CO7-1 Evaluation of Boron-containig Rare Earth Oxides Nanoparticles as an Agent for Boron Neutron Capture Therapy

T. Nagasaki, R. Kawasaki, S, Iizuka, H. Yukawa, Y. Tsujimoto, M. Kirihata[1], K. Tomita[2], K. Katagiri[3], K. Ono[4], S. Masunaga[4], and Y. Sakurai[4]

Graduate School of Engineering, Osaka City University,
[1] BNCT Research Center, Osaka Prefecture University,
[2]School of Science, Tokai University
[3]Institute of Engineering, Hiroshima University
[4]Research Reactor Institute, Kyoto University

INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a highly selective cancer therapy that can target single tumor cell without causing excessive radiation damage around normal cells. The success of BNCT depends on the delivery that ^{10}B compounds accumulate effectively inside the tumor cells. Clinically, boronophenylalanine (BPA) and borocaptate sodium (BSH) are currently used for BNCT as boron delivery agents but these agents have some disadvantages on accumulation or selectivity toward tumor tissue. In addition, the efficacy of boron neutron capture reaction is suppressed due to low neutron fluence. In this study, in order to improve the efficiency of neutron capture reaction, we used boron containing rare earth oxides nanoparticle ($SmBO_3$, $GdBO_3$, YBO_3). When their diameter is suitable sizes, these nanoparticles could be significantly accumulated in tumor tissue with EPR effect. Moreover, the particle doped with europium can possess the fluorescence. This fluorescent nanoparticles are expected as novel boron delivery drugs that can diagnose and treat cancer simultaneously and efficiently.

EXPERIMENTS: The cell colorectal cancer line colon26 were used in this study. Boron containing rare earth oxides nanoparticles were synthesized by homogeneous precipitation method. Cationic nanoparticle are coated with anionic chondroitin sulfate (XBO_3/ch, X: Sm, Gd, Y). Boron concentration was estimated by ICP-AES. Cytotoxicity of nanoparticle was estimated by WST assay.

RESULTS AND DISCUSSION: The TEM images showed that the particle haves spherical shape with relatively homogenous distribution and its diameter is about 100 nm (Fig. 1). The result indicates that its size is suitable for EPR effect. Moreover, we evaluated its pharmacokinetics by using BALB/c mice bearing colon26 murine carcinoma. The highest boron concentration of YBO_3 nanoparticle in tumor tissue was observed at 6 hours after administration by i.p. Then, BNCT was performed on tumor-bearing mice. Two hundred μl of nanoparticle solution (concentration of ^{10}B: 132 ppm with $SmBO_3$ and $GdBO_3$, 88 ppm with YBO_3) were injected by i.p. before 6 hr of neutron irradiation at Kyoto University Research Reactor (1 MW, 90 min, 4.1×10^{12} neutron/cm^2). By irradiation, YBO_3 nanoparticle showed strongest antitumor effect among used rare earth oxides nanoparticles (Fig 2).

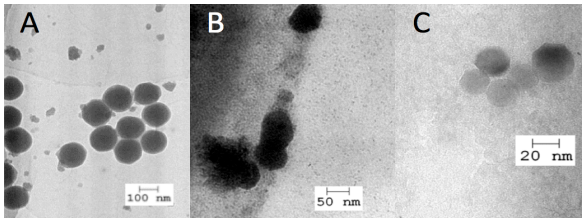

Fig. 1 TEM images of boron-containing rare earth oxide nanoparticles; (A) $SmBO_3$; (B) $GdBO_3$; (C) YBO_3.

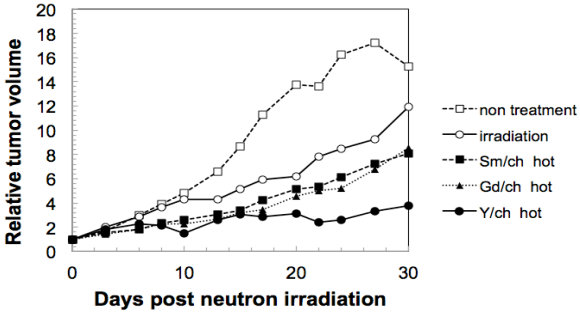

Fig. 2 Comparison of the antitumor efficacy with rare earth oxides/chondroitin sulfate complex.

As the nanoparticle of the rare earth oxide could contain huge boron atoms per particle, it can deliver efficiently a lot of boron toward tumor tissue with EPR effect. Especially, As yttrium had no shield effect toward neutron, YBO_3 showed highest BNCT effect. Therefore, this YBO_3 nanoparticle is promising toward next generation BNCT.

REFERENCE:

[1] K. Fujita *et al*., *J. Ceram. Soc. Jpn.*, **98**, 991 (1990).

採択課題番号 26005　　含ホウ素希土類酸化物のＢＮＣＴ薬剤としての評価　　通常採択

（阪市大院・工）長﨑　健、河崎　陸、飯塚俊輔、湯川寛子、辻本裕貴、櫻本昌士
（阪府大・21研究機構）切畑光統（東海大・理）冨田恒之（広大・工）片桐清文
（大阪府大BNCTｾﾝﾀｰ）服部能英（京大・原子炉）小野公二、増永慎一郎、櫻井良憲、田中浩基

CO7-2 *In vivo* Evaluation of Gd-DTPA-incorporated Calcium Phosphate Nanoparticles as Neutron Capture Therapy Agent

N. Dewi[1], P. Mi[2,3], H. Yanagie[1,4,5], Y. Sakurai[4], H. Cabral[2] , N. Nishiyama[3], K. Kataoka[2,6], Y. Sakurai[7], H. Tanaka[7], M. Suzuki[7], S. Masunaga[7], K. Ono[7], H. Takahashi[1,4]

[1]Dept of Nuclear Engineering & Management, Univ of Tokyo, [2]Bioengineering Dept, Univ of Tokyo, [3]Polymer chemistry division, Chemical Resource Laboratory, Tokyo Institute of Technology, [4]Cooperative Unit of Medicine & Engineering, Univ of Tokyo Hospital, [5]Dept of Innovative Cancer Therapeutics, Meiji Pharmaceutical University, [6]Materials Engineering Dept, The University of Tokyo, [7]Research Reactor Institute, Kyoto University

INTRODUCTION: Gadolinium-157 has been getting attention as alternative for neutron capture therapy (NCT) agent because of its high thermal neutron cross section (255 000 barns), which the highest among all stable elements. However, compared to short range secondary particles produced after neutron capture by ^{10}B isotope currently used for clinical trial of NCT, gadolinium neutron capture reaction (Gd-NCR) results in release of gamma rays, which reduce the localization effect of the treatment, which on the other side is increasing the possible additional effect if Gd-157 is accumulated to a bulk tumor cluster.

In this work, we performed in vivo evaluation of tumor growth suppression on multiple-injections Gd-DTPA/CaP nanoparticles mice as continued work from previous evaluation on single-injected group [1].

EXPERIMENTS: *In vivo* evaluation was performed on colon-26 tumor-bearing mice irradiated for 60 minutes at nuclear reactor facility of Kyoto University Research Reactor Institute with average neutron fluence of 1.8×10^{12} n/cm^2. Experimental procedure for multiple-injected groups is illustrated in Fig. 1. Antitumor effect was evaluated on the basis of the change in tumor growth and survival rate of the mice.

RESULTS: Higher gadolinium accumulation in tumor site was successfully achieved for multiple injections of Gd-DTPA/CaP nanoparticles, up to more than three times compared to single injection (data not shown). There was no acute toxicity in the treated mice, indicating the promising possibility of Gd-DTPA/CaP as Gd-NCT agent. However, we could not observe better tumor growth suppression after GdNCT treatment, which indicates the possibility of neutron depression on mice group with higher concentration of gadolinium. Nevertheless, further investigation is necessary to confirm the reason of moderate tumor growth suppression in multiple-injected mice group.

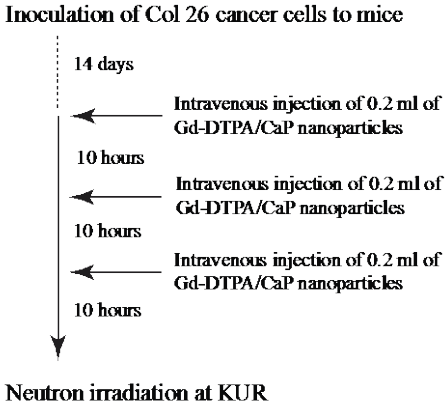

Figure 1. Experimental procedure for multiple-injected tumor-bearing mice.

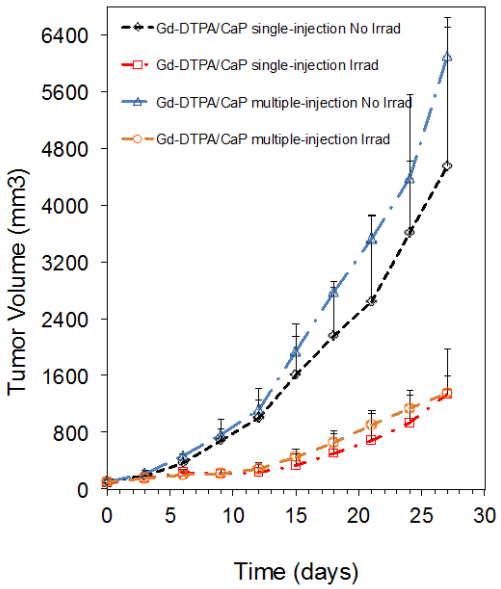

Figure 2. Tumor growth suppression comparison between single-injected and multiple-injected Gd-DTPA/CaP.

REFERENCES:

[1] Dewi N et al., KURRI Progress Report (2013) CO7-21.
[2] Dewi N et al., Biomed & Pharmacother (2013) **67**:451-7.
[3] Mi P, et al.: J Cont. Release (2014) **174**:63-7.

採択課題番号 26014　中性子捕捉療法法の一般外科領域癌への展開に向けた基礎的研究　通常採択

（東大・原子力国際専攻）高橋浩之、柳衛宏宣、Novriana Dewi（東大・獣医）柳川将志、飯塚智也（東大・心臓外科）櫻井由里子、毛利きくえ　（京大・原子炉）小野公二、増永慎一郎、鈴木　実、櫻井良憲

CO7-3 Development of a Phantom for Estimation of Beam Quality in BNCT

Y. Sakurai, T. Takata, N. Fujimoto, H. Tanaka, N. Kondo, Y. Nakagawa, M. Suzuki

Research Reactor Institute, Kyoto University

INTRODUCTION: After the restart of the operation of Kyoto University Reactor (KUR) in May 2010, 235 clinical studies of boron neutron capture therapy (BNCT) have already been carried out as of May 2015 [1]. Also, the BNCT clinical trial using Cyclotron-based BNCT Epi-thermal Neutron Source (C-BENS) started in November 2012 [2]. In the while, the research and development into several types of accelerator-based irradiation systems are underway by several research groups in the world at present time. With this situation in mind, it is important that the physical and biological estimations for dose quantity and quality are performed consistently among several irradiation fields, and that the equivalency of BNCT is guaranteed, even across BNCT systems. The individuality of irradiation fields can stand out for the beam-quality characteristic of the incident fast neutrons, such as the mixing ratio and neutron energy spectrum. The aim of this research is the development of the phantom made of specialized materials for the estimation of the beam quality. In 2014, a feasibility study on the estimation of relative biological effectiveness (RBE) for fast neutrons, based on the simulation and experimental data obtained for the proto-type "beam-quality estimation phantom".

METHODS: As a beam-quality estimation phantom, a phantom of 10% LiOH solution with 95%-enriched Li-6 was prepared [3]. The simulated thermal and fast neutron fluxes were converted into absorbed dose rates in normal tissue [4]. The composition for normal tissue was assumed to be H:11.1, C:12.7, N:2.0, O:74.2 in weight percent [5], and the density was assumed to be 1.0 g/cm^3. The operation power of KUR was 1 MW.

RESULTS: Figures 1 and 2 show the depth distributions of the total dose rate and its breakdown in the pure water phantom and the 10%-^{6}LiOH phantom, respectively. In the pure water phantom as shown in Fig. 1, the contribution of gamma rays is dominant and it becomes larger at depth, over the interior of the phantom. In the 10%-^{6}LiOH phantom as shown in Fig. 2, the thermal neutron dose rate decreases to below almost one-thirtieth of the fast neutron dose rate. In addition, the gamma-ray dose rate decreases to the same order as the fast neutron dose rate.

CONCLUSION: The condition for a larger contribution of fast neutron dose is realized in the 10%-^{6}LiOH phantom. It can be expected that the accuracy for RBE estimations in biological experiments would improve using the 10%-^{6}LiOH phantom as the contribution of fast neutron dose is increased to almost 50% greater than a pure water phantom for which the fast neutron dose contribution is at most 10%.

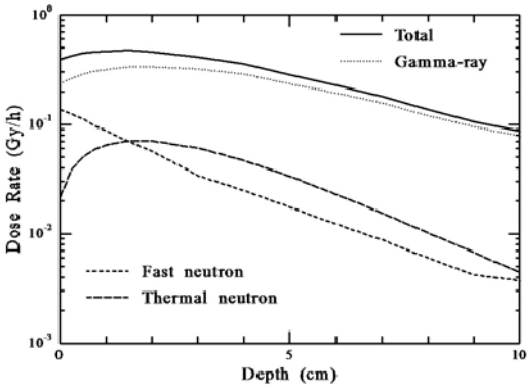

Fig. 1. Depth distributions of total dose rate and its breakdown in the pure water phantom.

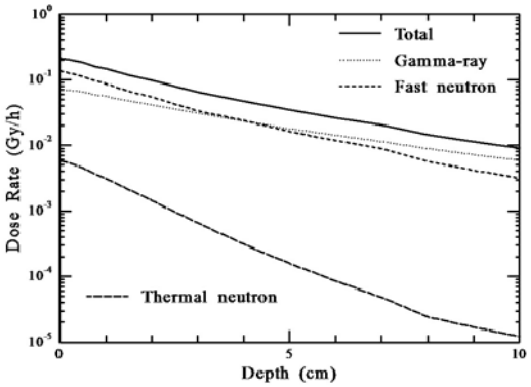

Fig. 2. Depth distributions of total dose rate and its breakdown in the 10%-^{6}LiOH phantom.

REFERENCES:

[1] Y. Sakurai *et al*., Nucl. Instr. Meth. A **453** (2000) 569-596.
[2] H. Tanaka *et al*., Nucl. Instr. Meth. B **267** (2009) 1970-1977.
[3] Y. Sakurai *et al*., Proc. 14th Int. Congr. Neutron Capture Therapy (Comision Nacional de Energia Atomica) (2010) 254-256.
[4] R.S. Caswell *et al*., Radiat. Res. **83** (1980) 217-254.
[5] W.S. Synder *et al*., Report of the Task Group on Reference Man (Pergamon Press, Oxford, 1975).

採択課題番号 26034　　ＢＮＣＴのための線質評価ファントムの開発　　通常採択
（京大・原子炉）櫻井良憲、田中浩基、藤本　望、近藤夏子、仲川洋介、鈴木　実

CO7-4 A Fundamental Experiment for the Measure Against the Activation of the Irradiation-room Concrete at BNCT Facility

Y. Sakurai, T. Takata, K. Kimura[1], H. Tanaka, K. Takamiya

Research Reactor Institute, Kyoto University
[1]Fujita Corporation

INTRODUCTION: At present time, the research and development into several types of accelerator-based neutron sources for boron neutron capture therapy (BNCT) are underway by several research groups in the world, with Cyclotron-based BNCT Epi-thermal Neutron Source (C-BENS) at the head of the list [1]. In near future, BNCT using the accelerator-based neutron sources may be carried out at several places in the world. Unlike the facilities for radiation therapy and charged-particle therapy, the neutron yield is larger at BNCT facility. Then, the activation of concrete, which is a main structure of the irradiation room, is larger. The use of low-activation concrete is prefer in the viewpoints of the decrease of exposure under the work in the irradiation room, the decommissioning of the irradiation room, etc.. This research is intended to perform the characteristic estimation for low-activation concrete and confirm its usability at BNCT facility. In 2014, a fundamental experiment for the measure against the activation of concrete using an Am-Be neutron source was performed, because the operation of Kyoto University Reactor (KUR) was limited.

METHODS: A characteristic estimation was performed for the measure against the activation using a neutron shield for a commercially available normal concrete. A resin containing B_4C, which is under development, was used as a neutron shield. This shield weakens neutron intensity to reach the concrete, by that high-energy neutrons are moderated by hydrogen in the resin and thermal neutrons are absorbed by boron in B_4C. As nine resin sheets of 10-cm side, 10-cm long and 2-mm thickness were stacked on the concrete surface, the shielding performance against the Am-Be neutron source was estimated by foil activation method. Indium foil was used as an activation foil. The shielding characteristic for fast neutrons was estimated by the activity change for In-113(n,n')In-113m reaction, and the generating characteristic for the secondary thermal neutrons was estimated by the activity change for In-115(n, γ)In-116m reaction.

RESULTS: Figure 1 shows the activity changes dependent on the thickness for the resin without B_4C. Figure 2 shows the activity changes dependent on the thickness for the resin with B_4C. In these figures, the activity changes for In-113(n,n')In-113m and In-115(n, γ)In-116m are drawn. From the comparison for the former activity, it was found that the shielding effect for fast neutrons was a little smaller for the resin with B_4C, as its hydrogen density was smaller. From the comparison for the latter activity, it was found that the generation of the secondary thermal neutrons was decreased to almost one fifth for the resin with B_4C. In the while, the activity, namely the thermal neutron intensity, was increased according to the thickness. This is due to the influence for the secondary thermal neutrons generated in the concrete.

CONCLUSION: From this experiment, it was realized again that the measure for the low activation of concrete itself, namely the development of low-activation concrete was important, even if the measure against the activation was performed using the neutron shield. The estimations for the important characteristics of low-activation concrete are planned, such as short-life activation, long-life activation, the shielding effect for neutrons and gamma rays, the generation of the secondary gamma rays, etc., when the KUR operation is restarted.

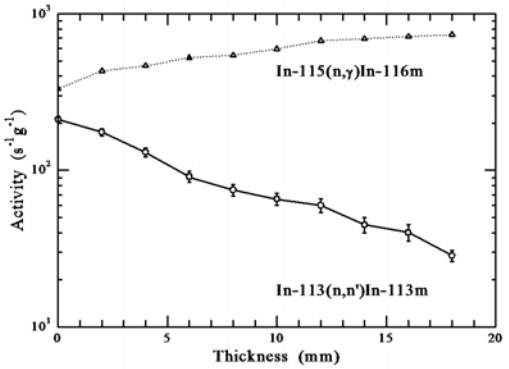

Fig. 1. Activity changes dependent on the thickness for the resin without B_4C.

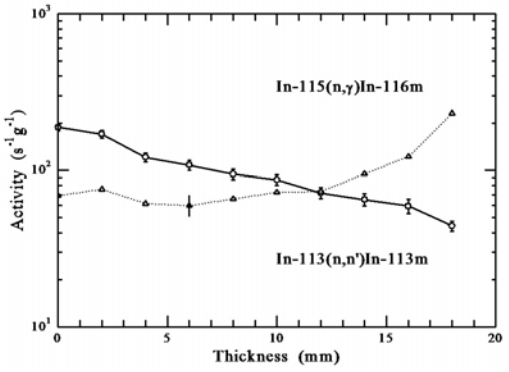

Fig. 2. Activity changes dependent on the thickness for the resin with B_4C.

REFERENCES:

[1] H. Tanaka *et al*., Nucl. Instr. Meth. B **267** (2009) 1970-1977.

採択課題番号 26035　　ＢＮＣＴ施設用低放射化コンクリートの特性評価　　通常採択
（京大・原子炉）櫻井良憲、田中浩基、高宮幸一

CO7-5

Radiosensitizing effect of purinergic antagonists on the damage of melanoma by BNCT

M. Tsukimoto[1], N. Mori[1], K. Nishino[1], T. Suzuki[1], K. Horie[1], K. Tanamachi[1], D. Kitahara[1], N. Kondo[2], S. Masunaga[2], Y. Sakurai[2], R. Abe[1]

[1] *Tokyo University of Sciences*
[2] *Research Reactor Institute, Kyoto University*

INTRODUCTION: Radiation exposure causes DNA damage, and DNA repair systems are essential to rescue damaged cells. Release of extracellular nucleotides, such as ATP, from cells plays a role in signaling via P2 receptors. We have reported that autocrine/paracrine signaling through P2X7-dependent ATP release and activation of P2Y6 and P2Y12 receptors serves to amplify the cellular response to DNA damage caused by γ-irradiation [1-3].

BNCT is one of the new radiation therapy that mainly use α-ray derive from $^{10}B(n,\alpha)^{7}Li$ reaction. ^{10}B is delivered to the cell as ^{10}B-bromophenylalanine (BPA). Since it is known that melanoma tend to incorporate phenylalanine, ^{10}B-BPA is well incorporated into melanoma. γ-ray is low-LET radiation, and BNCT is high-LET radiation. γ-Irradiation causes DNA damage by indirect action effect, which is mediated by reactive oxygen species. However, BNCT causes DNA damage by direct action. It is known that direct action effect is stronger than indirect action effect. Further, BNCT can irradiate cells from inside of the cell.

Recently, we have showed involvement of P2 receptors in DNA damage repair after γ-ray irradiation in A549 cells [2]. However, it has not yet been clear the involvement of P2 receptors in BNCT-induced DNA damage response. If the activation of P2X7, P2Y6, or P2Y12 receptor are involved in BNCT-induced DNA damage response, the antagonists of these P2 receptors enhance cytotoxicity of cancer cells by BNCT. Here, we investigated the radiosensitizing effect of P2 receptor antagonist on BNCT-induced DNA damage response and cytotoxicity.

EXPERIMENTS: Mouse melanoma B16 cells were incubated with 1 mM ^{10}B-BPA and P2Y12 receptor antagonist clopidogrel. B16 cells were irradiated with γ-rays and thermal neutron beams from a nuclear reactor (Kyoto University Research Reactor Institute;1 MW) at room temperature for a suitable time. After irradiation, the cells were incubated in humidified atmosphere of 5% CO_2 in air at 37 °C.

The irradiated cells were fixed in 4% paraformaldehyde in PBS for 10 min at room temperature and permeabilized in 0.1% Triton X-100 for 5 min on ice. After incubation in blocking buffer (10% FBS in PBS) for 1 h, the fixed cells were incubated with primary antibody against γH2AX for 24 h at 4 °C and with 2nd antibody conjugated with FITC for 1 h. Counterstaining with Hoechst 33258 was used to verify the location and integrity of nuclei. Fluorescence images were obtained with a laser scanning confocal microscopy.

On the other hand, cell viability of irradiated cells were measured by colony formation assay. 1.0 x 103 cells were seeded in a 100 mm dish. After incubation for 1 week, the cells were stained with 0.5% crystal violet. Colonies containing more than 50 cells were counted.

RESULTS: First, we tested the effect of BNCT on cell survival rate by the colony formation assay. Survival fraction of BNCT group decreased compared with group of γ-irradiated cells. In addition, the cytotoxic effect of BNCT was enhanced by pre-treatment with clopidogrel (Fig.1A). Next, we tested DNA damage response after BNCT by analyzing γH2AX focus formation. Formation of γH2AX foci that remain for 48hr was increased by BNCT. The BNCT-induced increase of γH2AX foci was suppressed by pretreatment with clopidogrel (Fig. 1B).

From these results, it was suggested that P2Y12 receptor participated in DNA damage repair caused by BNCT.

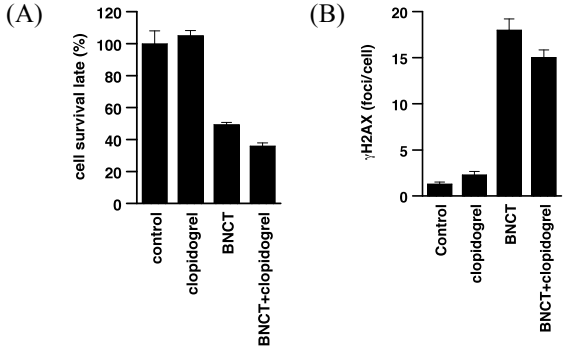

Fig.1 The effect of clopidogrel on BNCT-induced DNAdamage responce

REFERENCES:

[1] Tsukimoto M. et al., Radiat. Res. (2010) 173(3): 298-309.
[2] Nishimaki N et al., DNA Repair (Amst). (2012) 11 (8): 657-65.
[3] Ide S. et al., J Toxicol Sci. (2014) 39(1):15-23.

採択課題番号 26061　　DNA 鎖損傷修復抑制効果をもつプリン受容体阻害薬を用いた　通常採択
BNCTによる抗癌増強効果

（東京理科大・薬）月本光俊、森夏樹、西野圭祐、棚町圭祐、北原大輔、（東京理科大・生命研）鈴木利宙、堀江　和峰（京大・原子炉）櫻井良憲、増永慎一郎、近藤夏子

CO7-6 Feasibility Study for Establishing QA Method for Hospital- and Reactor-based BNCT

S. Nakamura[1, 2], A. Urushiyama[2], T. Nishio[3], C. Kurokawa[4], A. Wakita[1], M. Ito[1], M. Munechika[5], H. Okamoto[1], Y. Sakurai[6], H. Tanaka[6], N. Fujimoto[6] and J. Itami[1]

[1]*Department of Radiation Oncology, National Cancer Center*
[2]*Rikkyo University*
[3]*Graduate School of Biomedical & Health Sciences, Hiroshima University*
[4]*Department of Radiology, Juntendo University*
[5]*Department of Human Sciences, Tokyo Metropolitan University*
[6]*Research Reactor Institute, Kyoto University*

INTRODUCTION: In general, boron neutron capture therapy (BNCT) is currently performed using a nuclear reactor [1], [2]. Recent researches [3], [4], [5] have made possible to acquire an adequate amount of neutrons by accelerator and hospital-based accelerator-BNCT has come into reality. Therefore, National Cancer Center, Tokyo, Japan, is planning to install an accelerator-based BNCT system. Hospital-based accelerator-BNCT system has a lot of advantages. For example, there is no need to move a patient to the reactor. However, the hospital-based system also has disadvantages. In the hospital-based BNCT system, with compared with the reactor-based BNCT system, the measurement for a beam of BNCT is asked for the simplified method because the hospital-based system is restricted by an area of the institute. The purpose of this study is to establish the QA method for hospital-based BNCT.

EXPERIMENTS: In order to measure easily the neutron beam, new film was developed. The new film consisted of thermos luminescence phosphor. After exposure of the radiation, the output of the film with heated was red by CCD. Signal intensity was measured as the amount of lilght. The relationship of the film between the signal and doses were acquired with 6 MV photon beam of a medical linac in Juntendo University, Tokyo, Japan. The relationship between the signal and neutron fluence was analyzed in this study because the signal was evaluated as the dose. The experiment of neutron irradiation was performed in Kyoto University Research Reactor (KURR). In order to evaluate the film, the thermal neutron fluence was measured with the film and a gold foil, and these results were compared. However, the beam of the KURR contained both neutrons and gamma-rays. Therefore, the dose from gamma-rays was measured with the thermoluminescence dosimter (TLD) since the film also had sensitivity to photons. The contribution from the gamma-rays to the film was considered by the result of the TLD. In order to evaluate a relationship between the $TPR_{20,10}$ of a medical linac and the energy dependence of the film to gamma-rays, Monte Carlo simulation (BEAMnrc ver. 4.2.4. [6]) was used, and comparison of PDDs between measurement and simulation with the BEAMnrc were performed. Therefore, contribution of the gamma-rays was subtracted from the output of the film.

RESULTS: The comparison of PDDs between measurement and simulation was performed with a medical linac of 4, 6, 10, and 15 MV photon beam in National Cancer Center (NCC), Tokyo, Japan. The difference of those beams between the measurement and the simulation was within 0.59%. The relationship between $TPR_{20,10}$ and the calculated energy dependence which was normalized in the value of 6MV photon beam in NCC. With using those relationship and BEAMnrc, energy dependence of the film in the medical linac in Juntendo University and that in KURR was 1.002±0.003, and 0.986±0.002, respectively. Outputs of the TLD after irradiation in KURR were multiplied by a value of the ratio of energy dependence in KURR to that in Juntendo University, and the multiplied values were subtracted from the dose with the film. The relationship between the thermal neutron fluence and the dose with the film was shown in Fig. 1. The thermal neutron fluence was proportional to the dose with the film.

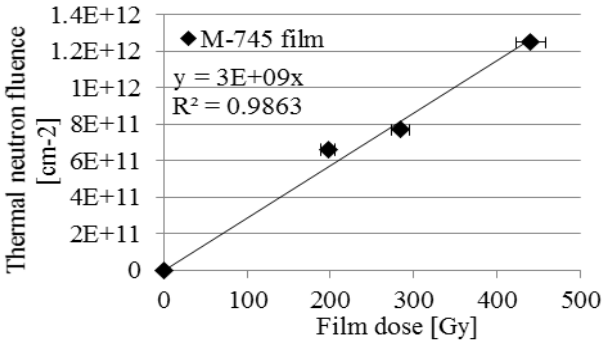

Fig. 1. The relationship between the dose with the film and thermal neutron fluence.

REFERENCES:

[1] K. Nakai *et al.*, Appl. Radiat. Isotopes., **69(12)**: 1790-1792; (2011)
[2] S. Masunaga *et al.*, Br. J. Radiol., **85(1011)**: 249-258; (2012)
[3] S. Kamada *et al.*, Appl. Radiat. Isotopes., **88**: 195-197; (2014)
[4] H. Kumada *et al.*, Appl. Radiat. Isotopes., **88**: 211-215; (2014)
[5] H. Tanaka *et al.*, Appl. Radiat. Isotopes., **69**: 1642-1645; (2014)
[6] DWO Rogers *et al.*, Med. Phys., **22**: 503-524; (1995)

採択課題番号 26068　　BNCT 照射場の QA/QC に関する基礎研究　　通常採択
（国立がんセ・放治）中村　哲志、岡本　裕之、脇田　明尚、伊藤　昌司、伊丹　純、（広島大院・医歯薬）西尾　禎治、（首都大・人間）宗近　正義、（京大・原子炉）櫻井　良憲、田中　浩基、藤本　望