Fabrication of Chitosan Microspheres absorbed with ^{166}Dy for an *in vivo* generator system

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1. Introduction

Chitosan, which is copolymer of a glucosamine and an *N*-acetyl glucosamine, is a natural cationic polysaccharide derived from chitin. Chitosan has been extensively used for development of biomedical and pharmaceutical formulations due to their hydrophilic, biocompatible and biodegradable properties of low toxicity. [1-2] Especially, chitosan microspheres are the most widely studies in drug delivery systems for the controlled release of drugs, antibiotics, antihypertensive agents, anticancer agents, proteins, peptide drugs and vaccines. [3]

At present, Radioactive Holmium-166(^{166}Ho) loaded microspheres are promising radiopharmaceuticals as an alternative approach to ^{90}Y-glass microspheres to treat of liver malignancies owing to its high-β radiation energy [$T_{1/2}$=26.6 h, E^{β}_{max}=1855 keV(51%), E^{β}_{av}=666 keV]. [4-6] Also, the high magnetic properties of ^{166}Ho can offer medical information of single photon emission computed tomography and magnetic resonance imaging that can track the therapeutic response.[7]

^{166}Ho can be produced by two approach using (n,γ) reaction and (n,γ)β reaction. [8]

(n,γ) reaction : ^{165}Ho(n, γ)^{166}Ho

(n,γ)β reaction: ^{164}Dy(n, γ)^{165}Dy(n, γ)^{166}Dy → ^{166}Ho

^{166}Ho produced by (n,γ)β reaction from ^{166}Dy [$T_{1/2}$=81.5 h, E^{β}_{max}=486.8 keV, E^{β}_{av}=130 keV] is a carrier free state and the relation of ^{166}Ho and ^{166}Dy can be applied on *in vivo* generator concept.

'*in vivo* generator' is a concept that long-lived parent nuclide *in vivo* decay into short-lived daughter nuclide with high decay energy at target tissue. [9] This system has been applied in a wide range of theranostics application. Especially, the ^{166}Dy/^{166}Ho pair show the good characteristic as *in vivo* generator system and are more effective in radiotherapy. Dy and Ho, as the neighboring elements in lanthanide, show the similar physical and chemical characteristics. [10]

Park et al(1996), Ho macro-aggregates have shown high *in vivo* retention(>99/5%) at 24 h and 10 days in the knee joint of rabbits.[11] P. L Martha et al(2004), ^{166}Dy-EDTMP complex has been accumulated in skeletal tissue and can be applied for marrow ablation.[12]

In this research, we will present here the preparation of Chitosan microsphere containing ^{166}Dy as parent nuclide for treatment of liver malignancies through ^{166}Dy/^{166}Ho *in vivo* generator system. We also present here separation of ^{166}Dy and ^{166}Ho by home-made HPLC and show the absorption and release profile of ^{166}Dy from microsphere.

2. Experiments

2.1 The preparation of chitosan microsphere

3%(w/v) chitosan solution was prepared by dissolving of chitosan in 7.5% (w/v) itaconic acid as water phase. The organic phase was 300 ml of paraffin oil with the addition of Span 40 as an emulsifier. To obtain the w/o emulsion, 30 ml of chitosan solution was added to the paraffin oil and stirred with speed of 2,000 rpm for 15 min. then, 18 ml of glutaraldehyde-satrurated toluene (GST) solution as crosslinking agent was dropped into the mixture, slowly. After 2.5 h, the obtained particles were washed 3 times with hexane, 3 times with D.W. and 3 times acetone, consecutively. The washed microsphere was dried at 50 ℃ with a convection oven.

2.2 Separation of Dysprosium and Holmium

Isotope-enriched $^{164}Dy_2O_3$ (100 mg, 96.8(±0.1)%, Isoflex, San Francisco, California, USA) was irradiated at a neutron thermal flux of 2.0×10^{14} $ncm^{-2}s^{-1}$ for 30 hr from POLATOM (Poland). After irradiation, the predominant radioisotopes were ^{166}Dy and ^{166}Ho due to their large cross section. The irradiated ^{166}Dy/^{166}Ho oxide was dissolved in 6 M HCl and evaporated at a 120 ℃ heat bath. The same process was continued in 0.1 M HCl and water. The final product was dissolved in water and 0.5 ml (approximately 78.8 mCi) of the product was injected to home-made automatic HPLC system and the separation process was carried out. The home-made HPLC detected a gamma-ray of ^{166}Ho and [^{166}Dy] Dy/^{166}Ho and the each isotope was separated by the difference elution times depend ionic form with an HIBA complexing agent.

2.3 absorption and release profile of Dysprosium from microsphere

The separated [^{166}Dy] Dy/^{166}Ho chloride was dissolved in D.W. (22.4 mCi/ml). 40 μl of the [^{166}Dy] Dy/^{166}Ho solution was treated to the 20 mg of chitosan microspheres and kept for 30 min. the chitosan microspheres soaked with [^{166}Dy] Dy/^{166}Ho were

washed 3 times with 1 ml of D.W. to remove unabsorbed [^{166}Dy] Dy/^{166}Ho. The activity of supernatant D.W (A) and microspheres (B) were measured by dose calibration and absorption (%) was calculated by following relation.

$$\%\ ^{166}Dy_{absorption} = \frac{Activity(B)_{microsphere}}{Activity(A)_{supernatant} + Activity(B)_{microsphere}} \times 100$$

For release profiles, 1 ml of PBS (pH 7.4) or Human serum was added to the chitosan microspheres soaked with [^{166}Dy] Dy/^{166}Ho and incubated at 37 ℃ under constant shaking for 24, 96 and 168 h. At each interval, the supernatant (C) and microspheres (D) were separated by centrifugation. The release profile of [^{166}Dy] Dy/^{166}Ho was calculated by below equation.

$$\%\ ^{166}Dy_{released} = \frac{Activity(C)_{Supernatant}}{Activity(C)_{supernatant} + Activity(D)_{microsphere}} \times 100$$

3. Result and discussion

The chitosan microspheres are spherical with smooth surface. The mean size of microsphere is increasing with the slower stirring speed, higher concentration of emulsifier and smaller volume of crosslinking agents.

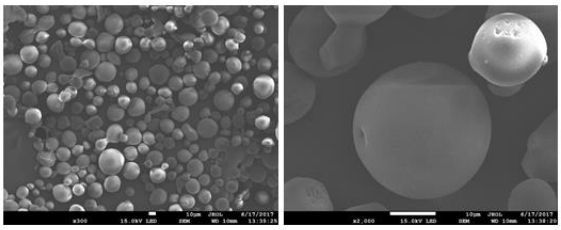

Figure 1. SEM image of chitosan microsphere

The mean size of microspheres dispersed in water was approximately 20.5 μm and it was reduced to about 11.5 μm after the water evaporated. This result represents the microsphere can absorb some solvents and show an 8-fold increase over their volume.

The absorption (%) of [^{166}Dy] Dy/^{166}Ho into chitosan microspheres were 53.89% ± 2.44 (confidence 95%). The release profile showed nearly constant value at each interval time and a higher value in human serum than PBS (pH 7.4).

These detailed studies showed that the biodegradable chitosan microspheres soaked with ^{166}Dy as a parent nuclide of ^{166}Ho exhibit potential theranostics medicinal application through *in vivo* generator system for treatment of liver malignancies.

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