# The Preparation of Dysprosium Microsphere for $^{166}$Dy/$^{166}$Ho *in vivo* generator system

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## 1. Introduction

The uses of radiolanthanide as therapeutic radiopharmaceuticals are increasing in these days due to their proper LET (Linear Energy Transfer) to induce cancer cell apoptosis.

Among these radiolanthanides, Holmium-166($^{166}$Ho) have been utilized in field of medical radiotherapeutic application such as radioimmuno-specific pharmaceuticals, bone marrow ablation and radiation synovectomy owing to its high-$\beta$ radiation energy [$T_{1/2}$=26.6 h, $E^{\beta}_{max}$=1855 keV(51%), $E^{\beta}_{av}$=666 keV].

$^{166}$Ho can be produced by two approach using (n,γ) reaction and (n,γ)$\beta$ reaction.

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

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

$^{166}$Ho produced by (n,γ)$\beta$ reaction from $^{166}$Dy [$T_{1/2}$=81.5 h, $E^{\beta}_{max}$=486.8 keV, $E^{\beta}_{av}$=130 keV] is a carrier free state and can be applied on *in vivo* generator concept. $^{166}$Dy as parent nuclide can be produced by double neutron capture reaction of stable $^{164}$Dy.

*in vivo* generator, first appeared in conference abstract by Mausner et al, is a concept that long-lived parent nuclide($^{166}$Dy) is delivered to target tissue and then *in vivo* decay into short-lived daughter nuclide($^{166}$Ho) with high decay energy.

$^{166}$Dy/$^{166}$Ho *in vivo* generator system has been reported since 1994. Suzanne V et al(1995) revealed that the *in vitro* and *in vivo* integrity of [$^{166}$Dy]Dy/$^{166}$Ho-DTPA complex had no translocation of the daughter nuclide after $\beta^-$ decay of $^{166}$Dy. Park et al(1996), Dy/Ho macro-aggregates have shown high *in vivo* retention(>99/5%) at 24 h and 10 days in the knee joint of rabbits. P. L Martha et al(2004), $^{166}$Dy-EDTMP complex has been accumulated in skeletal tissue and can be applied for marrow ablation.

Radionuclide loaded microsphere with a diameter of 20-50 μm are promising factor to treat of liver malignancies. Nijsen et al(1999), produced poly-L-Lactic acid microsphere including $^{166}$Ho for intra-arterial radionuclide therapy of hepatic malignancies.

In this research, we will present here the preparation of poly-L-Lactic acid microsphere containing Dy as parent nuclide for treat of liver malignancies through $^{166}$Dy/$^{166}$Ho *in vivo* generator system. We also present here microsphere surface status change depends on preparation conditions. Furthermore, the cytotoxicity against Liver hepatocellular carcinoma (Hep G 2) and Lung carcinoma epithelial cell (A 549) were studied.

## 2. Experiments

### *2.1 Materials and Instruments*

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin and phosphate-buffered saline (PBS, pH 7.4) were purchased from Gibco, Life Science. All other chemical reagents and solvents used for experiments were purchased from Sigma-Aldrich and used without further purification. Metal identification was performed by Atomic Adsorption spectroscopy (AA-7000, Shimadzu) The structure of synthesized particels were confirmed by Fourier transform infrared(FT-IR) spectroscopy (Jasco, FR/IR-460plus) with standard KBr disk method and Maldi-TOF Mass Spectrometer (Voyager DE-STR) from Applied Biosystems. Inc. The morphologies were characterized by 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-Poly-L-Lactic acid microsphere through O/W suspension polymerization*

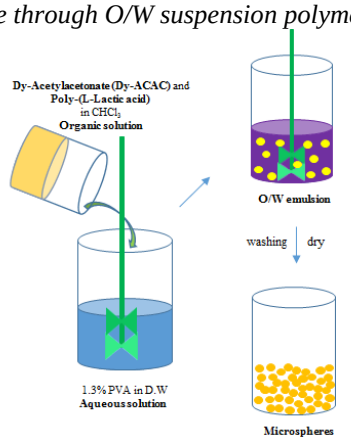


Figure 1. Process of O/W suspension polymerization

Before the suspension-polymerization process was performed, Dy coordinated complex was synthesized to reduce the ionic character of $Dy^{3+}$. The processes are like as bellows. 40 ml of acetylacetone(ACAC) was dissolved in 500 ml of distilled water (D.W) and 10% ammonium hydroxide was added to the solution to adjust the pH to 8.5. 4.9 g of $DyCl_3 \cdot 6H_2O$ dissolved in 15 ml of D.W was added to the solution and then pale yellow precipitation(ppt) was performed. The mixture continues to stir for 24hrs to facilitate the formation of ppt. The obtained Dy-ACAC was washed several times

with deionized water to remove the un-reacted materials. Each 0.05, 0.1, 0.5 and 1 g of Dy-ACAC and 1.5 g of Poly-L-Lactic acid (PLLA) were dissolved in 37 ml of Chloroform and then added to 300 ml of 1.3% Poly vinyl alcohol (PVA) solution. Each mixture was continuously stirred for 40hrs with 500rpm. In addition, the experiments with different speeds (300,400,500 rpm, 0.5g Dy-AcAc and 1.5g PLLA were used) were done. The final microsphere was washed with D.W-0.1 M HCl-D.W respectively and dry at 50 ℃.

*2.3 Cytotoxicity evaluation of Dysprosium ion against Liver and Lung cancer cell lines.*

To evaluate cytotoxicity of Dysprosium ion, EZ-CYTOX assay was performed with the Liver cancer cell line (Hep G 2) and Lung cancer cell line (A549). The cell lines were routinely grown at 37 ℃ in DMEM medium (10% FBS, 1% penicillin-streptomycin).

The $5\times10^3$ cells/well were seeded into 96 well plates and grown for 24 h. Then, the 200 μl of various concentration of $DyCl_3$ solution (2000, 1000, 500, 250, 125, 63, 31, 16, 0 μg/ml) were treated to the cell lines and incubated for 24, 48 h at 37 ℃. After incubation, the 100 μl of 10% EZ-CYTOX reagent was added and incubated for 4 h. The absorbance of orange formazan product by active mitochondria was measured by plate reader at 450 nm.

## 3. Result and discussion

The microspheres are spherical with the size of 20-30 μm. The size of microsphere 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 surface shape was affected by concentration of Dy-ACAC. When 0.05 g of Dy-ACAC was used, the microsphere with smooth surface was formed. As shown in Figure 2, when the contents of Dy-ACAC in microsphere are increasing (0.1, 0.5g and 1g), the surface becomes rough even irregular wrinkle

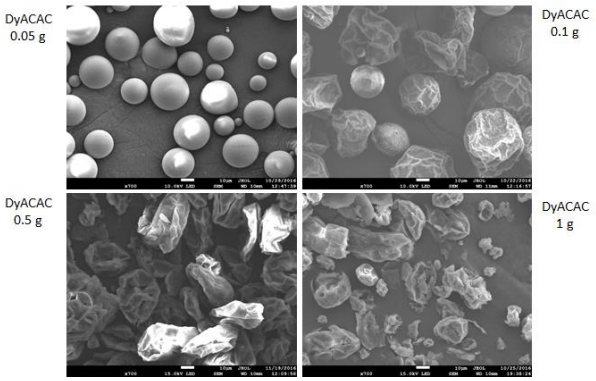


Figure 2. SEM image of microsphere against various DyACAC concentration.

The cytotoxicity of Dysprosium on Hep G2 and A 549 cell lines is shown in Figure 3. When high concentrated Dy ions(≥1000 μg/ml) are used, the cell viability is lowed to 30~45%. On the contrary, when the concentration of Dy is lowed((≤ 500 μg/ml), the cell viability are increasing to more than 100%. In case of Hep G 2 cell lines, the cell was activated by Dy ions and the viability is increasing as twice comparing with A549 cell lines.

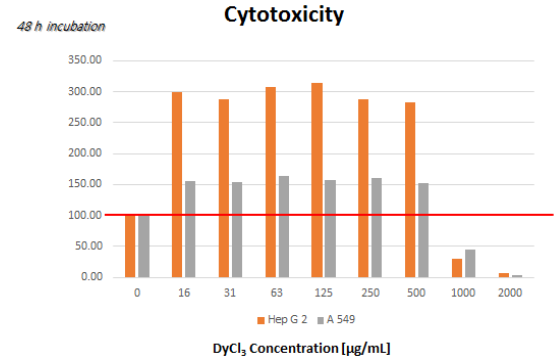


Figure 3. Cytotoxicity of $DyCl_3$ on Hep G 2 and A 549

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-microsphere and produce of irradiated $^{166}$Dy-microsphere will be done.

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