 \cdot 6H_2O$ were dissolved in ethanol and 30 μl of each solution was added to PLLA microsphere (20 mg) in e-tube. After 30 min, the each tube was washed with 1 ml of D.W and separated the microsphere and supernatant by centrifugation. To identify the contents of Dy in microspheres, the Dy concentration of supernatants was checked by AA spectroscopy.

3. Result and discussion

The size of Dy-PLLA-MS ① with spherical shape is increasing with the lower stirring speed (20 μm at 500 rpm, 27 μm at 400 rpm and 28 μm at 300 rpm) and the contents of Dy ion in the microsphere is identified with 8.75 mg of Dy/1g of microsphere.

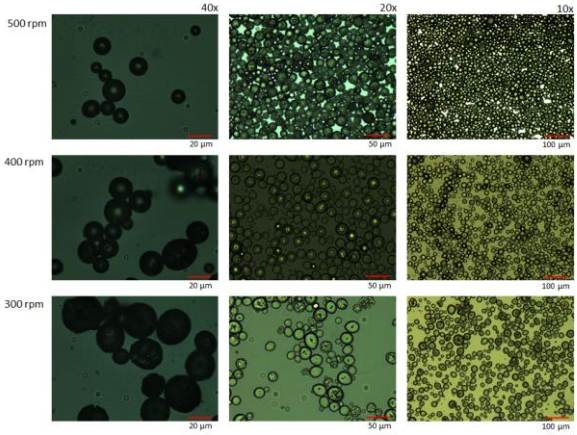

Figure 1. Microscopic image of Dy-PLLA-MS ①

The obtained PLLA microsphere (PLLA-MS) without Dy-AcAc are spherical with the size of 44.38 μm. The size of pure PLLA-MS is bigger than Dy-PLLA-MS ① obtained at same stirring speed with 300 rpm because the Dy ion in Dy-PLLA-MS ① cause a shrinkage of microsphere.

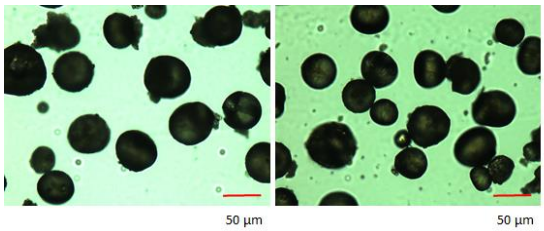

Figure2. Microscopic image of pure PLLA-MS

The absorption of Dysprosium solution to PLLA-MS is less than 50%. When the PLLA-MS was treated with 0.02 M Dy-AcAc solution, the absorption is about 25% and the contents value is 1.66 mg of Dy/1g of microsphere. In case of 0.5 M $DyCl_3 \cdot 6H_2O$ solution, Dy ion was absorbed in PLLA-MS about 13% and the value is 7.5 mg of Dy/1g of microsphere.

The aim of this project is to get the Dy-PLLA microsphere for application on Dy/Ho in vivo generator. After HANARO is re-operated, the study of double (n,γ) reaction to ^{164}Dy Source and produce of microsphere containing of ^{166}Dy will be done.

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