

I-1. PROJECT RESEARCHES

Project 7

Estimation for 3D Distribution of Biological and Chemical Doses for BNCT

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BACKGROUNDS AND PURPOSES: For the further advancement of BNCT, it is important to improve the estimation for biological effect due to neutrons. At Heavy Water Neutron Irradiation Facility of Kyoto University Reactor (KUR-HWNIF), the fundamental studies for BNCT in the fields of medicine, biology, chemistry and pharmacology, etc., have been performed. Through these studies, the difficulty in the estimation for neutron biological effects is being acutely realized. In the BNCT irradiation field, it is difficult to monochromatically generate neutrons with a specific energy, and neutrons with the various energy ranges such as thermal, epi-thermal and fast neutrons, and also gamma rays are mixed. Furthermore, the neutron energies and the types and energies of secondary charged particles due to the nuclear reactions in living body are different. The biological effect estimation for BNCT are being studied by multiple groups in the world, but the consistent estimations have not been possible due to the difficulty of estimation for the biological effects, particularly regarding neutrons. The final goal of this project research is to develop a consistent estimation method of the biological effects for BNCT. In 2024, the developments of the estimation method for the 3D dose distribution, focused on the biological and chemical effects were continued. For the biological dose estimation, the estimation methods using three-dimensional co-culture, which can model the structure of living tissues and organs, were mainly studied. For the chemical dose estimation, the estimation methods using chemical dosimeters such as gel dosimeters were studied.

RESEARCH SUBJECTS: The collaboration and allotted research subjects (ARS) were organized as follows;

ARS-1 (R6P7-1: “Establishment of beam-quality estimation method in BNCT irradiation field using dual phantom technique (VIII)”, Y. Sakurai, *et al.*.

ARS-2 (R6P7-2: “Biological effects of BNCT on glioma cells in 3D culture”, N. Kondo and Y. Sakurai.

ARS-3 (R6P7-3: “The response after BNCT on 3D oral cancer model using patient-derived cancer-associated fibroblasts”, K. Igawa, *et al.*.

ARS-4 (R6P7-4: “Verification of BNCT effect on hematological cancer cells and dosimetry for expansion of BNCT cases”, S. Yoshihashi, *et al.*.

ARS-7 (R6P7-7: “Development and evaluation of 3D gel dosimeter for the measurement of dose distribution in BNCT”, S. Hayashi, *et al.*.

ARS-8 (R6P7-8: “Establishment of three-dimensional dose distribution estimation method for BNCT using radiochromic gel dosimeter (II)”, Y. Sakurai, *et al.*.

ARS-10 (R6P7-10: “Measurement of neutron depth distribution for quality assurance in boron neutron capture therapy”, M. Takada, *et al.*.

ARS-11 (R6P7-11: “Measurement of wavelength-dependent luminescence for beam quality assurance of BNCT”, K. Tanaka, *et al.*.

ARS-12 (R6P7-12: “Feasibility of water/fat decomposition technique using multi-energy X-ray computed tomography for BNCT dose calculation”, T. Takata, *et al.*.

ARS-5 (R6P7-5), ARS-6 (R6P7-6), ARS-9 (R6P7-9), ARS-13 (R6P7-13) could not be performed due to the scheduling and preparation issues. So, the reports of these subjects are not appeared.

Establishment of Beam-quality Estimation Method in BNCT Irradiation Field using Dual Phantom Technique (VIII)

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INTRODUCTION: Before the start of treatment with BNCT, the relative biological effectiveness (RBE) for the fast neutrons incident to the irradiation field must be estimated. Measurements of RBE are typically performed by biological experiments with a phantom. Although the dose deposition due to secondary gamma rays is dominant, the relative contributions of thermal and fast neutrons are virtually equivalent in a water and/or acrylic phantom. Uniform contributions to the dose deposited from thermal and fast neutrons are based in part on relatively inaccurate dose information for fast neutrons. The aim of this study is to establish the accurate beam-quality estimation method mainly for fast neutrons by using two phantoms made of different materials [1]. In 2024, verification experiments for the dual phantom technique were continued using Heavy Water Neutron Irradiation Facility installed in Kyoto University Reactor (KUR-HWNIF) as in the previous year [2].

MATERIALS AND METHODS: One of the dual solid phantoms was made of polyethylene with natural lithium fluoride for 30 weight percent (LiF-polyethylene phantom), and the other phantom was made of polyethylene with 95%-enriched lithium-6 fluoride for 30 weight percent (^6LiF -polyethylene phantom). Glioblastoma cells were divided in two groups. One was treated group using p-boronophenylalanine (BPA, containing B-10) for B-10 concentration of 25 ppm, and the other was non-treated group. In addition to the conventional cell suspension, the cell in collagen gel was also prepared. These cell samples were placed at the depths of 0 cm, 2 cm, 5 cm and 8 cm in the phantoms. Figure 1 shows the arrangement of the cell samples within the phantom plate. The depth dose distributions for the thermal neutron, fast neutron and gamma-ray components were determined based on the simulation calculation results normalized referring to the measured values. The epi-thermal neutron irradiation mode was used for the phantom experiments.

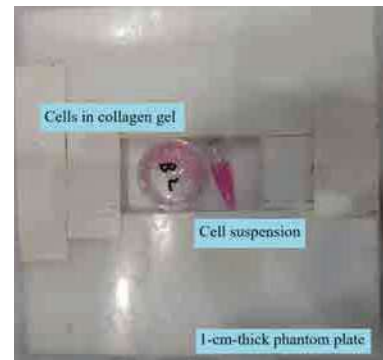


Fig. 1. Arrangement of the cell samples within the phantom plate.

RESULTS: It was confirmed that the survival fraction became smaller as the thermal neutron flux became larger in the LiF-polyethylene phantom. The survival fraction for the non-treated cell was higher than that in BPA-treated cell. In the ^6LiF -polyethylene phantom, the survival fraction showed no difference between non-treated and BPA-treated cells. These confirm the results from the previous years, with the same trends observed for the cells in suspension and gel. Incidentally, the differences were observed in the slopes of the dose-survival curves between these cell sample types.

ACKNOWLEDGMENT: This work was supported by JSPS KAKENHI Grant Number JP 24K03082.

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Biological effects of BNCT on glioma cells in 3D culture

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) have been applied to recurrent malignant glioma and even after standard therapy (surgery, chemo-radiation therapy) because of the selective damage to the tumor. Especially, glioblastoma (GBM) is the most miserable cancer, whose patient survival is 14.6 months and remarkably resistant to chemo-radiation and immuno-therapy. With BNCT, we achieved better local control and survival benefit in malignant glioma using thermal neutrons produced by the reactor in Kyoto University. However, the recurrence locally or distantly is inevitable after BNCT. To clarify the underlying mechanism in recurrence of glioma after BNCT is an urgent issue. On the other hand, 3D culture is more advanced and useful method than 2D culture to recapitulate the in vivo situation. Therefore, we firstly investigated the biological effects of BNCT on glioma cells in 3D culture and compared with 2D culture.

EXPERIMENTS:

Cells:

We used mouse glioma GL261 cells. They were cultured in DMEM with 10% heat-inactivated fetal bovine serum in 5 % CO₂ incubator. For the adherent 2D culture, cells were disseminated on the tissue-treated 6-well plates. For 3D culture, cells were suspended in the mixture of Cell matrix type 1A collagen (3mg/ml, pH 3), 5 x DME concentrated culture medium and the reconstitution buffer on ice, according to the manufacturer's protocol (Nitta Gelatin, Osaka Japan). Next, the suspended cells in the 500 µL of collagen solution were disseminated into each well of a 24-well plate and placed in the 5 % CO₂ incubator at 37 °C for 30 minutes for gel formation. Then, 500 µL of DMEM was poured over the gel. The DMEM was changed every three or four days.

p-Boronophenylalanine (BPA) treatments:

For 2D culture, BPA was added to the culture medium at 0 to 20 ppm final concentration, two hours before irradiation. For 3D culture, medium was changed to BPA containing DMEM (0 to 20 ppm), the day before irradiation. The medium was removed just before irradiation.

Neutron irradiation

We irradiated thermal neutrons for 5 minutes to the glioma cells in 2D culture, and 50 minutes to the glioma cells in 3D culture using the heavy water neutron irradiation facility in KUR. The cells was placed in 5 % CO₂ incubator with fresh DMEM after irradiation.

Sampling

One week after irradiation, we counted the survived cells. To suspend the cells, for 2D culture, we treated the cells with trypsin. For 3D culture, we treated collagenase to dissolve the collagen gel.

RESULTS:

The higher the BPA concentration, the lower the surviving rate. Compared with 2D culture, the glioma cells in 3D culture were resistant.

The Response After BNCT on 3D Oral Cancer Model Using Patient-Derived Cancer-Associated Fibroblasts

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INTRODUCTION: Cancer-associated fibroblasts (CAFs) are one of the most important components of tumor microenvironment (TME). Many studies have revealed that CAFs promote cancer progression, invasion, metastasis, and treatment resistance. In this study, we developed a three-dimensional oral cancer model with patient-derived CAFs and oral cancer cells, and to simulate the TME. We aimed to investigate radiation-induced effects on CAFs using X-ray irradiation and BNCT.

EXPERIMENTS: A 3D oral cancer model was fabricated using our previously reported protocol. The 3D oral cancer models were consisted of two layers; a stromal layer composed of either patient-derived normal oral fibroblasts (NOFs) or patient-derived cancer-associated fibroblasts (CAFs) (provided by Niigata University) and a cancer layer using the oral cancer cell line HSC-4 (JCRB0624). **X-ray irradiation:** 3D models were irradiated at single doses of 0, 5, 10 and 20 Gy using an X-ray desktop-operated machine at Okayama University. **Neutron irradiation:** Neutron beams were applied at the Heavy Water Neutron Irradiation Facility of KUR at 1MW operation for 0, 20 and 40 minutes. 10% BPA-supplemented medium was added 24 hours before irradiation. **Histological analysis:** The samples were collected 5 days after irradiation and stained with Masson's trichrome for histological evaluation.

RESULTS: Figure 1 shows the Masson's trichrome stained histopathological specimen including 0, 5, 10, and 20 Gy of X-ray irradiation (i) and 0-, 20-, 40-minute neutron irradiation (ii). After BNCT was conducted, a decline in the depth of cancer cell invasion in 3D cancer-CAFs model was observed (Figure 2 (i)), which didn't significantly change after X-ray irradiation (Figure 2 (ii)) [1].

Figure 1

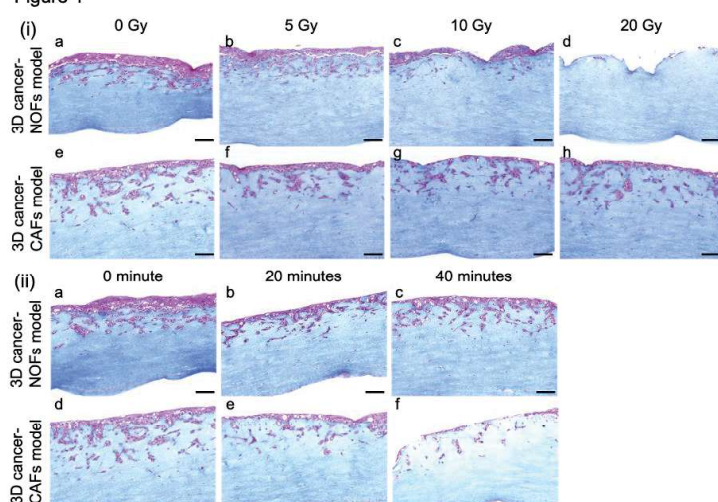
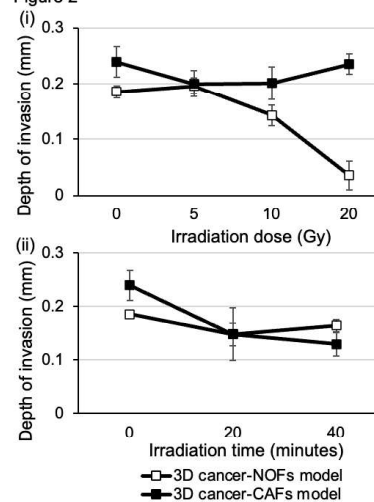


Figure 2



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Verification of BNCT Effect on Hematological Cancer Cells and Dosimetry for Expansion of BNCT Cases

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INTRODUCTION: The number of newly diagnosed patients with major hematologic cancers (malignant lymphoma, multiple myeloma, and leukemia) in Japan was 58,547 in 2019 [1], and 5,853 hematopoietic stem cell transplants were performed in FY2022 as the last treatment option for these refractory hematologic cancer patients [2]. While autologous transplantation is a desirable medical technique for patients because it does not require long-term administration of immunosuppressive drugs and prevents immune reactions (GVHD) caused by HLA incompatibility, the grafts contain tumor cells, which must be removed and treated with chemotherapy and radiotherapy, which are pre-treatment procedures. We are now conducting basic research to remove tumor cells in grafts by Ex Vivo BNCT. In this study, the response of PVA-GTA-I gel dosimeter to thermal neutrons, fast neutrons, and gamma-ray was evaluated to use the gel dosimeters for dose assessment in cell bags.

EXPERIMENTS: PVA-GTA-I gel is prepared by adding PVA as the main component, KI as the reactant, Fructose as the reducing agent, GTA as the gelator, and GDL as the gelation promoter by adding GTA. In this study, 100 mM of B-(OH)₃ was added to PVA-GTA-I gel to make it sensitive to thermal neutron beams. The prepared gels were sealed in an optical cell with a 1 cm optical path length.

Two irradiation systems were prepared: System A, in which an optical cell was placed in the beam direction, and System B, in which a Teflon plate containing LiF, which absorbs thermal neutrons, was placed in front of System A to remove the thermal neutron component.

In both systems A and B, irradiation was performed using the KUR heavy water irradiation facility, and the irradiation time was 60 minutes. The 486 nm absorbance of each of the neutron-irradiated gels was measured using a UV-visible spectrophotometer.

RESULTS: The distribution of absorbance in the depth direction in system A and B shows that the PVA-GTA-I gel with B-(OH)₃ is sensitive to thermal neutron radiation. The total dose was assumed to be the sum of thermal neutrons, fast neutrons, and gamma ray radiation, and the response of PVA-GTA-I gel to each dose was determined. The response to thermal neutron was calculated from the difference in sensitivity between System A and System B. The response to gamma ray was calculated using the results of calibration experiment using a ⁶⁰Co source. These results indicate the potential use of gel dosimeters for dose assessment within cell backing.

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Development and evaluation of 3D gel dosimeter for the measurement of dose distribution in BNCT

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INTRODUCTION: Three-dimensional (3D) gel dosimeters have been developed for the 3D dose measurement of the complex conformal dose distributions in radiation therapy [1]. These devices are composed of radio-sensitive substances and an aqueous gel matrix to preserve spatial information about the absorbed dose. The 3D absorbed dose distribution is deduced from the distribution of the reaction products measured by 3D imaging modalities such as MRI and Optical CT. In previous studies, we have developed a PVA-GTA-I radiochromic gel dosimeter that utilizes red color development due to the complex formation of polyvinyl alcohol (PVA) and iodide [2]. In this work, to compare the neutron sensitization effect with that of ¹⁰B, gadolinium contrast agent for MRI was added to the gel dosimeter in different concentrations and the response to a neutron beam from a nuclear reactor was evaluated.

EXPERIMENTS: The PVA-GTA-I gel dosimeter is composed of partially saponified PVA, potassium iodide (KI), glutaraldehyde, fructose, glucono- δ -lactone, and water. As a thermal neutron sensitizer, a Gd contrast agent (Gadovist[®]) was added into the gel dosimeters. The resulting solution was subdivided by pouring into PMMA cuvettes (4.5 mL, 1 cm path length). The neutron irradiations were performed using the HWNIF of KURR (1 MW) in the air at room temperature. The three different modes (mixed, thermal, and epi-thermal modes) of neutron beams made by heavy water spectrum shifter and cadmium thermal-neutron filters were applied to the samples. After irradiation, the response was evaluated on the change in absorbance at the absorption peak wavelength (486 nm) using a UV-Vis spectrophotometer.

RESULTS: Figure 1 shows the samples irradiated in each mode and the change in absorbance with respect to the Gd concentration. In all cases, the addition of a small amount of Gd caused a rapid increase in color development. For example, in the mix mode, even with 10 mM Gd, the response was approximately 6 times higher than that of the unadded sample. On the other hand, the increase in absorbance was almost saturated at 50 mM or more. ¹⁵⁷Gd has an absorption cross section for thermal neutrons that is about 60 times larger than that of ¹⁰B. These results indicate that ¹⁵⁷Gd contained in the contrast agent can be stably added to the radiochromic gel dosimeter, enabling it to detect thermal neutrons with higher sensitivity.

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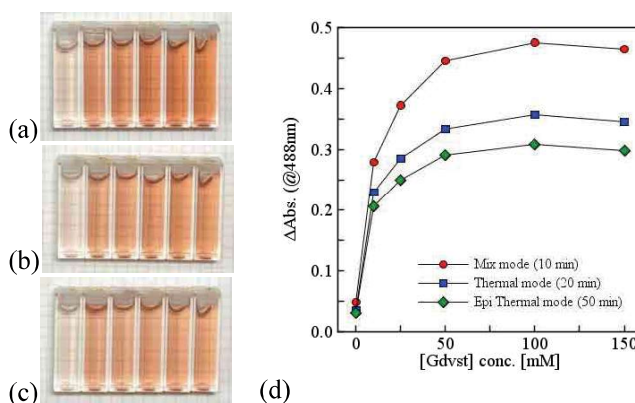


Figure 1 (a), (b), and (c) are PVA-GTA-I gel samples containing different concentrations of Gd (from left to right: 0, 10, 25, 50, 100, and 150 mM) irradiated in mixed, thermal, and epi-thermal modes, respectively. (d) shows their absorbance changes.

Establishment of Three-dimensional Dose Distribution Estimation Method for BNCT using Radiochromic Gel Dosimeter (II)

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INTRODUCTION: Development in accelerator-based irradiation systems for BNCT is underway. BNCT using newly developed accelerator systems is being implemented at multiple facilities around the world. However, there are still issues that need to be improved for further advancement of BNCT. The advancement of dose estimation is one of these, and the estimation of biological dose is particularly important. As part of the advancement of biological dose estimation for BNCT, we focus on chemical effects, which are the precursor to biological effects. We are studying on the estimation method using chemical dosimeters, especially gel dosimeters. Gel dosimeters are made of materials similar to living tissue and can be formed into any shape. Using a phantom made from gel dosimeter that models human body, three-dimensional dose distribution estimation becomes possible. Among the various types of gel dosimeters, we are especially focusing on “radiochromic gel dosimeters” that can be read out using optical CT, etc.. The purpose of this study is to establish three-dimensional dose distribution estimation method for BNCT using multiple types of radiochromic gel dosimeters. In 2024, the characterization for micellar gel dosimeters was continued.

MATERIALS AND METHODS: Leucocrystal violet (LCV) was selected as dye of micelle gel dosimeters, because it is extremely clear before irradiation, based on our previous study [1]. The micelle gel dosimeters were prepared based on 1 mM of LCV in the 96% water and 4% gelatin according to the method of Babic et al. [2]. For dose rate independency and temporal coloration reduction, the type and concentration of surfactant and sensitizer were changed. In addition, boric acid or urea was added for the estimation of boron or nitrogen dose components, referring to Gambarini et al. [3]. Furthermore, a dosimeter in which heavy water was replaced with light water was also prepared to estimate hydrogen dose component [4]. The amount of additives was based on our previous study. The characterization experiments were mainly performed using Heavy Water Neutron Irradiation Facility of Kyoto University Reactor (KUR-HWNIF) [5]. In the experiments, three irradiation modes, such as the standard mixed neutron irradiation mode, the standard epi-thermal neutron irradiation mode and the standard thermal neutron irradiation mode, were used. The absorbance of the gel dosimeters after the irradiation was measured at the peak wavelength of 600 nm using a spectrophotometer (V730-Spectrophotometer, Japan Spectroscopic Co., Ltd.).

RESULTS: From the experiment results, it was confirmed that the dose response of each gel dosimeter depended on the linear energy transfers (LETs) of the particles produced in each dosimeter. Using these results, the factors for dose-component discrimination method were determined and verified for different energy spectra, and the validity was confirmed.

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Measurement of Neutron Depth Distribution for Quality Assurance in Boron Neutron Capture Therapy

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INTRODUCTION: Thermal neutron depth distributions in acrylic and water phantoms have been generally measured using a gold neutron activation analysis for conducting quality assurance (QA) program in boron neutron capture therapy. For evaluation of thermal neutron distribution, radioactivity of gold wires are measured by using a high purity germanium detector, and gold wires are covered with and without a cadmium case to measure thermal neutron contribution in each measurement point. Hence, huge amount of medical staff manpower and time are required to perform the QA program. Thus, a simpler and less costly technique of measuring the depth distributions in the phantom is required to improve frequency and time of the QA procedure.

In this study, a technique of easy-to-use aluminum indirect neutron computed radiography was applied to measure the in-phantom 2D thermal neutron depth distribution to improve the BNCT QA procedure.

EXPERIMENTS: The neutron depth distributions were measured to verify this technique at heavy water irradiation facility. The aluminum sheet was inserted into an acrylic phantom with 300×300×300 mm. Neutron beam was irradiated to the aluminum sheet in the acrylic phantom for 10 minutes (5MW), then the radioactivated aluminum sheet was exposed to an imaging plate (IP). The neutron-induced radioactivity distribution in the aluminum was transferred to the IP. The radioactivity distribution on the IP was scanned by the IP reader. By just one neutron irradiation, the neutron depth distributions of 0-300 mm in depth and further offset neutron depth distributions were obtained. In addition, the neutron depth distribution was measured by the gold neutron activation method to verify this research result.

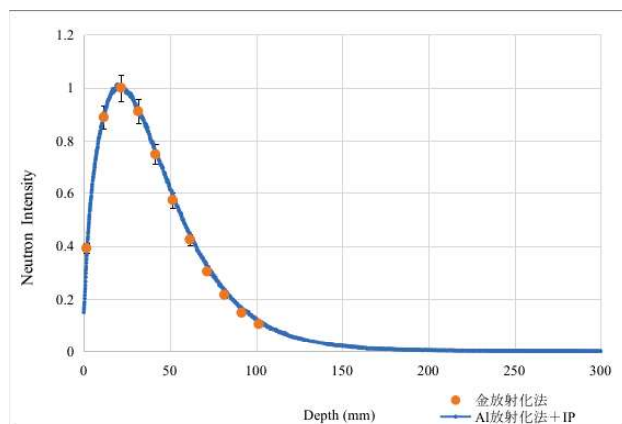


Fig. 1. Comparison of depth neutron distribution using aluminum- and gold-activation methods.

RESULTS: Figure 1 shows the measured neutron depth distribution at neutron beam center, as a solid line. The distribution is compared with the gold saturation radioactivity, shown as closed circles. The distributions were normalized at the peak around the 20-mm depth. The experimental depth distribution shows the neutron peak of 19.7 mm with FWHM of 54.5 mm. Both experimental results were in good agreement. The ratio of the aluminum to the gold neutron activation results showed 1.0–1.06 in the 0-80 mm depths. Based on the agreement within 0-6% difference, the aluminum neutron activation method was validated to measure the neutron depth distribution of BNCT

neutron beam in the acrylic phantom. Although relative neutron distributions were obtained using this method, an absolute neutron distribution could be obtained with the help of the gold activation method.

Measurement of wavelength-dependent luminescence for beam quality assurance of BNCT

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INTRODUCTION: In boron neutron capture therapy, measurement of beam fluence spatial distribution is required for quality assurance in the irradiation field. Previously, the imaging plate was tested for usage in this purpose[1]. The luminescence material for imaging plate, BaFBr:Eu, has multiple peaks in its excitation spectrum[2]. The dependence of the quantity of the luminescence on the wavelength of the excitation light, or possibly luminescence, will specify the beam quality. Thus, this study investigated the use of the luminescence for measurement of both quantity and quality of the radiation. A candidate of the luminescent material, BaFBr:Eu, has been proposed[3]. In this report, additional candidate was been surveyed to improve the measurement of beam quality.

EXPERIMENTS: As candidates, BeO, Al₂O₃:Cr, and Al₂O₃:CrMnCo were tested. The samples were irradiated with gamma rays from ⁶⁰Co, and neutrons and gamma rays at the standard mixed neutron irradiation mode of Kyoto University Reactor Heavy Water Neutron Irradiation Facility[4] at 1 MW of the power. In the latter, the samples were driven with Irradiation Rail Device. The dependence of the fluorescence quantity on the wavelength was measured with the fluorescence spectrofluorometer (Hitachi corporation, F-2700).

RESULTS: An example of the excitation spectrum is shown in Fig. 1 for Al₂O₃:Cr, and Al₂O₃:CrMnCo. The ratio of the luminescence quantity by excitation at 458 nm to that at 550 nm is about 10-20% lower for KUR than that for Co, where the uncertainty of the measured ratio is several %. Though the figure is not shown, the luminescence spectra for Al₂O₃:Cr and Al₂O₃:CrMnCo suggest the dependence of the quantity of the luminescence on the wavelength of the excitation light for Al₂O₃:Cr, and Al₂O₃:CrMnCo possibly indicates the quality of the irradiated beam. In this case, the spectrum does not show apparent difference between KUR and Co in this case. Also, the results for BeO did not show the spectrum with the peaks at proper wavelength. This may be because the samples are in glass tube, and the placement of the samples in the analyzer was not proper. Thus, the attempt to measure the beam quantity by using the luminescence dependence on wavelength is undergoing for usage of BAS-TR and fabricated BaFBr:Eu mainly, and Al₂O₃:Cr and Al₂O₃:CrMnCo as additional options to improve the identification.

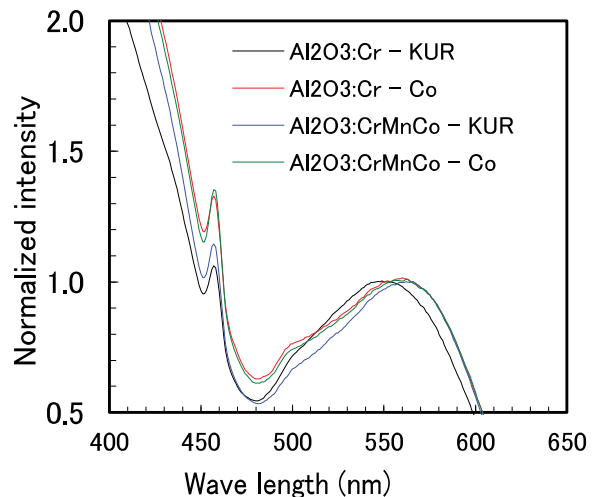


Fig. 1. Excitation spectrum

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Feasibility of Water / Fat Decomposition Technique Using Multi-Energy X-ray Computed Tomography for BNCT Dose Calculation

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INTRODUCTION: The Monte Carlo method is frequently used in dose calculations for Boron Neutron Capture Therapy (BNCT), where radiation transport calculations are conducted by specifying material information for each tissue in the body, such as density and elemental composition. Traditionally, reference values from literature, like those in ICRU Report 44 and 46, are assigned as fixed values [1, 2]. However, this approach fails to capture detailed material information, such as variations among patients or the precise distribution of water/fat content in tissues and organs. In this study, we aim to develop a method for quantitatively evaluating material density and water/fat content using data obtained from multi-energy X-ray Computed Tomography (CT), and to incorporate this material information into dose calculations. We examined the feasibility of water/fat decomposition by fundamentally estimating differences in attenuation coefficients by X-ray energy.

MATERIALS AND METHODS: The X-ray CT system (ECLOS-4, Hitachi, Ltd.) installed at KURNS is the subject of this study. The X-ray tube voltage can be selected from 100, 120 and 130 kV in the system. The linear attenuation coefficients of water, soft tissue, muscle, and adipose tissue were derived and compared at each of these tube voltages. The density and elemental composition of each material were obtained from the reference values in ICRU Report 44 [1]. The attenuation coefficients for the estimated X-ray energy spectrum of each tube voltage were derived from the mass attenuation coefficient dataset from NIST [3].

RESULTS: The estimated linear attenuation coefficients for each material are presented in Table 1. Due to the increased photoelectric effects induced by low-energy photons, the attenuation coefficients for all substances were higher at lower tube voltages. This difference is greater in adipose tissue compared to other tissues. When comparing the coefficients for water or soft tissue at 130 kV and 100 kV, the difference was 5.5%, whereas for adipose tissue, it was 4.2%. This system confirmed that a difference in CT values of approximately 1% is necessary between 100 and 130 kV tube voltages to differentiate between water and fat composition in tissues. Based on these findings, further studies on CT imaging conditions will be conducted.

Table 1 X-ray linear attenuation coefficients of the CT system.

Tube voltage	Linear attenuation coefficient [cm ⁻¹]			
[kV]	Adipose Tissue	Muscle Skeletal	Tissue Soft	Water Liquid
100	1.97×10^{-1}	2.32×10^{-1}	2.34×10^{-1}	2.22×10^{-1}
120	1.91×10^{-1}	2.23×10^{-1}	2.26×10^{-1}	2.14×10^{-1}
130	1.89×10^{-1}	2.20×10^{-1}	2.22×10^{-1}	2.10×10^{-1}

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