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Abstracts

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Welcome address from the president

On behalf of the organizing committee and the Scientific Council of IABERD it is my honor and pleasure to welcome you to the 2022 EPRBioDose meeting. We are pleased that you have chosen to join us for what promises to be a lively and enriching meeting. This is an especially exciting time for all of us as it has been four long years since our last meeting. Although this is not the in-person meeting in Japan we had hoped for, we at least have the opportunity to meet in a virtual format. Organization of this meeting has been no easy task, especially for the local organizing committee led by Shin Toyoda, as meeting plans were continually revised with the ebb and flow of the COVID pandemic. I am sincerely grateful to Shin's team and the Scientific Council for their patience and great effort in getting us together despite the challenges.

Over the three days of the meeting, we will learn about and discuss advances in new biomarkers, technological developments of established biomarkers, biological and EPR dosimetry in medicine, emergency situations and epidemiology, dosimetry networking, EPR dating and other related topics as presented in 5 invited talks and 73 submitted posters. We will have the opportunity within the virtual meeting format for our dosimetry community to build upon the discoveries and lessons of our members and connect with colleagues to develop relationships and collaborations that will help move the science of radiation dosimetry forward in the coming years. We hope you find your time with us during this 2022 meeting to be beneficial and invigorating.

With warmest regards,

Steven G. Swarts, PhD
President, IABERD

A special thank you to the local organizing committee members:

Shin Toyoda (Okayama Univ. of Science)
Tomisato Miura (Hirosaki Univ.)
Toshitaka Oka (JAEA)
Chihiro Yamanaka (Osaka Univ.)
Hiroshi Yasuda (Hiroshima Univ.)
Kanya Hamasaki (RERF)
Yumiko Suto (NIRS)
Gen Suzuki (International Univ. of Health and Welfare)
Mitsuaki Yoshida (Hirosaki Univ.)
Ichiro Yamaguchi (NIPH)
Atsushi Tani (Kove Univ.)
Seiko Hirota (Hiroshima Univ.)
Yoshiaki Kodama (RERF)
Seiji Kodama (Osaka Pref. Univ.)
Yukio Mizuta (JEOL)
Donovan Anderson (Hirosaki Univ.)

From the Chair of the local organizing committee

On behalf of the local organizing committee members, I would like to welcome you all, the participants of this exciting meeting, EPR BioDose 2022 online. We now have sakura full of bloom in Japan at my place, Okayama. We have chosen this period because of this, for you to enjoy sakura blossoms, but unfortunately this was not possible due to the covid-19 pandemic. However, in turn, we have another opportunity to enjoy our meeting in a cyber space. This would be a very special chance to meet everyone in the world without paying any travel expenses. I hope you enjoy this meeting and have contribution to scientific progress of our community. I would like especially thank Dr. Donovan Anderson for “constructing” the venues of our meeting and for organization of repeating tests.

Shin Toyoda, Dr.
Okayama University of Science
Chair of the Local organizing committee

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EPR BioDose 2022 online

Mar. 28-30, 2022

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***presenter**

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Session 1b Cytogenetic biomarkers

Session 1c New technical developments for established biomarkers

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Session 3 Biological and EPR dosimetry for emergency

Session 4 Biological and EPR/luminescence dosimetry for epidemiology

Session 5 Networking in biological and EPR dosimetry, QA & QM

Session 6 EPR dating and related topics

IN-1-D1

Role of biological and physical retrospective dosimetry in radiological incidents

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In cases of unknown or conflicting irradiation situations, biodosimetric approaches have proven to be a valuable tool for retrospective dose estimation of individual exposures. The techniques are mainly based on biological samples, but some physical methods using inert personal electronic devices are also applied to complement each other in different exposure scenarios. Cytogenetic testing has many years of experience and has become a standard component of radiation protection programs in many countries (IAEA 2011). Depending on the scenario and the number of people affected, different strategies for biodosimetry have been developed. In a large-scale accident or terrorist incident, the number of people could easily exceed the capacity of a single laboratory. Therefore, networking between well-trained laboratories has proven to be useful for providing the rapid and reliable dose assessments required in such circumstances (Kulka et al. 2018). Various biodosimetry laboratories worldwide have now joined forces and established regional and/or country-wide networks either on a formal or informal basis.
Between 2012 and 2015, RENEB, the European Network for Biological Retrospective Physical Dosimetry, was established in Europe, and 2017 the RENEB e.V. association was created as an independent legal entity. An essential element of such a network is its operational base, including various assays (Wojcik 2017). To achieve reliable and comparable results and apply robust standards across all network partners in the techniques used, quality-controlled interlaboratory comparisons (ILCs) are necessary to harmonize and improve performance (Oestreicher et al. 2017). The potential of biodosimetry for use in the context of a severe radiation accident was clearly demonstrated here and underlined the need for regular proficiency testing. With the help of biodosimetry, a classification of persons into medically relevant groups is possible and can support emergency management. Different aspects of biodosimetry and scenario-based options for clinical decision support in radiation accidents are presented (Blakely WF, Port M, Abend M., 2021).

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IN-2-D2

Basics and current status of cytogenetic dose estimation

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Japan has experienced a major radiation accident so far, i.e. the radiation exposure by atomic bombs dropped on Hiroshima and Nagasaki in1945, the exposure accident of fishing boat named Daigo Fukuryu Maru by hydrogen bomb at Bikini Atoll in 1947, JCO accident in Tokai village in 1999 and the accident of Fukushima Daiichi nuclear power plant due to Great East Japan Earthquake in 2011. Also, in Japan, a lot of institutions including hospital, university, and private company are handling radiological substances and registered in the Radiation Hazard Prevention Law revised in 2019. Considering the radiological accidents occurred in the past, most accidents are mainly due to human error. These facts mean that radiation exposure accidents will occur not only at nuclear power plants, but also at these institutions that handle the radioactive materials. Therefore, we have to establish and prepare the system to respond to the radiological accidents at any time. The most important thing in a radiation exposure accident is to save a life of the victims, and for that purpose, the evaluation of the radiation dose is extremely important. The radiation dose obtained by the biological estimation will be transmitted to the medical side, and appropriate medical treatment is taken for the exposed person. The most reliable method for evaluating radiation dose is the cytogenetic dose estimation called “Gold Standard”. Because, the chromosome abnormalities such as dicentric, translocation, ring and so on induced by radiation are direct evidence of the effects of radiation on living organisms. Many scientists have tried to find new dosimeters for dose assessment, but so far no new dosimeters have been discovered and chromosomal abnormalities have been used for dose assessment in the exposed person for over 60 years. The method of cytogenetic dose estimation is basically classified into four types: dicentric (DIC) method, translocation method, micronucleus (MN) method, and premature chromosome condensation (PCC) method. The application of these methods depends on the condition of the exposure accident. Basics and current status will be introduced in this presentation.

Keywords: Radiation emergency medicine; dose estimation; chromosome abnormality

IN-3-D2

EPR Biodosimetry in 2022: where are we now and where are we likely to go?

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Since first proposed in 1968, EPR biodosimetry has had an important role for the very important but difficult task of assessing not only the dose received but the health risks from unplanned exposures to ionizing radiation. Its value for dose estimation arises especially from its being a physically based technique that is relatively unaffected by biological variability. Several different, often complementary approaches have been developed using EPR, ranging from measurements made in vivo to measurements in inanimate objects that were located on or near the person at the time of exposure. Initially all biodosimetry studies made with EPR were based on measurements made in vitro at X-Band. Teeth, bones, fingernails, and hair were especially investigated. Later, measurements were made at other frequencies, especially lower frequencies which made in vivo measurements feasible.

Teeth in vitro This has become an important, perhaps the dominant technique for retrospective dosimetry for epidemiological studies of radiation released into the environment at levels that have the potential to a cause long-term health risk. The usual technique, in which the enamel is extracted and concentrated, has enabled reliable detection of exposure doses as low as 0.1 Gy. Because it relies on having an exfoliated tooth, it has limited applicability for measuring acute exposures from incidents involving large numbers of people. Recently, uses of small amounts of biopsied enamel make its sampling less invasive.

Bones in vitro Although the earliest reports showed the feasibility of measuring radiation dose in isolated bones, in general the low dose-response relationship limits its use to a few incidents with very high exposures and the availability of bone biopsies, e.g., from coincidental amputation associated with the radiation injury.

Fingernails in vitro This approach has great appeal because of the potential to collect samples by simple clipping of nails and analyzing them in a dedicated laboratory. Unfortunately, due to large overlapping signals from the cutting of the nails and the sensitivity of the EPR signals to environmental conditions, the technique may not be suitable for measurements of large populations. It has been useful in small incidents.

Hair in vitro Initially hair samples appeared to be very promising when studied in irradiated laboratory rats. But most hair has melanin, which has a very strong EPR signal that dwarfs the radiation-induced signals.

Inanimate objects in vitro There have been considerable interest and systematic efforts to use this approach with objects such as cell phones or buttons. While considerable progress has been made, the variability of the composition of the inanimate objects (such as components of cell phones) makes progress toward systematic application very challenging. This approach continues to be pursued to determine if it can be practical for biodosimetry.

Teeth in vivo This has the potential to be very useful. It has been shown to be implementable under field conditions with a sufficient dose resolution for initial triage, with measurements

made in less than 5 minutes. The principal factor that has prevented the implementation of this approach into official response schemes has been more administrative than scientific.

Nails in vivo In principle this would be the most useful EPR biodosimetry technique for use in mass casualty situations. It could rapidly provide both dose resolution and information on the homogeneity of the exposure with sufficient sensitivity to guide initial triage. While initial results have been very promising, the technique remains to be demonstrated to be feasible when applied under field conditions.

Overall EPR biodosimetry has characteristics and capabilities that make it likely to continue to be a very important component of the response to retrospective radiation dosimetry. It is especially valuable when used in combination with biologically-based approaches.

Keywords: EPR Biodosimetry, unplanned exposure, health risks

IN-4-D3

Development of high-throughput systems for biodosimetry

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Biomarkers for ionizing radiation exposure have great utility in biodosimetry to determine the dose of radiation to which an individual has been exposed when physical dosimetry is missing or in dispute. These dose measurements can provide important information for occupational and accidental exposures. They could also be used in medicine to track doses from medical exposures and even have the potential to identify an individual's response to radiation exposure that can help tailor treatments. The measurement of biomarkers of exposure in medicine and for accidents, where a larger number of samples would be suitable, is limited by the throughput of analysis, particularly for microscope-based methods, which tend to be labour-intensive. For emergency response biodosimetry, a large number of samples would need to be rapidly analysed. This would provide rapid dose estimates to medical practitioners allows timely administration of the appropriate medical countermeasures to help mitigate the effects of radiation exposure. In order to improve sample throughput for biomarker analysis, much effort has been devoted to automating the process from sample preparation through to automated image analysis. This presentation will focus mainly on biological endpoints traditionally analysed by microscopy, specifically dicentric chromosomes, micronuclei and γH2AX. These endpoints provide examples where sample throughput has been improved through automated image acquisition, analysis of images acquired by microscopy, as well as methods that have been developed for analysis using imaging flow cytometry.

Keywords: Biodosimetry, automation, image analysis, high throughput, imaging flow cytometry

IN-5-D1

ESR dating and the human evolution: contribution to the chronology of the earliest humans in Eurasia

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Electron Spin Resonance (ESR) dating method is used in the field of Prehistory and human evolution since more than 40 years. This method applied in Quaternary geology was introduced by Motoji Ikeya who dated for the first time some speleothem taken in the Akiyoshi cave, Japan (Ikeya, 1975). Arago cave in France was one of the first important sites to be dated by ESR. In 1981, an international colloquium gathered many physicists who applied different methods among them ESR dating on calcites, bones and quartz. The scattered results obtained by U-series, Thermoluminescence, ESR and other techniques, were debated pointing out all the difficulties to get reliable ages in a karstic environment in which taphonomic and erosional phenomena were predominant. This was the starting point giving an undisputable place to the dating methods in prehistory as a discipline in its own right. Since that time, many sites were dated in Africa though the chronology was mainly elaborated by Argon methods, palaeomagnetism and biochronology for the sites older than 2 Ma.
ESR method was applied on many famous archaeological sites in Eurasia which was reached by hominins around 2 Ma ago. Its versatility allowed possibilities for getting ages in different environments such as open air sites associated to river terraces, karstic and caves environments where hominins found shelters and also volcanic areas where ESR results could be compared to those obtained by Ar/Ar method. Combined ESR and U-series dating of fossil herbivorous teeth has proven that the results are reliable especially for the beginning of the Middle Pleistocene period. The multi-methods approach is applied as often as possible providing results which can be compared and which participate to the elaboration of a reliable framework for the last 2 million years.

1a-01-D1

Identification of radiation-induced changes in the miRNA of extracellular vesicles released by human lymphoblastoid cells

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Extracellular vehicles (EVs) are well-known mediators of the cellular response to different factors. Studies have found that ionizing radiation (IR) could induce the changes in composition of EVs released from irradiated cells, which play an important role in radiation-related communication between cells [1]. Identification of the dose-specific biomarkers enriched in EVs could be useful for accurate dose estimation after exposure to IR. Here we aim to investigate the miRNA profile of EVs released by irradiated human lymphoblastoid cells (AHH-1) and to identify the differentially expressed miRNA contained in EVs following radiation exposure. EVs were isolated by ultracentrifuge from cell culture media collected 24 h after irradiation of cells with a single 0, 2 and 5 Gy of ^{60}Co γ-rays at a dose rate of 1 Gy/min, and then the RNA was extracted for sequencing and bioinformatics analysis. miRNAs were quantified by qPCR. A total of 253 miRNAs whose abundance in EVs was significantly affected by IR, including 90 upregulated and 163 down regulated miRNA. Bioinformatic analysis indicated that the differentially expressed miRNA in EVs mainly enriched in the process of transcription regulation, negative chemotaxis, activation of GTPase activity and double-strand break repair. IR-induced miR-27a-3p、miR-18a-5p、miR-29a-3p、miR-30d-5p、let-7f-5p、let-7g-5p and let-7e-5p were significantly increased after expose to 0-5 Gy, while the expression level of miR-4729、miR-6790-5p、miR-7844-5p、miR-1229-5p and 6789-5p were down-regulated with dose. Our study suggested that dose-response miRNA of EVs released from irradiated AHH-1 cells have the potential to be used as biomarkers for dose estimation after radiation exposure.

Keywords ionizing radiation; extracellular vehicles; exosome; miRNA; biodosimetry

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1a-02-D2

Analysis of the acylcarnitines in plasma and small intestine of rats to find new candidate biomarkers for screening local abdominal irradiation

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Purpose: Previous studies show that the carnitine and acylcarnitines have the potential to be the new promising candidate indicators of radiation exposure. Here, our study aimed to analysis of the acylcarnitine in plasma and small intestine of rats with targeted metabolomics to find new candidate biomarkers for screening local abdominal irradiation and explore the related mechanism. **Methods:** In the present study, the abdomen of 20 male Sprague Dawley (SD) rats were irradiated locally with 0, 10, 15, and 20 Gy of ^{60}Co gamma rays to construct radiation enteritis model. The plasma and small intestine samples were collected at 72 h after exposure. The changed of acylcarnitines in the plasma and small intestine of SD rats irradiated ^{60}Co gamma-rays with 0 and 10 Gy were analyzed using liquid chromatography mass spectrometry based on targeted metabolomics. The expression levels and activity of the carnitine palmitoyltransferase 1 (CPT1) in fatty acid β-oxidation (FAO) pathway, which plays a crucial role in the changes of acylcarnitines were detected with qRT-PCR, western blot (WB) and related enzymatic activity kits. **Results:** Among thirty-seven kinds of detected carnitines, fourteen kinds of acylcarnitines in the plasma of 10 Gy group were significantly increased compared with those of 0 Gy group ($P < 0.05$). Eleven kinds of acylcarnitines in the 10 Gy group were significantly increased compared with those in the 0 Gy group ($P < 0.05$). Notably, three kinds of acylcarnitines (C16:2, C16-OH and C16:2-OH) were both elevated in the plasma and the small intestine samples. The mRNA and protein levels of CPT1A and CPT1B and the corresponding enzyme activities in the small intestine tissue of rats were increased significantly in all irradiation groups compared with those of 0 Gy group ($P < 0.05$). **Conclusion:** These results indicate that the elevated acylcarnitines have the potential to be candidate biomarkers of local irradiation. The elevated of acylcarnitines in rat plasma and small intestine samples may be relate to the activated CPT1 enzyme in fatty acid β-oxidation pathway after local irradiation.

Keywords: biomarkers, acylcarnitines, CPT1, local abdominal irradiatio

1a-04-D1

Identification of novel biomarkers for acute radiation injury using multi-omics approach and nonhuman primate model

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The availability of validated biomarkers to assess radiation exposure and to assist in developing medical countermeasures remains an unmet need [1]. We used a cobalt-60 gamma-irradiated nonhuman primate (NHP) model to delineate a multi-omics-based serum probability index of radiation exposure. Both male and female NHPs were irradiated with different doses ranging from 6.0 to 8.5 Gy, with 0.5 Gy increments between doses. We leveraged high resolution mass spectrometry for analysis of metabolites, lipids, and proteins at 1, 2, and 6 days post-irradiation in NHP serum.

A logistic regression model was implemented to develop a 4-analyte panel to stratify irradiated NHPs from unirradiated with high accuracy that was agnostic for all doses of gamma-rays tested in the study, up to six days after exposure. This panel was comprised of Serpin Family A9, acetylcarnitine, PC (16:0/22:6), and suberylglycine, which showed 2 – 4-fold elevation in serum abundance upon irradiation in NHPs, and can potentially be translated as a molecular diagnostic for human use following larger validation studies.

Taken together, this study, for the first time, demonstrates the utility of a combinatorial molecular characterization approach using an NHP model for developing minimally invasive assays from small volumes of blood that can be effectively used for radiation exposure assessments. In conclusion, the results of this study validate the use of metabolomics/lipidomics and proteomic signatures as biomarkers to evaluate the extent of radiation injury which may be used to assess the efficacy of potential radiation countermeasures.

Keywords: Biomarkers; metabolomics; nonhuman primates; proteomics; radiation injury

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Cross-platform validation of a mouse blood gene signature for quantitative reconstruction of radiation dose

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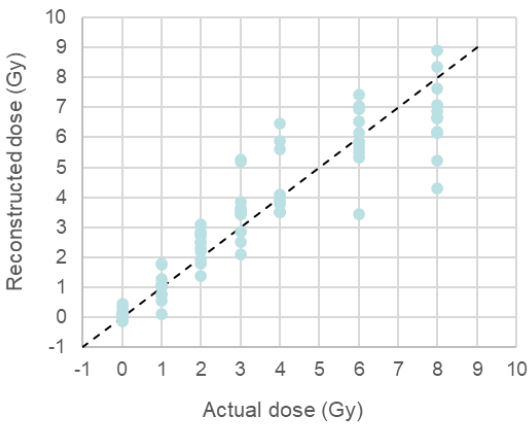
In the search for biological indicators of radiation exposure, gene expression is established as a promising translatable and sensitive endpoint that can lend itself to in-field high throughput applications after large-scale exposure of a human population. Healthy human blood irradiated ex vivo has been used extensively in this research and has yielded much of the knowledge and gene expression datasets available. The ex vivo model has limitations, however, such as lack of signalling from other irradiated tissues and deterioration of the health of blood cells in culture over time. Thus, we must use non-human in vivo models to test long-term responses and potential delayed effects of radiation, as well as realistic exposure modes such as partial-body irradiation and internal emitters. The conventional starting point is acute exposures (dose rate ~1Gy/min). Here we present a novel approach to define a gene signature in mouse blood cells that correlates with increasing radiation dose (using 1Gy/min dose rate) in a continuous manner. Starting with available microarray datasets, we selected around 30 top radiation-responsive genes and performed cross validation and training-testing data splits to downselect to 16 radiation responsive genes. We then tested these genes in an independent cohort of adult C57BL/6 mice (50:50 both sexes) and measured these genes by real time qRT-PCR in whole blood at 24h after exposure. The dose reconstruction in these samples using the Net Signal (difference between geometric means of top 3 genes positively correlated with dose, and top 4 genes negatively correlated with dose measured by qRT-PCR), was highly improved over that of the microarrays, with an RMSE (root mean square error) of ±1.04 Gy in male and female mice combined, with no significant sex specific differences in mRNA changes or cell counts after irradiation.



Keywords: Mouse, radiation, biomarker, gene expression

Mitochondrial damage as a biological marker for dose assessment

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Radiation dose assessment is indispensable to determine the need for appropriate therapeutic strategies and prognoses for severe radiation victims in radiological emergency situations. In particular, it is important to understand whether or not the entire body is exposed to over 1 Gy to induce acute radiation syndrome (ARS). However, a rapid and reliable dose evaluation method for a large population has not been established so far. Biodosimetry technique is beneficial to screen the highly radiation sensitive population in large nuclear incidence. A decrease in blood cell count following radiation exposure is the first quantitative bio-indicator. Haematological techniques can be also utilized to identify new biomarkers for dosimetry.

Other than nuclei, mitochondria are thought to be as radiation target. In response to radiation-induced DNA damage, mitochondrial oxidative phosphorylation (OXPHOS) is activated to conduct DNA damage responses and mitochondrial reactive oxygen species (ROS) generate as by-product of OXPHOS. ROS cause mitochondrial oxidative damage which was recognized by the E3 ubiquitin ligase, parkin, undergoing mitochondrial degradation to maintain the quality of mitochondria. On the other hand, the Nrf2 transcription factor activates transcription of antioxidant responses genes to protect against oxidative stresses.

The exposure conditions were whole body irradiation with mice *in vivo*. Mouse peripheral blood mononuclear cells (PBMC) isolated using Ficoll - Paque™ PLUS centrifugation were used for immunostaining with the marker of DNA damage γ-H2AX, parkin and Nrf2. Exposed to 5 Gy of X-ray significantly induces γ-H2AX foci in PBMC 1 day after irradiation. Oxidative stress markers of parkin and Nrf2 were more sensitive and persistent over time than nuclear DNA damage [1]. Mitochondrial damage (Parkin) was increased by radiation of 1 Gy or more. The oxidative stress response (Nrf2) was further observed to increase in fluorescence even at low doses of 1 Gy or less.

In conclusion, parkin and Nrf2 are potential biomarkers for use in radiation dosimetry. Identification of several biological markers which show different kinetics for radiation response is useful for radiation dosimetry that allows the assessment of radiation injury and efficacy of clinical treatment in emergency radiation incidents. Radiation-induced oxidative damage is useful not only for radiation dose assessment but also for evaluation of radiation effects on humans.

Keywords Biomarker; Mitochondria; Parkin; Nrf2; Mouse blood cells

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Employing gene expression and γH2AX focus assays for biodosimetry purposes after low-level irradiation

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Objective: Gene expression (GE) and the γH2AX-DNA double-strand break (DSB) focus assay are novel techniques for biodosimetry purposes after ionizing radiation exposures. Both assays are sensitive enough to detect radiation doses in the mGy range. In this study, we investigated the lower detection limit of both assays to further characterize these assays.

Material and Methods: Peripheral blood samples were obtained from six healthy donors (4 males, 2 females, age range: 28-34). They were placed in a water phantom, irradiated *in vitro* with 2.5, 5, 10, 20 and 50 mGy on a 3rd-generation dual source CT scanner (Siemens Somatom Force) and compared with a sham irradiated reference group. After incubation for 6 h at 37°C radiation-induced GE-changes of *FDXR* and *EDA2R* were examined using quantitative real-time polymerase-chain-reaction. After incubation for 20 minutes at 37°C radiation-induced DSB foci were quantified in 100 cells in the fluorescence microscope after γH2AX + 53BP1 immunostaining, by subtracting the average foci per cell values of non-irradiated aliquots from the irradiated ones. Statistical analysis was performed by t-test or Mann-Whitney U test, where applicable.

Results: A significant increase (2-fold, $p \leq 0.002$) in *EDA2R* GE relative to the unexposed control was observed after 5 mGy and copy numbers increased linearly up to 12-fold at 50 mGy. *FDXR* upregulation (2-fold) became significant after 20 mGy exposure ($p \leq 0.03$) and increased linearly up to 4-fold at 50 mGy. A significant increase in radiation-induced foci (RIF) compared to non-irradiated cells ($p \leq 0.02$) was observed from 10 mGy (RIF: 0.15 ±0.1) to 50 mGy (RIF: 0.59 ±0.1). There was a highly significant ($p < 0.001$) linear correlation between the mean values of differential gene expression/RIF and the applied radiation dose.

Conclusion: Our results show that both molecular assays are sensitive to detect very low dose ionizing radiation exposures. Our study indicates a lower detection limit of 5 mGy for GE and 10 mGy for the γH2AX focus assay. The current study implies a higher sensitivity for detection of low-level irradiation using *EDA2R* compared to *FDXR* GE changes.

Keywords: low dose radiation exposure; gene expression analysis; *FDXR*; *EDA2R*; γH2AX focus assay

Molecular indices for early biological dose assessment of radiation injury

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Introduction
Large-scale radiation events often occur without any advance warning, and easily expose many people to ionizing radiation. Rapid and early estimation of injuries is critical for identifying individuals who could potentially exposed irradiation. In this study, radiation-sensitive miRNAs were screened in human peripheral blood by high-throughput genomic assay and bioinformatics analysis, and providing diagnostic molecules for radiation damage assessment and detection indicators for rapid triage of large-scale radiation exposure.

Methods
We selected 7 men who have similar radiation sensitivity from 68 healthy adult men aged 20-30 years old, and peripheral blood was collected and irradiated with 0.2 Gy and 2.0 Gy γ rays. Total RNA was extracted from blood 6 h after irradiation. Gene sequencing technology was used to detect the expression changes of miRNA molecules in peripheral blood after irradiation. Differential miRNAs were verified by RT-PCR. To observe the dose-response relationship of different miRNA after γ rays, human B lymphocyte AHH-1 was irradiated with 0.1, 0.2, 0.5, 1.0, 2.0Gy γ rays.

Results
Compared with the control, 10 miRNAs were significantly changed after 0.2Gy γ rays, 21 differentially expressed miRNAs in the 2.0Gy group, and 6 miRNAs were positively correlated with the irradiation dose. Further RT-PCR results showed that the 4 miRNAs were consistent with the sequencing results. The expression levels of 4 miRNAs of AHH-1 cells were correlated with the irradiation dose, and miRNA -320d was significantly correlated with the irradiation dose.

Conclusion
The level of miRNA-320d was increased in a dose-dependent manner. We suggest that miRNA-320d may be a potential molecular indicator for early biological dose assessment of radiation damage.

Keywords
Ionizing radiation; Radiation damage; miRNA; Bioinformatics

Effects of radiation quality and dose rate on radiation-induced nucleoplasmic bridges in human peripheral blood lymphocytes

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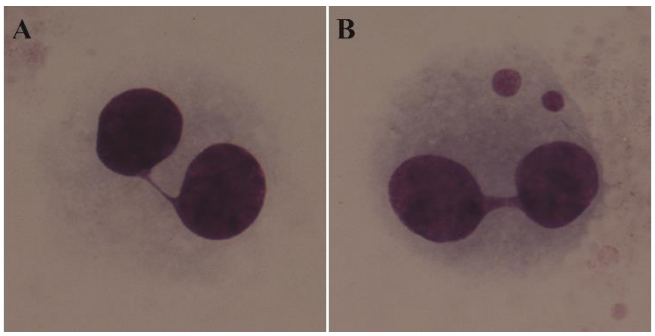
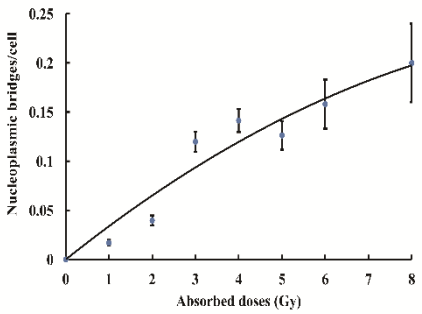
Previous studies showed that the yield of cobalt-60 γ-rays-induced nucleoplasmic bridges (NPB) in human peripheral blood lymphocytes is dose dependent. However, the influence of the radiation quality and dose rates on NPB frequencies has not been investigated. The present study aimed to investigate NPB frequencies in human peripheral blood lymphocytes induced by carbon ions and explore the dose rate effect on cobalt-60 γ-rays induced NPB. To establish dose-response curves, human peripheral blood samples were irradiated with 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 Gy of carbon ions at a dose rate of 3.0 Gy/min in vitro. To explore the dose rate effect, human peripheral blood samples were irradiated with 2.0 and 5.0 Gy of cobalt-60 γ-rays at dose rates of 0.2, 0.5, 1.0, 3.0, 5.0 and 10.0 Gy/min in vitro. NPB and micronuclei (MN) in binucleated cells were analyzed with the cytokinesis-block micronucleus cytome assay. Results showed that the dose-response curve of carbon ion-induced NPB frequencies follow a linear-quadratic model (R^2 = 0.934). The relative biological effectiveness (RBE) values of carbon ions to cobalt-60 γ-rays decreased with increased NPB frequencies (ranging from 2.47 to 5.86). Compared with group 1.0 Gy/min, the NPB frequencies in groups 10.0 Gy/min (2.0 Gy), 5.0 and 10.0 Gy/ min (5.0 Gy) were decreased significantly ($P < 0.05$). Carbon ion-induced NPB in human peripheral blood lymphocytes have a good dose-response relationship. Cobalt-60 γ-rays-induced NPB frequencies are affected by the specific dose rate.

Keywords Nucleoplasmic bridge; Carbon ions; Dose response; Dose rate; Micronucleus





Shortened 48 h cytokinesis-block micronucleus assay for triage dose assessment

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For radiological triage dose assessment with cytogenetic biodosimetry, ISO recommends both dicentric chromosome assay (DCA) and cytokinesis-block micronucleus (CBMN) assay. In a mass-casualty accident, individuals exposed to an equivalent whole-body dose of ≥ 2 Gy need to be quickly and reliably differentiated from a large population of worried-well for immediate medical treatment. Even though dicentrics are more radiation specific and the culture time is shorter than the conventional CBMN assay, dicentric scoring requires scorers trained to identify dicentrics and other chromosome aberrations. Manual scoring of 50 metaphases can also take up to 150 min. In contrast, manual micronucleus (MN) scoring can be quickly performed without prior knowledge of chromosome karyotypes.

In this experiment, CBMN assay performed in whole blood (WB) or isolated peripheral mononuclear cells (PBMCs) were compared in cultures of differing lengths (48, 72 h) and different Cytochalasin-B (Cyt-B) treatment times (at 24 h or 44 h). Giemsa-stained cells were manually scored. Preliminary results are shown as analysis is still ongoing.

Blood from 3 donors (26F, 34M, 52M) were irradiated with 0, 2 or 4 Gy X-rays. As expected, 48 h cultures showed lower nuclear division index (NDI) and percentage of binucleated cells (BNC) than 72 h cultures. Adding Cyt-B at 24 or 44 h also did not affect cell division for 72 h cultures. Despite the shorter 48 h culture time, > 1000 BNC were easily scored in donors with high NDI up to 2 Gy while > 500 BNC were scored for 4 Gy. Other than triage MN frequency, MN distribution could also easily differentiate between individuals irradiated with 2 and 4 Gy.

Another 3 donors (23F, 25M, 29M) were used for dose-response curve (DRC) construction of induced MN frequency for WB and PBMCs of shortened 48 h culture and conventional 72 h culture (Cyt-B added at 44 h). DRC construction with a pooled data set showed very similar DRCs between 48 h WB and PBMC cultures while 72 h WB cultures showed significantly higher MN frequency than PBMC cultures.

In conclusion, manual scoring with shortened 48 h CBMN assay is a feasible alternative to DCA for triage assessment for donors with high NDI or suspected to be exposed to < 2 Gy. With 200 BNC scored, unirradiated and 2 Gy donors could be identified within 10 min. For high doses of 4 Gy, 100 BNC scored was sufficient and scored within 15 to 20 min. Both MN frequency and MN distribution can be used for dose assessment.

Keywords Cytokinesis-block micronucleus assay; Cytogenetic biodosimetry; Triage; Dose estimation

Experimental assessment of cytogenetic damage formation after fractionated inhomogeneous irradiation

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Most modern radiotherapy schemes involve localized exposure and fractionation of the total radiation dose over time. In radiation cytogenetics the effects of partial exposure have been investigated rather intensively. However, in contrast to numerous dose rate studies involving continuous exposure, much less attention has been paid yet to the dose fractionation effect. To the best of our knowledge, the combination of inhomogeneous irradiation and fractionated dose has been never addressed experimentally to assess the net cytogenetic effect in human lymphocytes. To fill this gap, for the first time, the experiment was carried out to evaluate the combined action of the radiation dose inhomogeneity and fractionation.

Donors' blood samples were irradiated in vitro to 0, 2, 4 or 6 Gy acute γ-rays. The former three points were exposed to a second dose of 2 Gy in 2 h or 24 h after the first exposure. Also, the 0 Gy and 2 Gy points were repeatedly exposed to 2 Gy in 2 h and again to 2 Gy in 24 h after the first exposure. Inhomogeneous exposure modeling included partial, single dose exposure by mixing blood irradiated to 2, 4 or 6 Gy with uniradiated blood in a 1:1 ratio, and mixed dose exposures to 0, 2 and 4 Gy or 2, 4 and 6 Gy in 1:1:1 ratios. Amongst these simulations of inhomogeneous exposure, three points involving 2 and 4 Gy (i.e. two single doses and one mixed dose) were also irradiated with a second dose of 2 Gy in 24 h that was the closest imitation of radiotherapy conditions. In all series, blood was kept at 37,5 °C between dose fractions. Classic dicentric assay with fluorescent-plus-Giemsa staining was performed. Dicentric-plus-centric ring (Dic+CR) yields and per-cell distributions and resultant biodosimetry estimates by Dolphin method were compared between experimental points.

The additivity of Dic+CR yields showed that the repair of primary DNA damage and aberration formation in human lymphocytes in the split-dose irradiation scenario were completed in 24 h after the first dose fraction, as expected. Cytogenetic data were in a good agreement with the model of biexponential kinetics of DSB repair, but its' time constant t_0 appeared to be dependent on the first radiation dose and respective initial aberration yield. In simulations of inhomogeneous plus split-dose irradiation, the observed Dic+CR yields after the second dose fraction were 5-15 % lower than the expected additive baseline level, probably due to a higher rate of selective interphase death of those lymphocytes, which had been initially irradiated to higher doses. Biodosimetry estimates were compared between different experimental series that allowed to formalise the impact of the split-dose irradiation in the inhomogeneous exposure scenario. These results lead to a better understanding of the mechanism of cytogenetic damage formation in vivo during radiotherapy and may help in future practical applications of biodosimetric methods for radiobiological control in patients.

Acknowledgement: This study was supported by the Research Contract RC21066 in the framework of the Coordinated Research Project E35010 MEDBIODOSE of the International Atomic Energy Agency.

Keywords: Dicentric assay; inhomogeneous exposure; fractionated irradiation; radiotherapy.

The influence of repair temperature on DNA lesion repair and dicentric chromosome formation

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Hypothermia is known to influence biochemical reactions. It has been noted that hypothermia during in vitro irradiation of human peripheral blood lymphocytes is affecting the yield of chromosome aberrations. Low temperatures during irradiation, e.g. +0.8°C, +4°C or 20°C, were reported to act in a radioprotective manner with regard to the dose-yield relationship for dicentric chromosomes in human lymphocytes [1-3]. On the other hand, a hypothermic effect of the storage temperature after irradiation has not been detailedly analyzed so far with regard to DNA repair and dicentric formation.
Interestingly, the protocol of dicentric chromosome analysis (DCA) involves a two-hour time period at 37°C directly after radiation exposure and culture setting („repair time“) [4]. However, the influence of temperature changes during this 2h repair time have so far not been investigated. Therefore, we started to investigate the potential effect of different temperature settings (22°C vs 37°C) during repair time at the level of γ-H2AX foci as a marker for DNA double strand break (DSB) damage and repair, and dicentric formation.
Human peripheral blood lymphocytes were exposed to 0 Gy, 1.2 Gy or 3.5 Gy X-rays (240kV, 13 mA, 1 Gy/min, water kerma) immediately after venipuncture and kept for two hours either at 22°C or 37°C. Then, culture were set for DCA or cells fixed and subjected to DSB foci analysis. Foci numbers were determined 10 minutes and 2 hours after irradiation, while metaphases were fixed 48 hours after exposure for DCA.
First experiments indicate a decrease of γ-H2AX as well as a mild decrease of the dicentric frequency after incubation at 37 °C for 2 hours compared to 22°C. Further investigations are under way to investigate the reasons for this potential difference.

Keywords dicentric, γ-H2AX, hypothermia, DNA repair, temperature effect

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Blood culture volume affects mitotic index but not radiation-induced dicentric chromosome aberration frequency

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Purpose: The dicentric chromosome (Dic) aberration assay, the gold standard for biological dose assessment in radiation emergency medicine, requires an analysis of at least 500 lymphocyte metaphases spreads or 100 Dic aberrations. Therefore, peripheral-blood culture conditions able to obtain metaphases with high frequency for efficient dose evaluation should be optimized. However, the type of blood cultures and volume of cultured blood differ between biodosimetry laboratories. The purpose of this study is to investigate the blood volume at which a high mitotic index is obtained in peripheral whole blood (WB)-culture and isolated peripheral blood mononuclear cell (PBMC)-culture, and to examine the effect of blood culture volume on Dic aberration frequency.

Materials and Methods: Peripheral blood was collected from three healthy donors with their informed consent. The complete and differential blood counts were performed using an automated hematology analyzer. After blood count, peripheral blood was irradiated with 0 or 2 Gy X-ray. Culture conditions included use of phytohemagglutinin (180 µg/ml) and colcemid (0.05 µg/ml) for 48 hrs. The mitotic index (MI) and Dic aberration frequency were analyzed in 5, 10, 15, 20, 25, and 30 % WB-culture and 0.6, 1.2, 1.8, 2.4, 3.0, 3.6 and 4.2 ml WB-equivalent PBMC-culture.

Results and Discussion: In WB-culture, MI showed the highest value (~22 %) in 5-15% WB-culture and then gradually decreased to ~9 % with 30% WB-culture. In PBMC-culture, MI peaked (~ 36 and 31 %) at 1.8 mL and 2.4 mL-WB equivalent volumes respectively, and MI progressively decreased by ~30% as the amount of PBMC increased. Although individual differences were observed in the MI values among the three subjects, all the subjects showed the same tendency and PBMC-culture showed higher MI than WB-culture. There was no effect on Dic aberration frequency caused by differences in blood culture volume or types of culture. While blood volume induced variations were seen in MI between different donors, consistent Dic aberration results can be obtained. The estimated dose calculated based on the Dic frequency was equivalent to the absorbed dose of ex vivo X-ray irradiated blood from our dose-response curve. **Conclusion**: While MI was affected by the blood culture type and the volume of cultured blood, Dic frequency did not differ significantly between these conditions.

Keywords Whole blood-culture, PBMC-culture, mitotic index, Dic frequency, biodosimetry

Cytokinesis-block micronucleus assay performed in 0 and 2 Gy irradiated whole blood and isolated PBMCs in a 6-well co-culture system

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Back ground and Purpose: The cytokinesis-block micronucleus (CBMN) assay in cytogenetic biodosimetry uses micronucleus (MN) frequency scored in binucleated cells (BNC) for dose estimation. The nuclear division index (NDI), an indicator of cell proliferation and cell damage, is often reported together with MN frequency, although NDI alone is not recommended for biodosimetry. Whole blood (WB) or peripheral blood mononuclear cells (PBMCs) isolated from WB can be used for cell culture. In our previous study [1], CBMN assay performed in single cultures of 15 ml tubes showed a similar NDI between 0 Gy WB and PBMCs. However, higher NDI and lower MN frequency were seen in 2 Gy PBMCs than WB. In this study, CBMN assay was performed in mono- and co-culture systems of 0 and 2 Gy WB and PBMCs of various combinations with the 0.4 μm transwell membrane insert. We compared mono- and co-cultures to investigate the effect of soluble factors on NDI and MN frequency.
Materials and Methods: Peripheral blood was collected from four healthy donors with their informed consent. Mono- and co-cultures of different combinations of WB and PBMC (WB, WB-IR, PBMC, PBMC-IR) at 0 and 2 Gy in 6-well plates with 0.4 μm transwell inserts were compared with various CBMN assay parameters (NDI, %BNC, MN frequency).
Results and Conclusions: The same level of wells was compared for the same culture conditions, as there were significant differences between the upper and lower wells in some of the culture conditions. When PBMC or PBMC-IR were co-cultured with WB or WB-IR, respectively, the NDI of PBMC increased as compared to the mono-culture. The MN frequency of PBMC-IR did not increase when co-cultured with WB or WB-IR. MN frequency was consistently higher in WB-IR than PBMC-IR in both mono- and co-cultures. NDI, %BNC, and MN frequency were similar to those when WB or PBMC were co-cultured with PBMC-IR or WB-IR, respectively. There were also significantly lower NDI and %BNC, and higher MN frequencies in the 15 ml culture condition as compared to the 6-well mono-culture condition. In conclusion, it is necessary to keep the culture protocol of CBMN consistent when constructing dose-response calibration curves and estimating doses, because the type of cell culture (WB, PBMC), culture vessel, and interaction of blood cells could affect NDI and MN frequency.

Keywords
CBMN assay, biodosimetry, transwell membrane insert, co-culture, bystander effect

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Cytogenetic biodosimetry at ultra-high dose rates

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Testing and Validation of biodosimetry assays is routinely performed using conventional dose rate irradiation platforms, primarily orthovoltage irradiators, at a dose rate of approximately 1 Gy/min. In contrast the exposures from an improvised nuclear device (IND) will be delivered over a large range of dose rates with a prompt irradiation component, delivered in less than 1 µsec, and a protracted component delivered over hours and days.

It has long been known that dose rate effects are a strong modulator of radiation response: low dose rate exposures exhibit a significant partial damage repair, resulting in lower yields of, for example, dicentrics. Conversely high dose rate irradiations may overwhelm the repair processes resulting in higher yields. Extremely high dose rates, on the other hand, may exhibit saturation effects at high doses possibly due to oxygen depletion, effectively resulting in lower-than-expected late effects.

At the Radiological Research Accelerator Facility, we have recently developed a FLASH irradiator, based on a modified a medical linear accelerator. The FLASH irradiator allows delivering few-Gy electron doses in fractions of a microsecond, allowing irradiation of blood samples at dose rates mimicking those of an IND.

We present preliminary data from a large demographic study we have undertaken for investigation of age, sex and dose rate effects on dicentric and micronucleus yields. Our data demonstrates reduced dicentric yields but increased damage complexity at very high dose rates.

Additionally, we have seen that female donors tended to have slightly reduced dicentric yields than males as well as a slight dependence of dicentric yields on age.

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Keywords Dicentrics; High Dose Rate; Age; Sex;

Development of shortened chemical premature chromosome condensation assay for high-dose exposed patients

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Purpose: In the event of a high-dose radiation exposure accident, emergency medical treatment such as cytokine administration or stem cell transplantation should be performed within 72 hours. Dose estimation is also performed using the premature chromosome condensation (PCC) assay. Comparing between fusion PCC and chemical PCC assay, the chemical PCC assay is simpler and can be performed with minimum equipment. However, in reality, it is difficult to estimate the irradiated dose and fraction of the body exposed by chemical PCC assay within 72 hours after the accident as time is needed to transport patients to relevant medical facilities. The purpose of this study is to shorten the chemical PCC assay such that radiation exposure dose and fraction of the body exposed is able to be reported within 72 hours after the accident.
Materials and Methods: Peripheral blood was collected from 6 healthy adult donors using a heparin blood collection tube. After 0, 5, 10, 15, and 20 Gy X-ray irradiation, peripheral blood mononuclear cells (PBMCs) were isolated using CPT tubes and cultured under PHA stimulation calyculin A was added 30 minutes before the end of PBMC-culture [1]. PBMCs were cultured for 24-48 hours and the PCC index was analyzed at each culture time. Caffeine was also added in some experiments to examine if the PCC index could be improved.
Results and Discussion: In non-irradiated blood, the PCC index increased 38 hours after the start of culture. On the other hand, in 5 to 20 Gy X-ray irradiated blood culture, the PCC index increased 40 hours after the start of the culture, and the sufficient number of G_2/M-PCC cells required for dose assessment could be obtained. Furthermore, the G_2/M-PCC index was improved when a final concentration of 1.0 mM caffeine was added 6 hours before the end of the culture. In conclusion, the chemical PCC assay can be shortened to 40 hours instead of 48 hours for high-dose dose estimation.

Keywords Biodosimetry, chemical PCC assay, caffeine, G_2/M-PCC index, shortening

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Unique DNA breakpoint mechanisms in the formation of radiation induced structural chromosomal aberrations

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Purpose: Mechanisms underlying the formation of radiation-induced chromosomal aberration have already been extensively investigated. However, radiation-induced formation of dicentric chromosomes and translocations is still unclear regarding the implicated chromosomes and their breakpoint junctions. In this study, we assess (1) the frequency of dicentric chromosomes in comparison with that of translocations, (2) the involvement of each chromosome in the rearrangements, and (3) the nature of breakpoints with respect to the induced aberrations after *in vitro* and *in vivo* irradiation, respectively.
Materials and methods: This study was performed on *in vitro* radiated (4 Gy ^{60}Co) blood lymphocytes of eight healthy donors and on blood lymphocytes of 30 Hodgkin lymphoma patients after many years (more than 10 years) from radiation therapy (X-ray). Chromosomal aberrations were scored by sequential analysis using telomere and centromere staining followed by M-FISH.
Results: We demonstrate, for the first time, that the frequencies of radiation-induced dicentric chromosomes and translocations, respectively, are equal in cultured lymphocytes of healthy donors (44% for dicentric chromosomes vs 43% for translocations). Interestingly, 71.6% of the breakpoints involved in formation of the dicentric chromosomes were localized in pericentromeric (42.8%) or in telomeric (28.8%) regions. In contrast, 60% of the translocation breakpoints were localized within the whole-arm chromosomes. Surprisingly, the occurrence of rearrangements was not related to the size of the chromosomes. Thus, chromosomes 16, 17, 19, 20 and 22 were the most frequently involved. In lymphocytes of Hodgkin lymphoma patients after many years from radiation therapy, we identified specific configurations of dicentric chromosomes with both centromeres in close proximity and loss of 17p (including TP53). These configurations of dicentric chromosomes can be easily mistaken for translocations using conventional cytogenetics or conventional molecular approaches. Pericentromeric breakpoints were also associated with a high degree of chromosomal instability.
Conclusion: By using telomere and centromere staining followed by M-FISH we demonstrate (1) that induction of translocations and formation of dicentric chromosomes occur at similar frequencies following gamma-irradiation, and (2) that their formation is related to the localization of the breakpoint junctions. This study underscores a central role of pericentromeric breakpoints in the sustaining and the transmission of chromosomal aberrations during progression of chromosomal instability.

Keywords: dicentric chromosome, translocation, breakpoint, centromere, telomere

Applying Amnis® AI Software to Analyze the Imaging Flow Cytometry-Based Cytokinesis-Block Micronucleus Assay for Use in Large Scale Radiological/Nuclear Events

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Biological Dosimetry plays a key role in the medical management of patients for treatment following an accidental radiation exposure. In the case of a mass casualty radiological or nuclear incident, where hundreds to thousands of people would require biological dose assessment, rapid triage of these patients will be of utmost importance to produce accurate dose assessments as quickly as possible.

One accepted method of biodosimetry is the cytokinesis-block micronucleus (CBMN) assay. It is traditionally performed using microscope-based scoring which can be labour-intensive, time consuming and subject to scorer variability. Recently, the CBMN assay has been improved in several biodosimetry laboratories by using imaging flow cytometry technology. The imaging flow version of the CBMN assay allows for much higher throughput of samples while maintaining or increasing the accuracy of dose assessments.

In this study, we investigated the feasibility of improving the automated scoring of the imaging flow-based CBMN assay by applying Amnis® AI software to the analysis process. The software classifies image data using a type of deep learning neural network that takes advantage of convolution to interpret results. We input data from a 72hr dose-response curve and created a model to score the same image data as was initially used to create the curve. As compared with the previously used IDEAS® software, the AI software maintained the high rate of throughput and provided the same statistical robustness while showing a signal increase (steeper dose-response curve) across samples receiving a radiation dose. The more sensitive damage signal allows for more accurate triage dose estimates while still being timely. The model to score the assay was created much faster and required less technical expertise than past analyses of imaging flow cytometric data. This is where another advantage of using AI technology was realized; Amnis® AI offers more plasticity than traditional analysis methods for imaging flow cytometry and therefore the analysis could be more easily translated or adapted in non-ideal situations such as those which arise in an emergency situation. The increased flexibility and maintained accuracy and throughput of the new software is a useful addition to the biodosimetry tool kit.

Semi-automated Dicentric Chromosome Assay

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Dicentric chromosome assay (DCA) is the gold standard method used in cytogenetic biodosimetry to estimate the exposure to ionizing radiation. Centromere counting is part of the process to identify suitable metaphases for the DCA scoring, where the International Atomic Energy Agency (IAEA) recommends that only complete metaphases with 46 centromeres be recorded as part of the DCA score. However, this process of centromere counting contributes to the majority of the time spent, making it a time-consuming process. There have been alternative scoring techniques such as the DCA QuickScan method, commercially available automated software such as DCScore™ by Metasystems and other available automated techniques such as Rapid Automated Bio-dosimetry Tool II (RABiT-II) to reduce the scoring time. However, there may be trade-offs or extra requirements such as the use of fluorescence *in situ* hybridization (FISH) technique for RABiT-II, which may limit some laboratories that do not routinely conduct high-throughput analysis. Hence, we propose a modified open source software (DotDotGoose) [1], that was designed for manually counting objects in images, to aid in the DCA scoring process. The modification includes real-time optimized computer vision tool (OpenCV) [2] written into the source code of DotDotGoose to automatically identify and count centromeres. The code uses thresholding to identify individual objects in the image, followed by image dilation to group the arms of the chromosome as a single object and finally contouring the objects to identify and mark the centromere. With the software, a scorer could easily check each image for discrepancies or errors and proceed to identify any dicentrics in the metaphase. The scorer could also easily mark and delete multiple aberrations on the metaphase and export the scores into a .csv file or a .pnt file that can be transferred to another scorer to facilitate future discussions amongst multiple scorers. This software was used by our lab to build our dose response curve and for the Health Canada Biodosimetry Annual Intercomparison-INTC08 2021. It has reduced our DCA scoring time significantly for images with varying qualities.

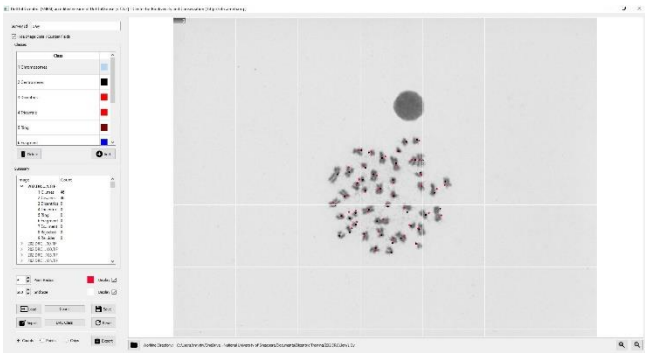

Image 1: User interface for modified DotDotGoose.

Keywords
Dicentric chromosome assay (DCA), Bio-dosimetry, Scoring, Computer vision, Simple

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Radiation Exposure Determination in a Secure, Cloud-based Online Environment

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In a major radiation incident, the speed of sample processing and interpretation of estimated exposures will be critical for triaging individuals. The dicentric chromosome (DC) assay is a gold standard in biodosimetry for assessment of absorbed radiation dose. The Automated Dicentric Chromosome Identifier and Dose Estimator System (ADCI) selects and processes cell images to identify DCs and determines radiation doses without manually reviewing images (Li et al. 2019). Previously, ADCI examined simulated population-scale exposures on a highly parallelized, multiprocessor supercomputer platform (Rogan et al. 2021). While execution of ADCI on high-throughput hardware can increase overall image processing speed, these systems are not widely available for biodosimetry laboratories, which are distributed worldwide. This study analyzed many samples with an array of lower throughput systems to achieve similar processing times. The goal was to both broaden accessibility and speed of this system with data parallelization while protecting both data and software integrity. ADCI_Online is a secure web-streaming platform that can be accessed worldwide from distributed local nodes storing metaphase sample data (Shirley et al. 2021). As a security precaution, data and software are separated until they are linked for estimation of radiation exposures. Performance is assessed with data from multiple biodosimetry laboratories. Dose estimates from ADCI_Online are identical to ADCI running on dedicated GPU-accelerated hardware. Image processing, automated image selection, calibration curve generation, and radiation dose estimation of a typical set of samples of unknown exposures were completed in <2 days. Parallelized processing and analyses using cloned software instances on different hardware configurations of samples at the scale of an intermediate-sized radiation accident (54,595 metaphase images) accelerated estimation of radiation doses to within clinically relevant time frames. While ADCI_Online is standardized for radiation assessment in biodosimetry proficiency testing, inter-laboratory comparisons, training, and research applications, the platform also has the capacity to mitigate analytic bottlenecks in intermediate-to-large radiation accidents or events.

Keywords Radiation protection in a mass casualty; Cytogenetic biomarkers; Cloud computing; Digital health informatics, Population health

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A Novel fluorometric method for dicentric chromosome assay using anti-CENP-C antibody

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Dicentric chromosome assay (DCA) is the most accepted cytological technique for the purpose of biological dosimetry in radiological and nuclear (RN) accidents, however, it is not always easy to evaluate Dicentric chromosome (Dic) because of the technical limitation of identifying Dics on Giemsa-stained metaphase chromosome samples. In order to strengthen the correct identification of Dics just after the RN accidents, it is critical to develop simple and easy but reliable technique to correctly evaluate its dose received. Here, we applied specific antibody against a centromere protein (CENP) C, CENP-C, whose antigenicity is resistant to the fixation with Carnoy's solution. Normal human diploid cells were irradiated with various doses of γ-rays, treated with hypotonic solution, fixed with Carnoy's fixative, and metaphase chromosome spreads were stained with anti-CENP-C antibody. Dose-dependent induction of Dic was confirmed from 0 to 5 Gy, whose results were comparable to those obtained by the analysis with Giemsa-stained chromosome samples. Visualization of the centromeres using anti-CENP-C antibody improved the accuracy of Dic up to 1.6 times compared with Giemsa staining. Thus, the innovative protocol for CENP-C-based DCA, which is simple and effective, should be an alternative method, avoiding uneasiness of the current DCA using Giemsa-stained metaphase chromosome samples. It is advantageous to the conventional methods, as immunofluorescence staining does not require heat denaturation, which destructs chromosome structures. In addition, because it uses antibody, several biological applications are expected. Therefore, our novel method using anti-CENP-C antibody should be recommended as a unique alternative to the methods for biological dosimetry.

Keywords CENP-C, Biodosimetry, Dicentric chromosome, Immunofluorescence staining

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NATO Science for Peace and Security (SPS) Project "*BioPhyMeTRE*" "Novel biological and physical methods for triage in radiological and nuclear (R/N) emergencies"

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The *BioPhyMeTRE* project (2020-2023) focuses on the use of innovative biological and physical methods for a fast evaluation of the radiation exposure level of people involved in a radiological mass casualty incident. The proposed biological method combines the two standardized Dicentric and Micronuclei assays into a single one, which allows the simultaneous scoring of chromosome aberrations and micronuclei on the same slide [1]. The physical method is based on the use of a low-cost, portable photo-stimulated luminescence (PSL) reader for the individual dose assessment by using personal items that people can have in their pockets or bags [2]. Both methods can be applied for a quick screening of the potential victims by using user-friendly analytical procedures. The multi-parametric approach by different techniques provides complementary information useful to overcome the limits (low radiation sensitivity, signal instability, etc.) that each method has, since the "ideal" dosimeter does not exist. From the beginning of the project (April 2020) important progress has been made to optimize and automate the biological procedure and very satisfactory results have been obtained with the PSL technique applied to salty crackers and beach sand. Inter-laboratory exercises for the validation of the analytical procedures are planned in the coming months. Next to the work for the improvement of the methods, other important activities have been carried out: online training for the transfer of knowledge and skills among the partners and disseminations of the results through the participation to several international conferences. The project, fully funded by the NATO Science for Peace and Security Programme (Grant G5684), is coordinated by the Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) of Italy and involves the Institute of Radiation Safety and Ecology (IRSE) of National Nuclear Center of Kazakhstan, the National Health Institute (ISS) of Italy and the Ruđer Bošković Institute (RBI) of Croatia.

Keywords Retrospective dosimetry; fortuitous dosimeters; photo-stimulated luminescence; radiation triage.

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Standardized and Automated Biological Dosimetry

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Large-scale nuclear disaster scenarios could involve hundreds, if not thousands, of casualties. Therefore, many studies have investigated ways to automate test evaluation, often with a focus on time efficiency and throughput optimization. We have examined optimization of biological dosimetry by using automation in combination with sample prep standardization. With this new approach, it is for the first time possible to dispense with individual calibration curves.
The cytokinesis-block micronucleus (CBMN) test is the second most frequently used test in biological dosimetry after the chromosome aberration assay. The CBMN test is characterized by the fact that evaluation can be fully automated. With the goal of achieving the highest level of reproducibility possible, we have optimized and tested both the sample preparation method and the automated evaluation to allow for rapid throughput of a high number of samples in a standardized manner.
To test our new approach, we collected samples from 24 human donors, irradiated those samples with doses ranging from 0 Gy to 8 Gy of gamma radiation, and processed the samples using our improved methods. The resulting calculated doses calculated with standard errors well below 1 Gy, had a linear fit across the entire range (R2=95%).
In an additional set of experiments, we assessed the instrument-to-instrument variability across 4 independent instruments; 144 slides were scanned 5 times per instrument. The results showed standard deviations of 0.2 Gy or lower for all doses. We will also show results from experiments assessing the operator, site, and day reproducibility with similar performance.
Our results show that it is possible to generate reliable dose estimates in a dose range of at least 8 Gy with errors below 1 Gy, without using in-house calibration curves. This was made possible by (a) the choice of an assay suitable for automation, (b) complete control and standardization of sample preparation, and (c) a reliable automated system for evaluation.
Our approach is easily scalable for accident scenarios with high case numbers. The results obtained in our tests demonstrate the transferability and standardization of the approach between different laboratories and users. This offers the potential for decentralized processing of large case numbers, for example in transnational biodosimetry networks.
This project has been funded in whole or in part with Federal funds from the Department of Health and Human Services; Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority, under Contract No. HHSO1002017000022C.

Keywords Biodosimetry;Analysis Standardization;Micronucleus Assay

Multi-parameter analysis of Cytokinesis Block Micronucleus Assay Calibration Curves

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As the lead laboratory of the Canadian Biological Dosimetry network, Health Canada (HC) is responsible for providing biodosimetry analysis after a mass casualty radiological or nuclear event. To process large numbers of samples, there is a need for high-throughput methods for biodosimetry analysis, best achieved through automation. The Cytokinesis Block Micronucleus (CBMN) assay assesses the damage from ionizing radiation in lymphocytes by quantifying micronuclei (MN) in binucleated cells (BNCs) which can be converted to dose through the use of calibration curves. This method has been adapted to, and optimized for, the imaging flow cytometer (IFC) [1,2]. In order to extend the dose range up to 10 Gy, a multiple-parameter approach was developed.

Whole blood samples, from healthy donors, were irradiated with 250 kVp X-rays (XRAD320, Precision X-ray) and cultured with 200 µL of blood in a 2:8 blood-media ratio at 37°C for 68 h. Samples were run automatically on 96-well plates with the ImageStream®^{X}-MKII (Luminex) imaging flow cytometer. Using IDEAS® (ISX-MKII software), images were analysed using several gates and features to identify key parameters. These included the number of total events, non-apoptotic cells, mono-, bi-, tri-, and quadra-nucleated cells, and MN. To improve the analysis based only on MN per BNC which peaks around 6 Gy, a multi-step analysis was performed using also the information given by all parameters. With this information, a two-step approach was used: (1) estimate whether the dose received was below or above 6 Gy, (2) use the corresponding curve to calculate the dose. Calibration curves were generated using 10 donors (each set containing 11 samples irradiated between 0 and 10 Gy). These curves were independently validated by irradiating 60 blinded samples in that dose range from three donors.

This multi-parameter approach to analysis of CBMN data from the IFC increased the dose range of applicability of the assay. Along with automated, hands-free analysis of CBMN, imaging flow cytometry has been demonstrated to be a useful tool for biodosimetry in mass casualty scenarios.

Keywords Biodosimetry; CBMN; X-rays; multi-parameter analysis; emergency response

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1c-26-D2

Development of a same-day dicentric chromosome assay

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Biodosimetry assessment of exposures to ionizing radiation using the Dicentric Chromosome Assay (DCA) takes a minimum of three days. This is due to the need to culture human lymphocytes for 48 hours before cells can reach mitosis and, hence, display a sufficient level of chromosome condensation and markedly visible centromeres. However, since the radiation countermeasures should be delivered in 24 h following exposure, a shorter time-to-answer is a highly desirable improvement.

Inhibitors of Protein Phosphatases, such as Calyculin A, have long been used to shift the balance between key molecular factors that control the cell cycle and, therefore, induce condensation of chromosomes earlier, outside of mitosis (Premature Chromosome Condensation (PCC)). However, multiple research groups have reported that the quality of PCC chromosomes induced with Calyculin A in resting (G0) lymphocytes were unsuitable for cytogenetic analysis: chromosomes were loosely condensed, entangled, and fuzzy, making it impossible to detect dicentrics.

Here we present an improved Calyculin A-induced G0-PCC assay in multiwell plates (a same-day RABiT-II DCA). Unlike previous studies, which were trying to achieve higher chromosome condensation using high concentrations of Calyculin A (50 to 5000 nM), we found that this approach is not effective, and much lower concentrations should be used instead. This results in an approximately 10-times improved index of PCC cells suitable for dicentric analysis, as compared to earlier reports. For a same-day dicentric analysis, we stained highly-condensed PCC chromosomes with centromere probes and performed preliminary scoring of dicentrics in samples exposed to 3 and 8 Gy.

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Keywords Dicentrics; Premature Chromosome Condensation; Calyculin A; RABiT-II DCA.

Interphase Fluorescence In Situ Hybridization (FISH) for interphase chromosomal aberration-based biological dosimetry

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Metaphase spreads stained with Giemsa or with chromosome specific paints utilizing fluorescent in situ hybridization (FISH) have been in use since long for the study of radiation induced chromosomal aberrations and radiation dose estimation following accidental exposure (biological dosimetry). However, in cases of accidental exposure to ionising radiation the culturing of lymphocytes to obtain metaphase chromosomes and analysis of chromosomal aberrations is time consuming. Similarly, analysing radiation induced chromosomal damage in G0/G1 cells or non-dividing cells by premature chromosome condensation (PCC) is difficult and time consuming. Following large scale radiological emergencies, the time required for analysis is more important than precision of dose estimate. Painting of whole chromosomes using chromosome specific probes in interphase nuclei by the FISH technique will eliminate the time required for the lymphocyte or cell culture and allow a fast dose estimate. This provided that a meaningful dose response can be obtained by scoring color changes between chromosomal domains visible in interphase nuclei.

In order to test the applicability of interphase FISH for quick biological dosimetry, we irradiated whole blood from a healthy donor with 8 Gy dose of gamma radiation. We kept the irradiated whole blood for 1 h at 37°C to allow DNA repair and processed the blood thereafter for FISH with probes specific for chromosome-1 and chromosome-2. In a preliminary result we observed damaged chromosomal fragments in interphase nuclei of lymphocytes irradiated with 8 Gy of ionising radiation. These fragments were efficiently detected and quantified by the FISH techniques utilizing both confocal microscopy and fluorescence microscopy. Further investigations are underway to validate and establish a dose response standard curve. It will be an interesting adoption of the FISH technique to further expand availability of this assay that may be more helpful for biodosimetry and to study the effect of radiation at chromosomal level in interphase nuclei.

Keywords Radiation; Biodosimetry; Chromosomal aberration; Interphase nucleus; fluorescent in situ hybridization (FISH)

Detection of DNA Damage from Ionizing Radiation Using Markers of DNA Double-Strand Breaks and Imaging Flow Cytometry

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The Ionizing Radiation Health Science Division (IRHSD) of Health Canada is responsible for assessing the biological effects of exposure to ionizing radiation. A critical portion of this work includes improving biodosimetry assays, which are methods that allow for accurate and efficient assessment of DNA damage. Existing assays can be lengthy on an individual basis, which would be problematic in the instance of a large-scale radiation event. The IRHSD is working to adapt traditional microscope-based assays to high-throughput methods for emergency response. One such assay, the γH2AX assay, is an effective tool for triage biodosimetry as it is suitable for rapid, high-throughput testing for ionizing radiation exposure. The functional properties of γH2AX and 53BP1 are well-established as biological markers of DNA double-strand breaks [1]. Using an imaging flow cytometer (IFC), that combines the sensitivity of microscopy with the statistical power of flow cytometry to allow for the visualization of cells [2], lymphocytes can be simultaneously stained for γH2AX and 53BP1 to rapidly analyze DNA damage caused by exposure to ionizing radiation.

To optimize the protocol, blood was obtained from healthy volunteer donors and diluted at a 3:7 ratio in complete media. The samples were irradiated with 250 kVp X-rays, fixed, permeabilized and stained, followed by a 2-hour incubation. The samples were run on the IFC and images of the cells were acquired using INSPIRE acquisition software. The images acquired were analyzed using IDEAS analysis software. The objectives were to determine the optimal conjugate for the γH2AX antibody, the effectiveness of incorporating the 53BP1 antibody as a co-locator of DNA damage to improve the specificity of the assay, and the optimal staining concentrations of the γH2AX and 53BP1 antibody fluorochromes.

The optimal conjugate for the γH2AX antibody was determined to be AlexaFluor 488, with an optimal staining concentration of 3.75 µL/100 µL of blood at a laser power of 150 mW. The effectiveness and concentration of the 53BP1 marker is still being determined. Once the 53BP1 staining concentration is established, dose response curves will be generated. This work has established some of the key components of the methods and materials used in developing the γH2AX assay for use with imaging flow cytometry that will be effective in the rapid detection of exposed individuals. In addition to biodosimetry, this assay will be used to estimate the number of DNA double-strand breaks from various doses and radiation qualities, which can be used to validate future studies in modelling DNA damage.

Keywords γH2AX; 53BP1; Imaging Flow Cytometer (IFC)

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1c-29-D2

Cytogenetic damage of human lymphocytes in humanized mice exposed to neutrons and X rays.

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Detonation of an improvised nuclear device highlights the need to understand the risk of mixed radiation exposure as prompt radiation exposure could produce significant neutron and gamma exposures. Although the neutron component may be a relatively small percentage of the total absorbed dose, their large relative biological effectiveness (RBE) can induce larger biological DNA damage and cell killing.
The objective of this study was to use the hematopoietically humanized mouse model to measure chromosome DNA damage in human lymphocytes in vivo exposed to neutrons (0.3 Gy) and X rays (1 Gy), 24 hours after exposure. The human in vivo dicentric (DIC) and cytokinesis-block micronucleus (CBMN) assays were performed to measure chromosomal aberrations in human lymphocytes from the blood and spleen, respectively. The mBAND assay based on fluorescent in-situ hybridization labeling was used to detect neutron-induced chromosome 1 inversions in the blood lymphocytes of the neutron-irradiated mice.
Cytogenetics endpoints, MN and DIC show that there was no significant difference in yields between the two irradiation types at the doses tested, indicating that neutron-induced chromosomal DNA damage in vivo was more biologically effective (RBE ~ 3.3) compared to X rays. The mBAND assay, which is considered a specific biomarker of high-LET neutron exposure, confirmed the presence clustered DNA damage in the neutron-irradiated mice but not in the X-irradiated mice, 24 hours after exposure.

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The project of another low-cost metaphase finder (Third Report)

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Biological dosimetry is used to estimate one's dose by biological phenomena. The most popular and “gold standard” phenomenon is the appearance of dicentric chromosomes in metaphases. The metaphase finder is a tool for biological dosimetry that finds metaphase cells on glass slides. It consists of an automated microscope, auto-focus system, X-Y stage, camera, and computer. It does the image analysis of the microscopic images of the glass slides, and displays the positions of metaphase cells. The metaphase finder was used for the personnel who worked at Fukushima nuclear plant to know how much dose they were irradiated. The author has already reported the project of this low-cost metaphase finder system at EPRBiodose 2015, and also the application of artificial intelligence (AI) at EPRBiodose 2018.

The author and a software company are now preparing to produce the system commercially. The reported system in 2018 consisted of an automated microscope, an auto-focus system, an X-Y stage, a camera, and a computer. To enhance the accuracy of the system, with the addition of deep learning was tested. The pre-selection of metaphases using mathematical morphology before the AI process enabled the AI classification of true or false metaphases. Then, a total of 1709 images of the metaphase finder detected as 'metaphases' were read into a nine-layer artificial neural network to detect true metaphases. A total of 456 images were used for training, and the rest of the images were used for validation. The false-positive rate of AI was 0.89 and the false-negative rate was 0.90.

At this time, the author is reporting that the prototype of AI implemented metaphase finder was combined with the microscope system, and that the metaphase finder system’s accuracy was compared with previous non-AI system, using the same samples. The next goals are to implement a new automated dicentric counter and then obtain a dose-response relationship using the new dicentric counter.

Keywords metaphase finder, biodosimetry, chromosome aberration

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Biodosimetry based on γ-H2AX quantification in human peripheral blood lymphocytes after partial-body irradiation

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Background: Recent studies have suggested that quantification of γ-H2AX foci can estimate exposure to ionizing radiation. However, individuals in nuclear and radiation accidents are mostly partial-body irradiation (PBI), when the dose estimation formula of total-body irradiation (TBI) is used to assess the dose of partial-body irradiation, the dose obtained is often lower than the actual radiological dose. At present, there is no one method to evaluate the PBI dose widely. Therefore, there is an urgent need to establish a method for PBI dose assessment. **Objective:** The purpose of this study was to evaluate the dose-response relation of γ-H2AX foci in human peripheral blood lymphocytes after PBI, establish a simple and high throughput model to estimate PBI dose. **Methods:** Fluorescence-based quantitation of γ-H2AX foci was evaluated in human peripheral blood lymphocytes exposed ex vivo to ^{60}Coγ- rays in a dose range of 0.5 to 8 Gy. We analyzed the response of γ-H2AX to ionizing radiation in relation to dose, time after exposure, and individual variability. Here, we introduce a partial-body exposure analysis method, Q γ-H2AX, which is based on the number of γ-H2AX foci damaged cells. Results from TBI study were used to establish Q γ-H2AX dose-response calibration curves to assess acute partial-body exposures. **Results:** The results show the promise of automatic-H2AX scoring for a reliable assessment of radiation doses in a dose range of 0.5 Gy to 8 Gy at 1 h after exposure. Dose-response curves were fitted: $YF_1=18.532x^2-3.444x+3.109$, $R^2=0.92$ ($YF \leq 27.91$); $YF_2=7.949x+15.693$, $R^2=0.71$ ($YF > 27.91$). YF means the average fluorescence intensity of each lymphocyte after correction, and X means the dose of irradiation. **Conclusion:** The quantitation of γ-H2AX by flow cytometry method may provide a robust biodosimeter for analyzing partial body exposures to ionizing radiation in humans.

Keywords: γ-H2AX; biodosimetry; partial-body irradiation (PBI); ^{60}Co γ-rays

Inter- and intraindividual response to alphas, X-rays and mixed beams analysed at exon-level gene expression and chromosomal aberrations

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Introduction
In preparedness to a radiological emergency, further validation of transcriptional ionizing radiation biomarkers, including isoform expression, require a better understanding of inter- and intraindividual variability to exposures of different pure and mixed radiation qualities. Mixed beams of alphas and X-rays leads to inter- and intraindividual variable responses at the gene expression level [1]. Here, we investigated the interindividual and seasonal stability of the response after the same pure and mixed beam exposure conditions at the exon-level. In parallel, the stability of chromosomal aberration frequency was evaluated.

Methods
Blood from two donors was drawn in triplicate during three different seasons and exposed to 0-2 Gy of X-rays, alphas or 1:1 dose of X-rays and alphas. Differential variant expression of *FDXR*, *CDKN1A* and *MDM2* was analysed by qRT-PCR at 24 h after exposure. For chromosomal aberration analysis, blood cultures were set up and harvested 48 hours post-exposure by standard cytogenetic procedure.

Results
The magnitude of the dose-dependent upregulation of *FDXR*, *CDKN1A* and *MDM2* alternative transcripts varied inter- and intraindividually, with the exception of a stable response after alpha particles for the most strongly upregulated *FDXR* variants. Despite the seasonal variability, the response of each set of targeted alternative transcripts seemed very consistent between donors. *FDXR* variants 1-8 showed an overall synergistic response after mixed beams in both donors. On the contrary, when *FDXR* variants 4 and 7 were excluded by primer design, the overall interseason response was subadditive in both donors. All tested *MDM2* alternative transcripts showed an additive response to mixed beams, while *CDKN1A* variants varied between additive and subadditive depending on the level of fold change selected for the construction of the envelope of additivity.
Scoring of chromosomal fragments is currently on-going. In the first season, a synergistic interaction of alphas and X-rays was generally observed, with a higher frequency of extrachromosomal fragments in one of the donors.

Conclusion
Inter- and intraindividual seasonal variability occur at the expression level of alternative transcripts in human lymphocytes, but overall, there is good agreement in induction of *FDXR*, *CDKN1A* and *MDM2* transcripts between the two donors tested in the present study. The mixed beam effect was alternative transcript-dependent. Conclusions from aberration analyses are pending.

Keywords Gene expression, alternative transcripts, aberrations, mixed beams, variability

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Preliminary Study on Radiation-sensitive genes of Neutron/gamma Mixed Radiation Field

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Introduction

Nuclear reactor operation, tumor radiation therapy, nuclear leakage, and nuclear explosion accidents can all result in exposure to mixed neutron/gamma（n/γ）radiation. Radiation damage assessment based on changes in radiation biomarkers has recently become an effective emergency classification method for radiation emergencies. This study aimed to screen radiation-sensitive mRNA molecules in human peripheral blood which had been exposed to mixed neutron/gamma radiation, and identify early-stage diagnostic indicators of radiation damage risk assessment in mixed neutron and gamma radiation exposure.

Methods

The peripheral blood of three healthy adult males were collected, and 2.5 MeV monoenergetic neutrons (dose rate 1 Gy/h) and 60 Co source (dose rate 3.37 Gy/min) was administered to the monoenergetic neutron 1.42 Gy, gamma1.42Gy and neutron/gamma (n:γ=1:1) mixed radiation field, respectively. Differentially expressed mRNA were identified by whole transcriptomic sequencing technology, and the expression levels of differentially expressed genes were verified using qRT-PCR testing and bioinformatics analysis.

Results

Co-expression analysis of differentially expressed mRNAs in each irradiation group revealed 34 differentially expressed co-expressed mRNAs, all of which were up regulated, Including AEN, PHLDA3, FDXR,CD70, BBC3, BAX, DDB2,FCNA, DDB2, POLH, MDM2, etc. Human lymphocyte AHH-1, which was irradiated with the 252Cf source.was used for qRT-PCR verification. The results showed that the differential expression of BAX, FDXR, FCNA, DDB2, POLH, MDM2 and AEN was consistent with the sequencing results, and DDB2, FDXR, PCNA, BAX genes showed an obvious dose-response relationship within 0 and 1 Gy. Bioinformatics analysis had indicated that these differential genes may participated in the regulation of P53 signaling pathway, nucleotide excision repair, apoptosis and other signaling pathways which would affect cell proliferation, apoptosis, DNA damage repair, etc,.

Conclusion

The differentially expressed genes DDB2, FDXR, PCNA, BAX, etc. identified by our research are expected to be developed as potential radiation biomarkers for neutron/gamma mixed radiation field exposure, and these genes could provide data regarding biological biomarkers for health risk assessment of mixed radiation exposure fields.

Cytogenetic and hematologic changes in breast cancer patients after partial-body and fractionated radiotherapy

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Various radiation biodosimetry tools have been studied in preparation for nuclear and radiological accidents. However, direct data of *in vivo* response in various exposure scenario would still be necessary to make an appropriate strategy for dose estimation and medical treatment in the radiological accidents. We assessed changes in chromosomal aberrations and blood cell counts before, during, and after localized fractionated radiotherapy. Twelve patients who received adjuvant radiotherapy after breast conserving surgery were recruited for this study [1]. Their blood samples were taken at various time points during radiotherapy. Blood cell counts were measured and dicentric chromosome assay and translocation assay were performed at each time point. Frequencies of dicentric, dicentric plus ring, excess acentrics, and translocation were increased with cumulative irradiated dose. Lymphocyte counts were decreased and neutrophil to lymphocyte ratio were increased during radiotherapy, but there were no significant changes in hemoglobin, platelet, and neutrophil. Their lymphocyte counts, NLR and chromosomal aberration frequencies were significantly correlated during radiotherapy. However, there were individual differences in radiation response and recovery kinetics between patients. Taken together, our study suggests that cytogenetic and hematologic markers could be useful to assess radiation exposure in localized and fractionated irradiation scenario and individual physiological background and previous radiation exposure history should be considered for accurate radiation biodosimetry in radiological accidents.

Keywords Biodosimetry; Radiotherapy; Lymphocytes; Dicentrics; Translocation

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Differentiating exposed vs. unexposed persons following ^{131}I internalized exposure using transcriptional analysis: Biodosimetry using a high-risk neuroblastoma patient cohort

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Over the last two decades, genome-scale tools have identified large-scale changes in gene and protein expression profiles within minutes to days after exposure to ionizing radiation (IR). We have previously demonstrated that gene expression analysis on DNA damage and DNA repair transcripts (*CDKN1A*, *BAX*, *DDB2*) in the peripheral blood can be used to estimate internalized ionizing radiation exposures up to 96 hours after treatment. We now expand our biomarker panel to include 7 transcripts (*CDKN1A*, *FDXR*, *BAX*, *BCL2*, *BCL2L1*, *DDB2*, and *PRKDC*) which can accurately differentiate between ~2 Gy of exposure and untreated baseline samples 72 hours after ^{131}I exposure without relying on a time-dependent equation. At 72 hours post-exposure, we were able to use our gene-expression derived model for predicting dose of exposure (R^2 >0.89 compared to kinetic decay model) as well as sorting exposed from unexposed samples with over 98% specificity and 92% sensitivity. Here, we expand upon our previous findings and validate a partial-least squares discriminant analysis (PLS-DA) linear regression model with leave-one-out cross-validation (LOO-CV) for predicting exposures up to ~2 Gy based on gene expression findings alone. We also utilized this 7-part transcript panel to expand our timeline and investigate gene expression fluctuations up to 15 days following ^{131}I exposure. In this study, peripheral blood was drawn from patients before IR therapy, as well as 72 hours and 15 days post ^{131}I-metaiodobenzylguanidine (^{131}I-mIBG) treatment. Total lymphocyte RNA was extracted before (untreated) or after ^{131}I-mIBG (treated). At 15 days post-treatment, we utilized PLS-DA and LOO-CV to determine the specificity of exposure prediction compared to untreated controls. Our panel was able to differentiate exposed from unexposed samples with 87.5% sensitivity and 87.5% specificity at 15 days. Our studies highlight our expanded biodosimetry transcript panel as a predictive biomarker tool for both early and late internalized ^{131}I exposures in children. It also demonstrates the utility of our transcript panel to differentiate exposed from non-exposed individuals to radioactive ^{131}I out to 15 days, which can aid in triaging individuals in the event of a mass exposure scenario. Ongoing analyses aim to identify additional biomarkers predictive of treatment outcomes for children with high-risk neuroblastoma.

Keywords: ionizing radiation; mIBG; neuroblastoma; gene expression; biodosimetry

2-03-D3

Chromosome aberrations outcome peculiarities during radiotherapy course followed by previous radiation exposure

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With the cancer treatment development, cancer patients became subsequent part of the human population. In case of large-scale radiation accident the knowledge about confounding factors influencing the background aberration level is of importance for biological dosimetry.

The aim of the study was to determine the peculiarities of aberrations outcome during radiation treatment in cancer patients who have had previous radiotherapy.

Unstable chromosome type aberrations were analyzed in lymphocytes of 30 patients with lung and head and neck cancers were studied before, in the middle and at the end of radiotherapy course. Part of the patients (primary patients) had no previous radiation or chemical treatment, the others (secondary patients) went through radiotherapy in 0.5 – 7 years before study. The treatment was provided with linear accelerator, dose per fraction was 1.8 – 2 Gy.

It was demonstrated that the pace of chromosome aberrations enhancement depended on the irradiated body volume. For the patients with higher irradiation volume (lung cancer patients) chromosome exchange level raised from the beginning to the end of radiotherapy course both in patients with and with no previous treatment. In patients with lower irradiation volume (head and neck cancer patients) the pace of chromosome aberration level raise during radiotherapy course was lower in a group of patients with previous irradiation. The frequency of chromosome aberrations before radiotherapy was higher than background in patients with both tumor localizations. For head and neck cancer secondary patients the dicentric level before radiotherapy was higher than background and higher than that of in primary patients. The values of chromosome aberration level before radiotherapy in secondary patients was comparable with the value in the middle of radiotherapy course in primary patients. Thus, the dicentric ratio in primary patients before to the middle of radiotherapy course was 1 : 28.4, that of in secondary patients was 1 : 1.26.

The peculiarity of biodosimetry data treatment in cancer patients studied because of suspected accidental radiation exposure will be discussed.

Keywords: chromosome aberrations, biodosimetry scenario, cancer patients, previous radiotherapy.

Association Between SNPs of DNA Damage Repair Genes and Radiosensitivity in Healthy People

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Objective At present, radiotherapy remains the mainstay of modern cancer treatment. About 50% of all cancer patients require radiotherapy at some stage of their illness. However, Patients which were treated by the same dose of treatment experience different toxicity. The reason is mainly related to the difference in sensitivity to radiation damage[1]. A minority have no observable effect, most have clinically mild or moderate changes, and a few suffer serious normal tissue complications that may even be life-threatening. In this study, we screened and verified single nucleotide polymorphism sites (SNPs) related to radiation damage, analyzed the impact of this site on radiosensitivity. It will provide molecular targets for radiosensitivity prediction, which is to be able tailor radiotherapy for individual patients to improve outcome.
Methods A 60Co long-distance treatment machine was used to irradiate the blood from 68 healthy donors with a dose of 0 or 2 Gy γ-rays (dose rate 0.5 Gy/min). Healthy donors were divided categorically into tertiles（resistant, normal, sensitive） based on 2 Gy γ-irradiated chromosomal aberration score. Genomic DNA from 0 Gy irradiated samples was extracted, then exome sequencing and bioinformatics analysis were used to analyze the differential SNPs of the resistant donors and sensitive donors, which were verified by MALDI - TOF MS. Goodness of fit to the Hardy-Weinberg equilibrium (HWE) was assessed by the $\chi 2$ test for each SNP. Different genetic models, such as codominant, dominant, and recessive models, were tested for their association with radiosensitivity.
Results The healthy donors were classified into three groups by the tertile: "resistant" (chromosomal aberrations rate <11), "normal" (chromosomal aberrations rate 12-15), and "sensitive" (chromosomal aberrations rate >16). The 0 Gy irradiated samples were subjected to exome sequencing and bioinformatics analysis. A total of 2077 differential SNP sites were initially selected, and 35 sites were verified by MALDI-TOF MS. All the studied SNPs followed Hardy‑Weinberg equilibrium (P>0.05). Using the normal group as a reference, the genotype distributions of rs13018957 (TRIP12), and rs2022302 (POLN) were found to be significantly different in the dominant genetic model of the sensitive group (rs13018957: p=0.00; rs2022302: p=0.01). The genotypes CT and CC of rs13018957 (CT: p=0.00, OR=26.13, 95%CI=3.03-225.30; CC: p=0.03, OR=15.97, 95%CI=1.27-200.44), AA of rs1700490 (p=0.03, OR=32.22, 95%CI=1.33-781.25), and AG of rs2022302 (p=0.01, OR=13.98, 95%CI=2.09-93.36) were risk factors for radiosensitivity.
Conclusions Current study suggests that 2 SNPs are associated with radiosensitivity. It will provide molecular targets for radiosensitivity prediction, which is to be able tailor radiotherapy for individual patients to improve outcome, and it will help to understand the genetic basis of radiosensitivity .

Keywords Ionising radiation; Radiosensitivity; Chromosome aberration.

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Dosimetric characterization of an ultra-high dose rate beam for FLASH radiotherapy through alanine EPR dosimetry

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Experimental evidence is growing, supporting the evidence of a considerable normal tissue sparing effect when treatments are delivered with dose rates much larger with respect to the conventional ones [1]. In particular, an increasing of the therapeutic window has been demonstrated for dose rates over 50 Gy/s, over a large variety of in-vivo experiments [2]. If confirmed, the 'FLASH effect' has the potential to re-shape the future of radiation treatments, with a significant impact on many oncology patients [3]. Ultra-high dose rate (UHDR) beams for FLASH radiotherapy present significant dosimetric challenges [4]. Ionization chambers are affected by ion recombination effects, although novel approaches for decreasing or correcting for this effect are being proposed [5]. Passive dosimeters, as radiochromic films and alanine [6], could be used for UHDR measurements, although dose determination is typically time consuming. Solid state detectors, such as diamond or Silicon Carbide (SiC) detectors, have been also recently investigated, as a valuable alternative for real-time measurements.

In this work we analysed the response of alanine pellets to UHDR electron beams. Irradiations of alanine pellets with electron beams at 7 and 9 MeV, accelerated by a SIT-Sordina ElectronFlash Linac, at conventional and UHDR regimes have been carried out. Average dose rates up several hundreds of Gy/s were used for the experimental campaign, with instantaneous dose rate even more two orders of magnitudes larger. Indeed, pulse structure of the used accelerator is characterized by a pulse duration between 1-4 us and a frequency up to hundreds of Hz. The analysis of the depth dose profile performed by stacking alanine pellets along the electron beam direction allowed to evaluate whether a dependence on the dose rate is present for these UHDR beams. The experimental results were aided by computational analyses. The results will be presented and discussed in details.

Keywords Alanine, FLASH radiotherapy, ultra-high dose rates, EPR dosimetry

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A Tiered Biodosimetry Approach for Triage after Radiological/Nuclear Mass Casualty Incidents

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Large-scale radiological or nuclear incidents can potentially expose several hundreds and thousands of people to substantial doses of ionizing radiation. Timely assessment of absorbed radiation dose is critical for making an appropriate “life-saving” clinical decision for the exposed human population. Our laboratory has been undertaking a tiered multiparametric biodosimetry approach for an effective triage. In our tiered approach, high sensitive and low false positive biodosimetry assays will be used in tier I for a rapid exposure dose categorization for triage. In tier II, cytogenetic validation of exposure doses will be performed for segregated individuals whose exposure doses are at or above 2 Gy. Our tiered approach will be useful for rapidly segregating the exposed individuals from “worried well” population thereby prioritizing the medical intervention process. A number of minimally invasive dosimetry assays (Pseudo Pelger-Huet anomaly, neutrophil to lymphocyte ratio, interphase chromosome breakage assay, PenDoc device for lipid profiling and smart phone components) are currently being optimized and validated for specificity and sensitivity. Once optimized and validated, appropriate assay/tool will be chosen for development of automation in high throughput platforms. Concurrently, efforts to promote automated high throughput sample processing for Dicentric Chromosome Assay and Cytokinesis Blocked Micronucleus Assay from initiation of lympchyte culture to absorbed dose estimation are also underway. Our ongoing multidirectional approach [1-5] using automated high throughput robotic platforms will constitute an efficient radiation emergency response to fulfil the biodosimetry needs for Rad/Nuc mass casualty incidents in the future. The status on the developments of various assays in our laboratory and the importance of a tired approach for triage will be discussed.

Keywords: Radiation biodosimetry, triage, mass casualty incidents, automated high throughput platforms and cytogenetic assays

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Signal detected by in vivo EPR tooth dosimetry in a nurse with many years of experience in endoscopic retrograde cholangiopancreatography

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A positive signal was detected in in-vivo electron paramagnetic resonance measurements using a tooth performed with the help of a nurse who was engaged in endoscopic retrograde cholangiopancreatography over 10 years. The estimated dose was 0.13 ± 0.03 Gy (mean ± standard deviation). The equivalent dose to the lens of the eye of this nurse was at least 66.8 mSv in the period from June 2004 to March 2011, which was confirmed by the official records of the hospital, and the maximum monthly dose was about 10 mSv in this period. However, it was considered necessary to examine the effects of aesthetic dental treatment since this treatment might also produce EPR signals.

In a survey conducted in Japan, half of the nurses in the endoscopy room had an annual equivalent dose of 20 mSv or more to the lens of the eye. Although the doses to workers are now considered to have been reduced by improving radiation protection techniques, it was thought that the signals could still be detected among medical workers by this method.

Keywords EPR; tooth enamel; occupational radiation exposure; ERCP; nurse

The importance of the National biodosimetric laboratory of the Republic of Kazakhstan for ensuring national and regional preparedness and response in radiation¥nuclear emergencies

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The National Radiation Safety System of the Republic of Kazakhstan is a complex and resource-intensive task that requires time, money, energy, coordinated efforts and interdepartmental interaction at different administrative stages for its development and implementation. The high value of emergency preparedness and response, strengthening emergency planning capabilities is a critical priority of national importance.

As recent events in the world show, geopolitical turbulence, natural disasters or technogenic disasters can affect a large number of people. The consequences of these disasters often cross borders and can threaten the security and stability of entire regions.

In the case of a radiation/nuclear emergency, when the details of the events are little known, and there are no physical measurements or dose calculations, biological dosimetry may be the only means of determining the individual quantitative absorbed radiation dose. In the early period after a radiation/nuclear emergency or a terrorist attack, where a large number of people are irradiated, cytogenetic biodosimetry is a method of "first sorting" of victims at the premedical level. It will determine the speed and reliability in making decisions related to the priority of medical care, save lives, minimize health consequences, increase the duration and quality of life of victims [1].

Therefore, a lot of attention is paid to the development and improvement of cytogenetic biodosimetry at the Institute of Radiation Safety and Ecology of the National Nuclear Center of the Republic of Kazakhstan. From 2020 to 2023, Kazakhstan has a project fully funded by the NATO Program "Science for Peace and Security" (Grant G5684) "BioPhyMeTRE”: “Novel biological and physical methods for triage in radiological and nuclear (R/N) emergencies” in collaboration with the Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) Italy, the National Institute of Health (ISS) of Italy and the Ruger Boskovic Institute (RBI) of Croatia [2].

Keywords: biological dosimetry, triage, radiation safety, preparedness and response

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Multi-harmonic Signal Acquisition for L-band EPR Tooth Dosimetry

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Electron paramagnetic resonance (EPR) tooth dosimetry is one of the well-known retrospective dosimetry methods. It has high stability of radiation induced signal and unnecessity of additional dosimeter. In vivo tooth dosimetry using L-band EPR spectrometer has been recently developed without tooth extraction and additional sample preparation [1]. Therefore, it is considered as a suitable method for first screening of field triage for mass casualty radiation emergency.[2] In SNU, a homebuilt L-band EPR spectrometer was fabricated for in vivo tooth dosimetry based on previous research in Dartmouth EPR center, and its performance was verified [3]. In the established EPR spectrometer, only 1st harmonic signal was acquired during the demodulation steps using Lock-in amplifier. However, independent multi-harmonic signals exist in the modulated signal which could improve dose estimation accuracy. Therefore,
digital signal processing was applied for the signal demodulation step to improve accuracy of dose estimation using multi-harmonic signals.

In the developed spectrometer, modulated signal was directly transmitted to the high-resolution data acquisition board and converted to the digital data. After the signal conversion, LabVIEW based software demodulates the signal at the multiples of modulation frequencies to acquire multi-harmonic signals.

The accuracy of dose estimation was verified for 1st and 2nd harmonic signals using one upper central incisor. The standard error of inverse prediction (SEIP) of each dose response curve were 0.166 Gy and 0.309 Gy for 1st and 2nd harmonics. Theoretically, two harmonics are independent, and the calculated correlation value of two harmonics was 0.071. Therefore, optimally weighted average could improve the dose estimation accuracy. The SEIP was minimized when the weight factor of harmonics was 0.78 and 0.22 for 1st and 2nd harmonics, respectively. This was same as theoretical optimal value with an assumption that harmonics are mutually independent. As a result, SEIP was reduced to 0.151 Gy.

Keywords In vivo EPR tooth dosimetry; Digital Signal Processing, Multi-harmonic signals

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Recent findings regarding PSL detection on salt-containing fortuitous dosimeters

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The security risk of R/N events have increased through the years due to the raised use of ionising radiation in hospitals, the nuclear research or industry, and, unfortunately, also to the threat of malicious or terrorist attacks with radiological materials. Radiation victims, i.e. people not carrying conventional dosimeters can be exposed to doses of ionising radiation which requires individual dose estimates to support classifications for convenient medical triage in large scale accidents. The current golden standard is the biological dosimetry, that is based on high cost techniques taking at least several days for the dose estimation. Therefore, the development of physical methods is the focus of the research to complement biodosimetry. Significant efforts have been made on the dose assessment from the objects that could be found at the location of the event, i.e., on fortuitous dosimeters. The optical luminescence technique has been successfully applied to various materials. Before establishing protocols regarding such objects and materials, they should be studied by determining their dose-response and reliability.

In this purpose, we combine the use of a low-cost portable Photo-Stimulated Luminescence (PSL) reader with the selection of cheap materials that contain salt: salty snacks and coastal sand [1]. The proposed method for determining the dose from such dosimeters is based on the use of a reader also applied for determining irradiated food [2] and therefore widely spread in many countries, and available for mass application.

Until now, the two laboratories involved in the *BioPhyMeTRE* project, fully funded by NATO Science for Peace and Security Programme (Grant G5684), collected interesting and promising results on salty snacks and coastal sand [2]. These results encourage the use of blue light stimulation which provided a better response compared to infrared light and were not influenced by the difference in dose rates applied by the two Institutes. Furthermore, the different approach for determination of the detection limit were used and discussed.

Keywords retrospective dosimetry, photo-stimulated luminescence, radiation triage, salty snacks, sand

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Gamma irradiation effect on some asthma drugs: EPR detection of radiosterilization

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Among many sterilization methods, radiosterilization is a preferred method in the pharmaceutical industry due to its advantages. However, the detection of radiosterilization of drugs is a growing concern for many governments regulatory agencies around the world due to changes in the drug's structure as a result of exposure to radiation. Electron Paramagnetic Resonance (EPR) proved to be a very sensitive technique to discriminate between irradiated and non-irradiated drugs and detect radicals for days even to years. The drugs with the trade names Airfix (Neutec Pharmaceuticals, Turkey) Ventolin tablets 2 mg concentration (GlaxoSmithKline, UK), and Prednol (Gensenta Pharmaceuticals, Turkey) which are the subject of this article, are the primary drugs used alone or in combination in the treatment of asthma. In this study the focus is on the detection of radiosterilization of these three irradiated drugs by using EPR spectroscopy. For this purpose, powdered tablets were irradiated with ^{60}Co gamma source and EPR spectra of natural and 25 kGy irradiated samples were recorded with JEOL JesFa-300 X-band EPR spectrometer. EPR analyzes of both irradiated and non-irradiated samples were recorded under the same spectrometer conditions at room temperature and the temperatures of the -150 ^{o}C and +50 ^{o}C. Regarding the commercial aspects of drugs, it is expected that radicals can be detected up to 2 years after irradiation. Therefore, paramagnetic centers formed by the effect of radiation in the samples were determined and the stability of these centers was examined for two years by performing the fading study.

Keywords. EPR spectroscopy; gamma irradiation; asthma drugs; radiosterilization

EPR dosimetric properties of X-ray irradiated potassium bitartrate

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The electron paramagnetic resonance (EPR) of potassium bitartrate (PB) as a new potential EPR radiation sensitive material was investigated. PB samples were exposed to X-ray at room temperature in the range of absorbed dose, 1-9 Gy. The EPR spectra were investigated through variation of signal intensity with respect to absorbed dose, magnetic field modulation amplitude, and microwave power. The g-factor obtained in this study for PB sample was 2.00942 ± 0.00130. The dose-response curve indicates linearity up to 9 Gy. PB also present radiological properties close to bone at an energy of less than 200 keV. Therefore, PB is a promising material for EPR dosimetry at specific applications.

Keywords : Potassium bitartrate; Electron paramagnetic resonance (EPR); Radiation; X-ray; Dosimetry.

EPR investigation of gamma irradiated Famoser drug: For dosimetric purposes

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Applications related to radioactivity gain importance in many fields as time passes in parallel with the developments in science and technology. Today, in many fields of technology (industry, medicine, security, agriculture, animal husbandry, research, education, etc.), ionizing radiation sources are used intensively. Radioactive pollutants and radiation accidents can cause genetic changes in living organisms, deterioration of DNA structure, cancer disease, and even death depending on the absorbed dose [1]. Measurement of radiation exposure doses and their monitoring can be performed using Electron Paramagnetic Resonance (EPR) dosimetry. It is very important to measure the doses that people are exposed to, especially in accidents, accurately and quickly. Considering that the public does not use a standard dosimeter especially in radiological or nuclear accidents, it is important to investigate the dosimetric properties of the materials next to and/or on the victims to evaluate the exposed radiation doses during the incident retrospectively [2]. For this reason, studies on new dosimeters (drugs, plastics, glass materials etc.) are in the focus of attention of researchers. In this study, Famoser 20 mg, one of the gastroenterology drugs, was irradiated with a ^{60}Co gamma source in the dose range of 10-900 Gy and examined with the EPR technique. EPR spectra of natural and irradiated powder samples were recorded under the same spectrometer conditions, using JEOL JesFa-300 X-band EPR spectrometer located in Selçuk University Advanced Technology and Research Center (ILTEK). Dose-response, kinetic (isochronal and isothermal annealing) and fading studies were performed in order to investigate the potential use of the drug as a retrospective/accident/emergency radiation dosimeter in possible radiological/nuclear events.

Keywords Electron Paramagnetic Resonance (EPR), EPR dosimeter, gastroenterological drugs, Famoser, irradiation

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EPR dosimetry on touch screen of smartphones: dosimetric investigations of the latest generation of glass

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In the context of malevolent or accidental use of radioactive materials, there is an identified need for a fast and reliable method to sort out the individuals actually exposed to ionizing radiation and needed immediate medical cares. A proposed method uses the smartphone touchscreens analysed by EPR as a radiation dosimeter in order to determine the dose of exposure to a person [1]. EPR has been used to estimate dose of exposure to radiation with a wide variety of materials collected on exposed individuals [2]. However, the huge advantage of tempered glass of touchscreen is that everyone has currently a smartphone and therefore a fortuitous dosimeter in their pocket. The work here is focused on the study of the latest generations of Corning® Gorilla® Glass. Gorilla® glasses are known to be alumino-silicate glasses but the specific compositions are not published. By being able to identify in the glass of the touch screen, the stable induced defects caused by irradiation along with the detection of the type of glass generation, the exposure dose can be quantified. The EPR spectra are complex in their analysis as radio induced defects could overlap with defects present already in the glass (Figure 1) or generated during their manufacturing process or even due to exposure to UV irradiation.

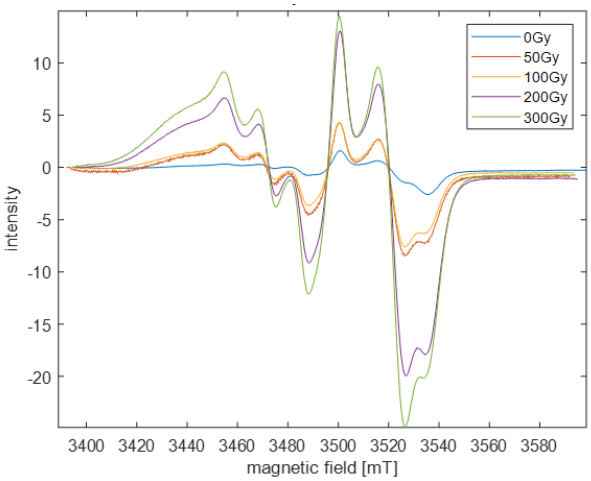

Figure 1. EPR spectra of Gorilla® Glass 4th generation according the irradiation dose

Keywords EPR spectroscopy, accident dosimetry, touchscreen glass, radio-induced defects.

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3-11-D2

Heat-induced EPR signals of human fingernails

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With consideration of the various high-temperature situations in normal daily activities and certain working environments, the potential confounding influence of heat on the radiation-induced EPR spectra of human fingernails has been investigated. The samples were heated at different temperatures between 20–160 °C for 1 h before or after irradiation with ^{137}Cs γ-rays. Changes in the overall visual shape of the EPR spectra were observed in the irradiated and unirradiated samples heated above 100 °C, thereby showing a heat-induced signal was present in the fingernail spectrum. The effect of heating showed an evident bulge in the spectra (between g = 2.014 and 2.024), which was more pronounced in both the irradiated and unirradiated samples heated at higher temperatures, with reduced peak-to-peak intensities and broadened spectra. The heat-induced signal was found to be unstable, decayed for about a few days, and was easily removed upon treatment with water. Similar thermal features were seen in the EPR spectra of both the irradiated and unirradiated samples, which suggests that the heat-induced signal and RIS were overlapped, but probably independent. Overall, these findings will be useful for more reliable dose assessment in case of accidental hand exposure under different thermal conditions.

Keywords EPR; ESR; fingernail; temperature; hand exposure; radiological accident, heat-induced signal, retrospective dosimetry

A preliminary report on retrospective dose assessment by FISH translocation assay in FDNPP Nuclear Emergency Worker Study (NEWS)

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Purpose: For emergency restoration work in the Fukushima Daiichi Nuclear Power Plant (FDNPP), the upper dose limit was raised from 100 to 250 mSv from March 14 to December 16, 2011. During this period, approximately 20,000 workers engaged in emergency work, out of which 174 workers were exposed to radiation exceeding 100 mSv, which is a limit on effective dose of 100 mSv in 5 years for normal occupational exposure. A national project of long-term longitudinal health care and epidemiological research analyzing the effects of radiation on human health for these workers (NEWS) has been conducted since 2014 in Japan. The biodosimetry team is currently performing retrospective dose assessments in order to confirm/assist physical dose assessment and clinical findings.
Materials and Methods: In 2018, peripheral blood for chromosome translocation analysis was collected from 62 workers. Retrospective dose assessment was performed by fluorescence *in situ* hybridization translocation (FISH-Tr) assay using whole-chromosome painting probes for chromosomes 1, 2 and 4 (ISO 20046, 2019). The exposure dose was estimated with the “radir” package in R, using the individual donor’s age-matched dose-response curve constructed by Abe *et al*. and Goh *et al*. [1, 2].
Results and Discussion: The range of estimated dose by FISH-Tr assay was 0-635 mGy and 23 workers showed reliable estimated doses of more than 189 mGy. Workers with physically measured cumulative dose recordings above 70 mGy in 2011 estimated doses up to 5 times higher in biologically estimated doses in 2018. Furthermore, smoking and medical exposures are potential factors that could increase the estimated biological dose. The regression line slope between the estimated and cumulative dose was higher in smokers and ex-smokers than in non-smokers. Medical exposure from CT scans also showed a more significant effect on the discrepancy between cumulative and estimated biological doses, highlighting that previous medical exposures could potentially over-estimate retrospectively estimated occupational exposures.

Keywords Retrospective biodosimetry, FISH translocation assay, FDNPP, nuclear emergency worker, NEWS

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Usage of enamel as a dosimeter below 200 mGy

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Releases of the substantial amounts of radioactive materials from the Fukushima Daiichi Nuclear Power Plant due to its accident result in an external exposure to human and animals. From the point of view of radiation safety, the precise estimation of the external exposure dose from those radioactive materials is important. We utilise electron spin resonance (ESR, or electron paramagnetic resonance, (EPR)) dosimetry for the dose reconstruction of human/animals [1]. On the other hand, from the viewpoint of dosimeter, tooth enamel can be one of the powerful materials which can measure from the order of mGy to Gy. In the present study, we evaluated the performance of tooth enamel as a disposable dosimeter below 200 mGy.

Dentine of Japanese molar tooth was ground away with a dental bur until only the hollow shell of the harder enamel was left. The separated enamel was crushed into small grains with nippers and distributed into three quartz tubes. Three sets of a tube and a commercial pocket dosimeter (PD) were put in a plastic box and exposed to the environment in high, medium, and low air dose rate areas in Fukushima Prefecture, Japan, from April to October (avoid the snowing season) for two years (the exposure period is total one year). Before the exposure and after the collection, ESR spectra were measured by a model JES-RE2X ESR spectrometer (JEOL, Tokyo, Japan) and the CO_2^- radical intensity was estimated by using 'EPR dosimetry' programme [2].

The absorbed dose estimated by both methods linearly increase with increasing days, and they matched within error bars for all the areas. It indicates that the tooth enamel can be used as a dosimeter as well as the commercial PD below 200 mGy. We are going to evaluate the performance of the tooth enamel at more low/high absorbed dose by providing more pure enamel or using synthetic hydroxyapatite crystals.

Keywords electron spin resonance (ESR); electron paramagnetic resonance (EPR); Fukushima nuclear power plant accident: external exposure dose

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4-03-D3

EPR-based validation of the uncertainties of calculated external doses for population exposed in Urals region

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EPR measurements of teeth are widely used in retrospective dosimetry to validate theoretically derived external doses. Particularly, it was used as a method for external dosimetry in the territories contaminated by PO Mayak (Urals region) in the 1950s to validate the mean dose estimates predicted by the Techa River Dosimetry System (TRDS). The current version of TRDS includes stochastic modelling to predict both mean doses (point estimate) and the corresponding uncertainties (interval estimate) for individuals exposed. Stochastic modelling includes assumptions and simplifications and the interval dose estimates need validation too. We propose an approach on how to use the results of tooth EPR dosimetry for validation of both individual dose and the dose uncertainties calculated. The analysis of the calculations with the previous version of TRDS shows an underestimation of the uncertainties of external exposure doses.

The purpose of the study is to compare the interval dose estimates obtained with EPR in tooth enamel and model predictions obtained with the improved version of TRDS.

In total, 493 EPR measurements of teeth from 220 people were suitable for the analysis. Twenty-five percent of teeth measured by EPR were investigated in parallel for ^{90}Sr activity concentration in the tooth tissues to exclude the internal dose component. The internal dose fraction for other teeth was modeled and excluded too. The overall uncertainty of EPR-based dose includes measurement errors and uncertainty of internal dose estimation, as well as the uncertainty of background dose subtracted. The majority of the individuals lived in areas with low levels of pollution or had short-term contacts. Small EPR doses were measured for them at the limit of the method's capability with a large uncertainty; the width of the 90% confidence interval (CI) could exceed 200-300 mGy. In contrast, 90% CI of TRDS-predictions for the same individuals did not exceed 100 mGy. Therefore, the data available were grouped according the width of 90CI, viz.: (1) 90%CI of EPR-based dose ≤ 90%CI of TRDS prediction (38 individuals); (2) 90%CI of EPR-based dose > 90%CI of TRDS prediction (182 individuals).

In total 91% of interval dose calculations (90%CI) overlap with those based on EPR. In the first group, this overlapping is 100%. Looking at the first group, one can expect that the calculated uncertainty is more reliable the more the proportion of the interval estimate based on measurements overlapped with the calculation result. The average fraction of the overlapping was found to be 80%. Looking at the second group, we expect that if the calculation uncertainty interval is completely within the measurement uncertainty interval, the calculation uncertainty does not contradict the measurements. The fraction of non-contradictive results was found to be 80%. We conclude that current predictions of external dose uncertainties are reasonable.

EPR studies of soils in vicinity of the former testing site in RK and used approaches

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The environmental problems of the former nuclear weapon test sites remain relevant, as does the study of impacts to environmental and population. Estimates of the irradiation of environmental mineral objects of the region become more difficult due to longtime decay of radionuclides – products of nuclear explosions. The EPR method was first used to investigate the effects of an explosion on the environmental objects in [1].

At the study of the Semipalatinsk test site (STS) objects in RK with EPR method there were found and studied the signals of paramagnetic centers (PMC) of the E_1' type in the soil fractions and correlation of the radiation PMC signals with mapping of point pollution by radionuclides as was shown on the terrain along South-East trace STS [2, 3]. Studies carried out later on soils and bottom sediments in regions related to storage and recycling of wastes of nuclear industry showed correlation of signals with storage duration [4].

Provided in the given work studies with EPR method of underground testing site are devoted to finding out the applicability of EPR –dosimetry methods to studying irradiance of mineral natural objects. As a result, we found EPR spectrum features for soil fractions. Approaches being proposed to estimation of radiation exposure of test area objects are based on signals of radiation paramagnetic centers of soil probes fractions and layers [5], as the small fractions were the most informative.

PMC concentration dependencies on external factors and on soil properties have been studied. It has been shown that it is possible to use the influence of modifying factors – annealing and irradiation to detect PMC of type concerning to possible radiation impact.

Keywords EPR, soils, fractions, annealing, irradiance.

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Twenty-two years later: Consistent dose estimation of an accidental overexposure by retrospective biological dosimetry

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As the chromosomal translocation rate increases with age in the non-exposed population, the translocation-based dose estimation of an external radiation exposure victim needs to take into consideration such background. We sought to retrospectively estimate the dose of exposure of a victim from the Lilo radiological accident in Georgia twenty-two years afterwards and compare it to the original biological dosimetry-based dose calculation performed in our laboratory. Similar types of studies have retrospectively estimated a radiation dose, notably involving victims of the Chernobyl, Goiânia and Tammiku accidents [1][2][3]. Nevertheless, their estimations were done after shorter periods of time post-exposure and in some cases, the exposure might not have been exclusively of an external nature [1][2].

In this study, we used Fluorescence In Situ Hybridization (FISH) to detect and score chromosomal translocations in lymphocytes from a recent blood sample of the victim. We performed the analysis using our laboratory's updated FISH dose-effect curve and taking into account translocation data from a large panel of unexposed individuals. We found the mean exposure dose to be similar to the original assessment obtained by the dicentric chromosome assay (DCA) more than 22 years ago. Furthermore, the confidence interval from the DCA analysis was contained within our FISH confidence interval, which as expected, was slightly larger. Altogether these observations confirm a comparable dose estimation.

In conclusion, retrospective biological dosimetry by FISH allowed us to estimate a dose that is consistent with the original assessment 22 years prior. This suggests that our current dose-effect curve could be used for relative dose estimations long time after external exposure.

Keywords Retrospective biological dosimetry; FISH; Chromosomal translocations; Accidental overexposure.

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Assessment of the Cytogenetic Abnormalities in Blood of Local Inhabitants Due to Continuously Exposed to High Background Radiation in Mamuju

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Mamuju municipal in West Sulawesi has known as the high background radiation area (HBRA) [1]. The elevated dose of ionizing radiation in this area might cause damage to the cells and tissues of the inhabitants [2] that its safety should be evaluated. It can be known by assessing cytogenetic abnormalities through observation of biomarkers such as micronuclei (MN), nucleoplasmic bridge (NPB), nuclear bud (NBUD) and 8-shaped in human peripheral blood lymphocytes. This research aimed to analyze the effect of high natural radiation on cells through the alteration of their nuclear division index (NDI) and formation of biomarkers which may influenced by age and sex factors. The study was done on 15 blood samples that were taken from inhabitants living in NBRA (Ahu and Salletto villages) and 15 blood samples from those of control area with normal radiation exposure (Topoyo village). The feasible method of cytokinesis block micronuclei (CBMN) [3] was used to do this assessment. The results showed that NDI and frequency of all biomarkers in study group were higher than those of control, where high natural radiation did significantly induce MN and 8-shaped formation ($p<0,05$) but not for NPB and NBUD. The frequencies of all cytogenetic abnormalities assessed were still within normal range. The age and sex factors did not influence towards all the cytogenetic abnormalities. It was concluded that chronically exposure of high background radiation affected on cell proliferation and certain biomarkers in human lymphocyte.

Keywords: cytogenetic abnormality; high background radiation; NDI; micronuclei; Mamuju.

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Preliminary study of chromosome aberrations using two-color FISH and giemsa assays in lymphocytes of individuals living in elevated radon concentration areas

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Takandeang village in Mamuju, West Sulawesi, was known as a high background radiation area (HBRA) in Indonesia. Takandeang village is also known to have high levels of indoor and outdoor radon concentrations [1]. People living in this area are exposed to high levels of radon in their daily lives. Radon emits alpha-particle radiation that could damage DNA and increase the risk of lung cancer in this population. Cytogenetic damage has been shown to be a reliable biomarker of cancer risk [2]. The aim of this initial study was to investigate the chromosome aberration frequencies in Takandeang village inhabitants by using fluorescence in situ hybridization (FISH) and giemsa staining analyses. Chromosome analysis using FISH cocktail of whole chromosome paints for chromosomes 1 and 4 together with giemsa techniques was performed on four residents of the Takandeang village and four individuals as controls from the Topoyo village, Mamuju Tengah (Middle Mamuju), West Sulawesi, Indonesia. The result showed that no dicentric and translocation chromosomes were found in the Topoyo village inhabitants. In inhabitants of Takandeang village, four chromosome translocations were found in one subject (Figure 1). Overall, our preliminary study discovered the increased of translocation frequencies in Takandeang village inhabitants. More research, however, is needed to have a better understanding of the effects of prolonged exposure to high levels of radon concentration.

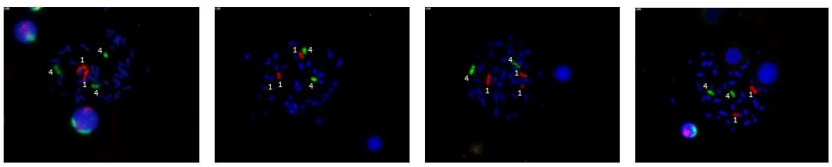

Figure 1. Translocations in peripheral blood lymphocytes of one donor from Takandeang village inhabitants were detected by FISH using whole human chromosome specific paints for chromosomes 1 and 4.

Keywords Chromosome aberrations; FISH; Giemsa; Mamuju; Radon

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Investigating Manganese Concentrations in Shelled Species and the Effect on EPR Spectra

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In electron paramagnetic resonance (EPR) spectroscopy, high intensity Mn2+ peaks are known to impact the lower limit of detection for low-dose studies. An investigation into the manganese concentrations in various shelled species was carried out in order to quantitatively compare the amount of Mn present in shells and how they influence the EPR spectra. Inductively coupled plasma – optical emission spectroscopy (ICP-OES) was used to determine manganese concentrations in shells of terrestrial and aquatic snails, scallops, crabs, lobsters, zebra mussels, pond mussels, blue mussels, and oysters. The same species were then analyzed using EPR spectroscopy in order to compare the concentrations of manganese in the shells with the intensity of the manganese peaks in the EPR spectra. Species that had low manganese concentrations, and corresponding low-intensity manganese peaks in their EPR spectrum may be suitable to use as low-dose environmental dosimeters. Additionally, Mn detection may be used as a method to select applicable species for low-dose EPR dosimetry.

Keywords Inductively coupled plasma spectrometry, electron paramagnetic resonance spectroscopy, environmental dosimetry, manganese, shelled species

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Electron Paramagnetic Resonance for Dosimetry of Dreissenid Mussels

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Exposure of freshwater ecosystems is possible as a result of accidents involving facilities of the nuclear fuel cycle. Electron Paramagnetic Resonance (EPR) spectroscopy was identified as a methodology worth investigating for the purpose of environmental radiation measurements since it is widely established for radiation measurements in calcified tissues.

To this end the feasibility of detection of radiological contamination in calcified components of aquatic environments through EPR spectroscopy is investigated. Dreissenid mussels from the Great Lakes showed a radiation induced signal under the dose of 20 Gy using a Bruker EMX micro EPR Spectrometer. Sample preparation and measurement protocols were developed. In the dose range under 20 Gy a linear relationship of the peak-to-peak height of the line at g = 2.0034 was established. EPR spectra were analysed using a program written to aid the assessment of a large number of spectra in batches of samples and measurements eliminating the need for manual peak-to peak measurement [1].

Varying background EPR signals were identified in different sampling groups, with samples gathered in winter having a markedly lower background signal. Irradiations Were performed with Cs-137, a medical LINAC and Co-60. The sample group with the lowest background was irradiated with a Co-60 source. It was possible to resolve doses as low as 0:2 Gy, thus reducing the value of 2 Gy, previously reported in literature [2]. Optimization of errors introduced through experiment and normalization was performed and peak-to-peak heights were measured with a relative error of 12% (0.2Gy) to 8% (5 Gy).

This work provides further validation that EPR dosimetry of shelled species has the potential to contribute to better characterization of absorbed doses in aquatic environments.

Keywords EPR spectroscopy; radioecology; dosimetry; dreissenid mussels

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External exposure dose estimation of wild Japanese macaques captured in Fukushima Prefecture: Decomposition of electron spin resonance spectrum

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Radioactive nuclides released by Fukushima Daiichi Nuclear Power Plant accident have contaminated the environment especially the eastern part of Fukushima Prefecture. We have been conducting dose assessments for Japanese macaques captured in Fukushima to reveal radiobiological effects on the low-dose expose animals. CO_2^- radicals, a stable chemical species produced by radiation exposure in teeth, can be sensitively detected by an electron spin resonance (ESR) measurement. The intensity of CO_2^- radicals in teeth can be utilized as an individual indicator of cumulative exposure dose [1]. To accurately determine the external exposure dose, it is desirable to examine the analysis of the CO_2^- radical intensity and improve its detection limit (DL). We recently reported a dose response curve in 0-200 mGy and estimated the external exposure dose for 7 wild macaques captured in Fukushima [2]. In this work, for further improvement of this method, we examined ESR spectra of teeth of these 7 and additional 3 macaques captured in Fukushima by two spectrum-decomposition algorithms.

Based on a protocol for enamel preparation reported in [1], the dose response curve was made by additive dose method for granule of teeth. The obtained ESR spectrum was decomposed into the CO_2^- radical, native radical and 3 gaussian-type background components. We examined the same spectra by (i) ”EPR Dosimetry” [3] in which all components as well as the linear dependence of the spectrum were freely optimized to reproduce the observed spectrum, and (ii) a step-by-step stochastic optimization. In the first step of the method (ii), the CO_2^- radical and native radical components were optimized using a linear dependence determined in wider range than the method (i). Second, the 3 background components were optimized within small amplitudes. Third, all of the components were fine-tuned around the optimized values of the first and second step.

We performed more than 7 individual measurements for each sample and the all spectra were analysed by the algorithms (i) and (ii). The DL was 33.5 mGy [2] for the algorithm (i) and 122.1 mGy for the algorithm (ii). While some of the ESR spectrum of the teeth of the additional 3 wild macaques failed to converge in decomposition by the algorithm (i), the algorithm (ii) suggested a converged CO_2^- intensity. Since the algorithm (ii) determined the linear dependence of the spectrum in the wider range than the algorithm (i) and generated an appropriate initial guess of the primary native radical component, it showed a higher numerical stability in the decomposition than the algorithm (i).

Keywords EPR (ESR) dosimetry, FDNPP, tooth, wild animal, CO_2^- radical

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Alanine/EPR dosimetry in magnetic fields

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A recent development in radiotherapy (RT) is magnetic resonance guided RT (MRgRT) using MR Linac devices which combine an MR scanner unit for imaging with a linear accelerator for tumour irradiation. Quality assurance on these devices requires dosimetry in presence of the static magnetic base field of the MR scanner unit. This work investigates the characteristics of the alanine/EPR dosimetry system [1] in such magnetic fields.

Pellets of polycrystalline L-alpha-alanine powder (4.8 mm diameter, approx. 2.9 mm height, Harwell) were used. Alanine detectors were composed of a stack of four alanine pellets placed within a PMMA holder. Sub-millimetre air gaps around detectors can have significant effects on dose measurements in magnetic fields [2]. The effect of air gaps around alanine detectors in magnetic fields was, therefore, investigated in the first step. Alanine detectors were irradiated in a water phantom in a clinical 6 MV photon beam (Elekta Precise treatment system at PTB, $TPR_{20,10}$=0.683) in the presence of a static magnetic field with 1 T flux density generated by an electromagnet (Bruker ER073W). The water phantom was positioned between the pole shoes of the electromagnet. The air gap surrounding the detectors was set to (0.0, 0.1, 0.2, 0.5, 1.0) mm by using PMMA holders constructed accordingly. In the second step, the response of the alanine dosimeter to the photon beam in the presence of magnetic fields with (0, 0.3, 0.6, 0.9, 1.2, 1.4) T flux density was investigated by means of irradiations performed using the same setup (PMMA holder with vanishing air gap). After the irradiations, the dose information resulting from the radical formation in the alanine under the impact of ionizing radiation was read out using a Bruker EMX 1327 EPR spectrometer in the X-band.

The first part of the investigation revealed no measurable effect of the surrounding air gaps on the indication of the alanine dosimeter. The second part of the investigation revealed no significant effect of the static magnetic fields with (0.3, 0.6, 0.9, 1.2) T flux density on the indication of the alanine dosimeter, while an effect of (0.58+-0.16)% was measured in the presence of the 1.4 T magnetic field.

The results suggest, that practically relevant air gaps (below 0.05 mm) around alanine detectors can be neglected in the presence of the clinically relevant magnetic fields, and that the alanine dosimeter can be used without further correction factors for photon beams with radiation qualities close to $TPR_{20,10}$=0.683 in the presence of static magnetic fields up to 1.2 T while the indication has to be revised upwards by approx. 0.6% in the presence of 1.4 T.

Keywords alanine dosimetry; EPR spectrometry; MR-guided radiotherapy; dosimetry in magnetic fields

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5-03-D3

The NIAID Biodosimetry Development Program: An Overview

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Established in 2004, the Radiation and Nuclear Countermeasures Program (RNCP), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) now has a thriving medical countermeasures (MCM) program and robust biodosimetry research portfolio. The mission of RNCP-funded research is to advance MCM products, and biomarkers and technologies to assess and triage to guide medical management of patients experiencing acute radiation syndrome (ARS) and/or the delayed effects of acute radiation exposure (DEARE), in the event of a radiation public health emergency.

The RNCP biodosimetry mission includes: 1) basic mechanistic research to elucidate novel approaches for rapid and accurate assessment of radiation exposure, 2) studies to support advanced development for eventual U.S. Food and Drug Administration (FDA) clearance of promising triage or treatment devices/approaches; 3) identification and characterization of biomarkers and/or assays to determine degree of tissue/organ dose that can predict outcome of radiation injuries (i.e., organ failure, morbidity, and/or mortality), and 4) pro-active outreach efforts to facilitate interactions between emerging leaders developing cutting edge biodosimetry approaches.

Small and large animal models, as well as human ex vivo studies of promising biomarkers of radiation injury, bioassays, and devices are facilitated by a variety of funding mechanisms (cooperative and inter-agency agreements, small business and other research grants and contracts,) with the end goal of advancing products and technology toward clearance under the US FDA's Radiation Biodosimetry Medical Countermeasure Devices Guidance Document [1]. RNCP accomplishments include the development of novel biomarker assays leading to promising pathways to clearance developed in conjunction with the FDA, as well as publication of a recent journal special issue devoted to biodosimetry. The overarching goal of the NIAID RNCP biodosimetry program is to develop and establish accurate and reliable biodosimetry tools that will improve radiation preparedness and ultimately save lives during a radiological or nuclear incident.

Keywords Radiation biodosimetry, triage, definitive dose, predictive biodosimetry, biomarkers.

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Monte Carlo Modelling of Experimental Setup Used for Biodosimetry Intercomparison

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Exposure to ionizing radiation (IR) can lead to adverse health effects and therefore accurate dose quantification is essential. Biodosimetry (BD) techniques use an individual's biological response to IR, e.g. quantification of cellular damage, to estimate the dose received. The BD program at Health Canada (HC) generates dose-response curves for different radiation types and assays to translate biological damage into absorbed radiation doses [1]. These curves are produced by irradiating biological samples in custom-designed phantoms using a cabinet X-ray machine. To verify the accuracy of these curves for different radiation types, a Monte Carlo (MC) model of this system can be used and validated with laboratory measurements. MC computational methods simulate the radiation tracks based on probable interactions to model a given process. To ensure consistency, it is important to investigate the difference in dose between the regular and custom-designed phantom setups. The objective of this project is to create an MC model of the experimental setup at HC and evaluate the impact of using different phantoms.

At HC, blood samples taken from healthy volunteer donors are irradiated using a cabinet X-ray machine at 250 kVp, calibrated with a cylindrical ion chamber. The ion chamber and blood vial(s) are placed within a 30 cm x 30 cm x 9 cm slab of RW3 (water equivalent solid material). This setup is simulated with the EGSnrc Monte Carlo toolkit [2] with the radiation source derived from the X-ray spectrum of the machine calculated using the SpekCalc software. A single slot phantom, as well as a specially designed phantom capable of irradiating 7 blood vials plus the ion chamber simultaneously, were modelled and compared to the experimental setup.

Preliminary simulations calculated the ratio of absorbed dose per number of incoming X-rays to the sensitive area of the ion chamber with and without air gaps in the phantom for a few different filtration settings. Using the same setup, measurements were made in the lab and the ratios of the doses measured in each phantom were calculated for each filter. Both sets of data predicted ratios relatively close to unity for all filters considered, indicating minimal difference between the two phantom setups. To further improve agreement with laboratory measurements, a more complex and complete model of the cabinet X-ray machine is being created. This will enable calculating dose-response curves for more complex radiation environments, such as the space radiation environment or a large-scale nuclear emergency.

Keywords:
EGSnrc, Orthovoltage Irradiator, Monte Carlo Modelling, Dosimetry

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Monte Carlo simulation of the energy released by neutrons on organic compounds for EPR dosimetry

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In this work we report the analyses of the energy released per unit mass in organic compounds used for EPR dosimetry exposed to neutron beams in order to predict the increase in dose achievable by addition of gadolinium (Gd) inside the pellets. In particular, Monte Carlo (MC) simulations were carried out for alanine, ammonium tartrate and phenolic compounds irradiated with neutron beams with different energy spectra at various depths inside a water phantom. The addition of gadolinium increases sensitivity of these dosimeters to neutrons thanks to the high gadolinium cross section for neutron capture and to the large number of secondary particles (mainly Auger and internal conversion electrons) which are able to release energy inside the sensitive material layers. For small depths in water phantom and low energy neutron spectra the increase in dose due to gadolinium is large. The enhancement is smaller in case of epithermal neutron beam, whereas the increase in dose for fast neutrons is less than 50%. The sensitivity improvements for the various compounds considered were compared. In particular, the sensitivity enhancements were calculated for each neutron energy range (therrmal, epithermal and fast neutrons). The results are explained and discussed on the basis of the compositions of the compounds considered. All results here found suggest that considered organic compounds added with gadolinium could be fruitfully used for dosimetric applications in Neutron Capture Therapy.

Keywords: EPR dosimetry, organic compounds, Neutrons, Monte Carlo simulations

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Interlaboratory comparison of EPR tooth enamel dosimetry with investigations of the dose responses of the standard samples

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Interlaboratory comparison studies are important for radiation dosimetry in order to demonstrate how the technique is universally available. There have been quite a number of intercomparison studies where each laboratory was asked to prepare their own standard samples and also to process the samples with unknown doses. The differences of the reported doses from the actually given doses were analysed in the aspect of such as measurement conditions [1].

As the set of standard samples, aliquots of crushed tooth enamel with a series of given doses, say several ten to several hundred mGy, are essentially important for determining the given (accidental in the application to incidents) doses, the set of standard samples are also examined in each participating laboratory in the present study. After a set of standard samples together with the samples with unknown doses, which were prepared in the same laboratory as the standard samples, are measured at a participating laboratory, those samples are sent to another participating laboratory for next measurement.

There is some small difference observed in the sensitivity (the slope of the dose response line) of the standard samples while the differences in the obtained doses for the samples with unknown doses are rather systematic, implying that the difference is mostly due to the samples but not to measurements. Detailed results of analysis will be reported.

Keywords EPR; ESR; tooth enamel; intercomparison; standardization

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The success of ESR for dating of fossil mollusc shells: Samples from Old Konya Lake

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The Konya Closed Basin is one of the important center of Turkey in terms of agricultural and industrial productions. Old Konya Lake, located in the Konya Closed Basin, has expanded and narrowed several times due to climatic changes during the Quaternary period and completely dried up about 6 ka before. Due to the uninterrupted and well-preserved lacustrine deposits of the Quaternary period in the basin, it has attracted the attention of many scientific researchers. It is very important to precisely date the lake, which was affected by the climate changes experienced during the Quaternary period. In this study, for dating of the Eastern Part of the Old Konya Lake fossil mollusc shells were chosen as dating material because they are very well preserved and abundant in the study area. Electron Spin Resonance (ESR) was preferred as main dating method since it has wide dating range covering the whole Quaternary period and many other advantages [1]. After sedimentological and paleontological evaluations, lacustrine shells (*Dreissena iconica, Valvata piscinalis, Bithynia tentaculate, Unio pictorum*) in the coastal sediments taken from the four different locations (Adakale, Sazlıkpınar, İsmil, Hotamış Lake) of the basin were analyzed and dated using ESR spectroscopy. ESR ages were obtained well stratigraphically matched between 6.3 ± 0.5 ka and 138 ± 38 ka, and these ages were used to interpret the paleogeographic and paleoclimatic characteristics of the basin. The periods (the warm climate of D-O-2, D-O-5, D-O-6 and D-O-14 events and the cold climate of H2, H3, H4 or H5 and H6 events) corresponding to the climatic changes during the last glacial periods, which were found in good agreement with the ESR ages, were revealed by interpreting the geochemical, paleontological and isotope analysis data of the Hotamış Lake location [2]. In addition to ESR method, both ^{14}C and Th/U methods were also used for dating of some mollusc shells however the ages were stragraphically inconsistent. This problem may be due to young calcite contamination and also because the mollusc shells are open systems. In the study, the reliability and advantages of the ESR technique as a dating method for mollusca shells were once again demonstrated. This study was supported by TÜBİTAK project, with the number 114Y237.

Keywords: ESR dating; mollusc shells; Konya Closed Basin; Old Konya Lake; Quaternary period

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PARAMAGNETIC CHARACTERIZATION OF FOSSIL MOLLUSC SHELLS AT EASTERN PART OF THE OLD KONYA LAKE: ITS IMPORTANCE FOR EPR DATING

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EPR is a very important spectroscopic technique widely used in many different application areas, such as in geological studies, as it is a unique technique used in the direct detection and characterization of paramagnetic systems having unpaired electrons. With this feature, EPR can be used for determination of radicals existing or formed by natural radiation in the structure of geological materials, and even as a dating method [1]. Spectroscopic splitting factor (g), which is highly sensitive to environmental conditions, is an important EPR parameter because it has known as the fingerprint of the radical. Also, there are additional features such as temperature behaviour, line width and microwave power dependence of the signals that can be used to characterize the radicals [2]. In this study for the first time, the radicals in the structure of fossil mollusc shells (*Dreissena iconica, Valvata piscinalis, Bithynia tentaculate, Unio pictorum*) collected from the Eastern Part of the Old Konya Lake in Turkey were identified by EPR technique. All EPR experiments were carried out using JEOL JesFa-300 X-band EPR spectrometer located in Selçuk University Advanced Technology Research and Application Center (ILTEK) at different modulation field adjustments between the temperatures of 123-293 K and the microwave powers of 0.01-200 mW. The dependence of the radicals in these shell structures on the microwave power, temperature and the absorbed radiation dose was investigated and the presence of radicals that could be used in EPR dating studies was investigated. Characteristic features of intrinsic and impurity related radicals occurred by natural radiation were determined and compared by considering the species of mollusc shells. The importance of radical characterization at the EPR dating studies, where different species of mollusc shells used as dating material, was emphasized. This study was supported by TÜBİTAK project, with the number of 114Y237.

Keywords: EPR spectroscopy, Old Konya Lake, Fossil mollusc shells.

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The behaviour of organic radical species and atomic hydrogen in gamma-irradiated chibaite at low temperature

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Chibaite is one of the silica clathrate minerals and has a framework structure of silica with cage-like voids occupied by guest species [1]. The structures of silica clathrates are very similar to those of gas hydrates, suggesting that chibaite is isostructural with gas hydrate structure II. Chibaite can include alkanes of methane, ethane, propane, and isobutane (2-methyl-propane) as guest species in the cage. It means that methyl, ethyl, *n*-propyl, isopropyl, isobutyl and *tert*-butyl radicals could be observed in gamma-irradiated chibaite. The preliminary electron spin resonance (ESR) measurements of the gamma-irradiated chibaite showed that characteristic radicals variable in types and the amounts depend on sample temperatures between 110 K and 290 K. It suggests that hydrogen picking process occurs in chibaite. The hydrogen picking process is an intermolecular reaction where atomic hydrogen is transferred from guest organic species to radicals formed by gamma-ray irradiation. This process was observed in clathrate hydrates such as propane hydrate with structure II [2]. If this process happens in chibaite, the dynamics of hydrogen picking process could be discussed by comparing the phenomena observed in gas hydrates and chibaite. In this study, we have investigated the variation of the amounts of organic radicals using natural chibaite samples through isothermal annealing experiments.

We gently crushed chibaite samples in a mortar into several pieces with about 2-3 mm in diameter and irradiated them by gamma-rays with ^{60}Co source at 77 K. ESR signals of six gamma-irradiated samples were measured at 120 K. Isothermal annealing experiments of three irradiated samples were performed at 200 K, 230 K and 260 K for 150-180 min. As a result of ESR observation at 120 K, methyl, ethyl, *n*-propyl, isopropyl and *tert*-butyl radicals and hydrogen atoms were observed in every sample. The integrated intensity ratios of the signals were almost the same among the samples. This suggests that the content ratios of gas molecules in chibaite samples are almost the same. In the isothermal annealing experiments at 200, 230 and 260 K, methyl, ethyl, *n*-propyl, isopropyl radicals and hydrogen atom decreased significantly, whereas *tert*-butyl radicals increased. The signal intensities of *tert*-butyl radicals as against the sum of the other organic radical species were reversed at 260 K. This fact will indicate that hydrogen picking process also occurs in chibaite. These processes both in gas hydrates and chibaite were observed in the similar temperature range. This suggests that the hydrogen picking process observed in chibaite at 260 K is not strongly affected by the host molecules forming the framework structure.

Keywords: silica clathrate, chibaite, hydrogen transfer reaction, organic radical species

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Effect of high-velocity friction on ESR signal in quartz

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A fault dating using electron spin resonance (ESR) is a developing method to estimate the age of the last seismic fault activity. This method assumes that natural radiation-induced ESR intensity, which is proportional to trapped charge concentration in the interseismic period, was annihilated by fracture, stress, and frictional heating during the fault slip [1]. The high-velocity friction (HVF) experiments using a rotary friction apparatus mimicking seismic fault slips proved this annihilation, called ESR signal zeroing [2]. However, the sensitivity of ESR can be reduced by high-dielectric materials, hence, the apparent zeroing might occur due to titanium oxide debris mixed into the gouges by wear of titanium alloy host blocks during the HVF experiments. Therefore, we have re-examined the zeroing by seismic fault slips near the surface by performing HVR experiments using low-dielectric mullite cylinders as host blocks and ESR measurements for simulated-fault gouges.

HVF experiments were performed for course artificial quartz grains as the starting material, at an equivalent slip rate of 1 m/s, a normal stress of 1 MPa, and displacements of 10–40 m. ESR measurements to detect the E_1' centre in quartz (≡Si$^\bullet$, where − is an electron pair and $^\bullet$ is an unpaired electron) were performed for the starting material and simulated-fault gouges.

The ESR spectra showed the presences of the E_1' centre (g=2.001) and partially overlapped signals, which could be pyrolytic carbon radical [3] (g=2.003) in Teflon contaminants. To obtain the more accurate shape of the E_1' centre, we subtracted the overlapped signals from the ESR spectra using “Easyspin” MATLAB toolbox. For quantitative comparison of ESR intensities, mass normalization of quartz in gouges is required. Hence, we removed the effect of Teflon contaminants in the gouges using calorimetric techniques with thermal analyzers.

The intensity of the E_1' centre tended to increase with displacement and were more than 2 orders of magnitudes higher than that of the starting material. The E_1' centre is generally thermally stable up to about 200°C [4]. Moreover, the maximum temperature measured at the gouge surface was 160°C at a displacement of 30 m. Hence, the decreasing effect of frictional heating on the E_1' centre was negligible and the grain fracture is the dominant effect for the ESR intensity as reported in the rock fracture experiment [5]. In contrast, the maximum measured temperature at a displacement of 40 m recorded 270°C. In this experiment, although the decreasing effect of frictional heating on the E_1' centre is expected, grain fracture affected the centre enough to surpass it, resulting in the increase in the centre with displacements. On the contrary to previous consensus [1], this implies that seismic fault slips near the surface can increase the E_1' centre due to grain fracture.

Keywords Electron spin resonance; High-velocity friction; E_1' centre; Teflon.

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Investigation of the origin of Okinose sand using ESR signals of quartz in granite around Osaka Bay

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Osaka Bay, located in the west part of Japan, has shallow eastern area (5-20 m in depth) and deep western area (20-70 m). A submarine dune is found in Okinose area apart from ~12 km southeast of the Akashi Strait, where the water depth is 25-45 m and shallower than the surrounding area [1]. On the sand dune of Okinose, sand waves with a wavelength of 20-100 m have been observed. The average particle size of the sand sample obtained from the dune was ~0.65 mm and the sand almost consisted of quartz, revealed by X-ray power diffraction analysis. According to the study of the origin of the quartz in the river bed sediments using the ESR signals of quartz, Al and Ti centers could be used to identify the origin [2]. In this study, we have measured electron spin resonance (ESR) of quartz contained in the sand of Okinose together with granite and granodiorite around the area of Osaka Bay and compared the signal intensities of Al and Ti centers to discuss whether it is possible to approach the origin of the sand deposited in Okinose.

Granite and granodiorite near Osaka Bay were collected from Rokko area (6 samples), Kakogawa area (2 samples), and Awaji Island (8 samples). They were crushed using a hand crusher, and sieved. The samples with a particle size of 500-1000 mm were immersed in 10 wt% HCl solution to remove carbonates, and then 30 wt% H_2O_2 solution to remove organic matters. After washing and drying, magnetic minerals were removed with a neodymium magnet. Furthermore, they were immersed in 40 wt% HF solution for 2 hours to obtain quartz samples. In addition, the Okinose sand collected in the survey of Fukae Maru in 2018 was treated with the same procedures. The treated samples were irradiated with γ-rays at room temperature for 9 kGy, and ESR measurements were performed using an X-band (9 GHz) ESR spectrometer at 77 K and room temperature. It was confirmed by XRD that all the samples were mainly quartz.

ESR spectra at 77 K showed Al-center and Ti-Li-center signals in all samples. The intensities of the Ti-Li center varied among the samples, whereas those of the Al center were almost the same. When the intensity ratio of Ti-Li center to Al center was compared among the samples, the ratio in Okinose sand was almost the same or smaller than that in quartz samples from granite and granodiorite except for one sample from Awaji Island (Nojima granodiorite). Because the large ratios were observed especially in Nunobiki granodiorite and in granites in Kakogawa area, the contribution from those area to Okinose should be small. This means that granodiorite in the central part of Awaji island, granite in the northern part of Awaji Island, and granite in the Rokko area near Kobe city will be the main source of the sand in Okinose.

Keywords ESR; quartz; Al center; Ti center; granite

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Thermal stability of the ESR signals in tephra quartz and evaluation of equivalent doses

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Electron spin resonance (ESR) dating of quartz has still problems, one of which is to obtain correct equivalent doses for older samples [1]. Some studies have indicated that the signals have stable and unstable components with different thermal characteristics (e.g., [2] - [5]) where it was shown that those thermal stabilities can be different from sample to sample. In the present study, we systematically investigated the characteristics, the thermal stability and the dose response, of the Al and Ti centers in quartz of several origins and ages, also of the signals in natural and irradiated samples.

The results of isochronal annealing experiments indicate that there are two components both in Al and Ti-Li center with different thermal stabilities and saturation doses (D_0). The less stable (low temperature) component has higher sensitivity to dose. The stable (high temperature) component of Al center was observed in natural samples and hardly reproducible after annealing unless the sample has been irradiated to a high dose of several ten Gy.

The equivalent dose of tephra sample was evaluated with preheating, the condition of which was determined due to the results of these heating experiments results. With the preheating procedures, the results indicated that the ages with Al center are still underestimated while those with Ti-Li center (preheating around 260°C) are well matched to expected ages. For these particular samples, ESR dating of tephra should use Ti-Li center with preheating around 260°C.

Keywords ESR; quartz; thermal stability; preheat

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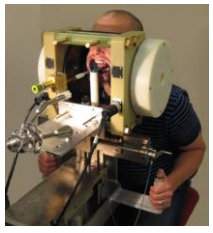

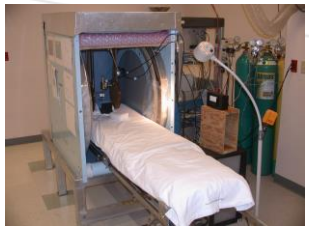

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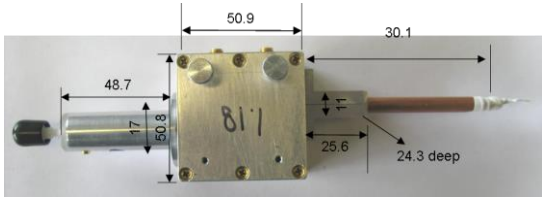

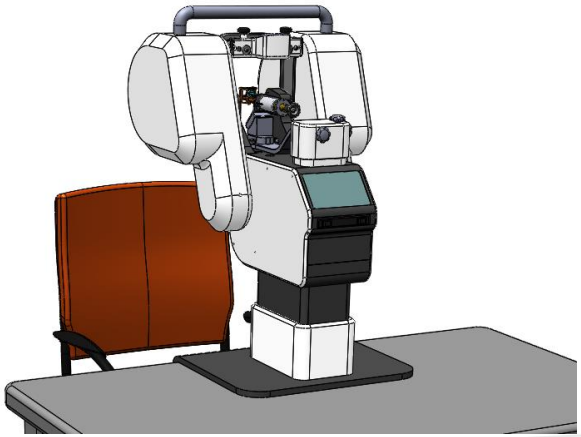

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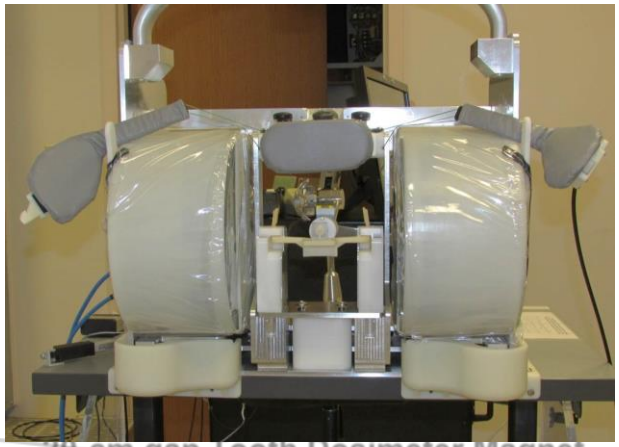

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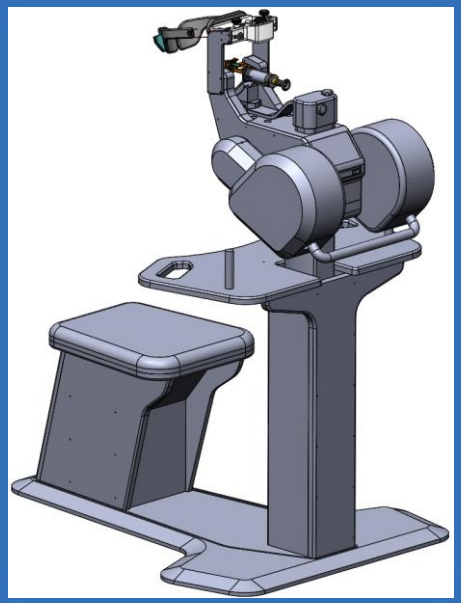

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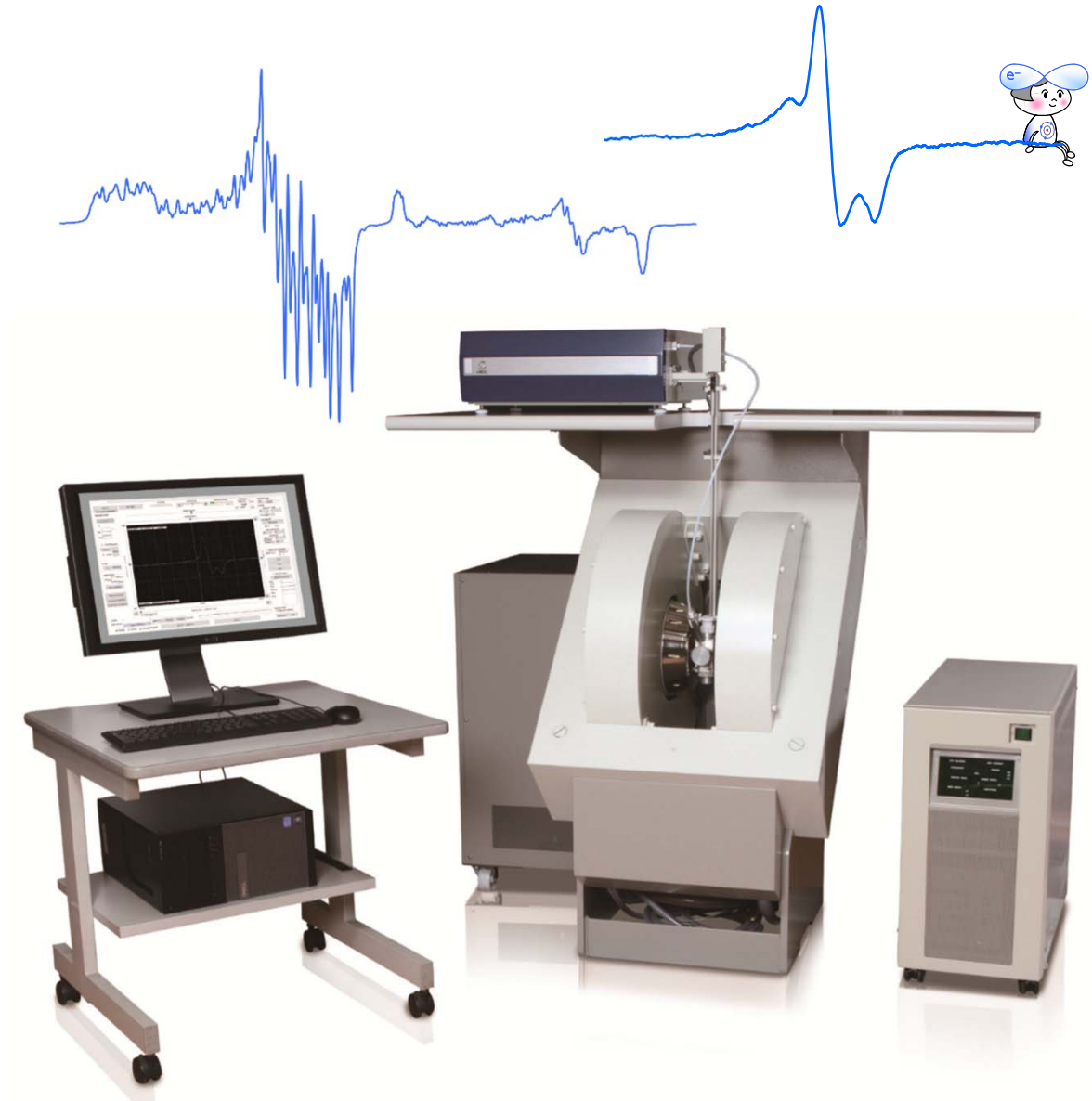

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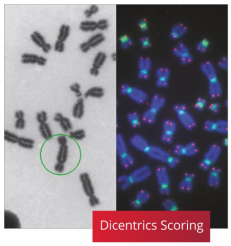
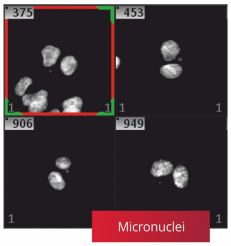
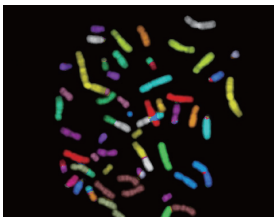
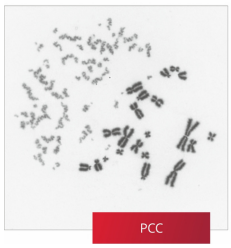
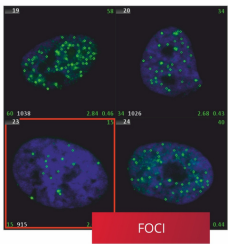


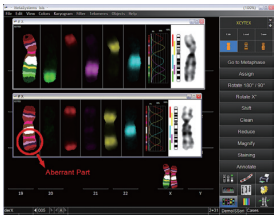




Human Chromosome Multicolor FISH(mFISH) , Probe 24XCyte







High Chromosome Color Banding Probe (mBAND) (analyzed by Isis software)

The samples here are for research use only. They cannot be used for clinical diagnosis.
For more information, please contact us.

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