Concern over the health effect of ionizing radiation began right after the discovery of X-ray in 1895. Two general principles for radiation protection are to prevent acute exposure and to limit chronic exposure to acceptable levels. Follow-up study of atomic bomb survivors exposed at Hiroshima and Nagasaki in 1945 showed that very high dose of acute radiation exposure have increased cancer risks among general public.

It is important to protect human health from unnecessary exposure to ionizing radiation and to have the benefits through various applications such as uses in medicine, industry, agriculture and research. Frequent medical use of X-ray raises significant concern among general public. Annual number of medical use of X-ray devices has been reported to have positive correlation with increase of risk causing cancer incidences.

Lead plate is commonly used to shield X-ray in clinics and laboratories, however, shielding is not usually perfect and small amount of X-ray penetrate through the shield. Penetrated X-ray raises considerable concern among general public and radiation workers. For development of PPE materials shielding toxic effects of ionizing radiations, establishment of a new evaluation system based on bioassay is necessary, since physical shielding of ionizing radiations with high penetration ability, such as X-ray and gamma ray, is practically difficult. We, however, have realized that it is possible to reduce the biological effectiveness of high energy photons by filtration technique in our preliminary experiments. When mammalian cells, including Chinese hamster V79 cells and human normal fibroblast cells, were exposed to the high energy photons such as X-ray and gamma ray with or without shielding materials made of metal, such as lead, steel, or tungsten, survival rate of the exposed cells were significantly affected (data not shown). These observations lead us to the systematic examination described in this study.
To investigate the biological effect of ionizing radiations that penetrated a metal plate shield, we have developed the colony assay system using Chinese hamster V79 cells. V79 cells have higher than 80% of a plating efficiency and a generation time of 12 to 14 hours, and have been employed in many toxicological bioassay experiments. We speculated the high energy photons that penetrated metal plates made of lead (Pb) or tungsten (W) may be biologically less effective, since highly interactive photons would be filtrated by a metal shield. Here we demonstrate the usefulness of the bioassay system using V79 cells for evaluating the biological effectiveness of X-ray with or without filtration. Changes in the biological effectiveness of X-rays were clearly distinguished by the bioassay system described here.

Chinese hamster V79 cells were maintained in growth medium composed of MEM medium (Thermo Fischer Scientific, Japan) containing 10% of foetal bovine serum (FBS, Biowest, France), using plastic petri dishes (BD Falcon 353003, USA). Cells were cultured using CO₂ incubator (Tabai Espec, Japan) in the atmosphere containing 5% CO₂ and 100% humidity.

Exponentially growing cells in a petri dish were washed with phosphate-buffered saline (PBS), treated with 2.5% trypsin, and suspended in 10 ml of the growth medium. 1 ml of cell suspension containing approximately 10⁶ cells was aliquoted to 15 ml test tubes (Biologix 10-9152, USA). X-ray was generated using PANTAK HF320-S X-ray System (Shimadzu, Japan) at the energy of 200 kV-20 mA. Generated X-ray was filtrated by using 0.5 mm copper (Cu) + 0.5 mm aluminium (Al) filter to remove secondary electrons and low energy photon in the braking X-ray generated. 10 Gy of X-ray was exposed to the cells were equivalent to the one with the lethal dose of X-ray exposure. The cell suspension exposed to the equivalent dose of X-ray but filtrated with a 1 mm thick Pb plate formed 80, 83, and 97 colonies, respectively. It was calculated to be 87 colonies on average with standard deviation of 4.5, showing more than 3 times enhancement of cell survival compared to the experiment carried out without a shielding plate. Thus, the X-ray filtrated with metal plates seemed to be biologically less effective than the non-filtrated X-ray, although the dose of X-ray exposed to the cells was equivalent to the one without filtration.

There was a possibility that the difference in the biological effectiveness observed in the above experiments may be caused by the dose rate of X-ray exposure. To examine the correlation between the dose rate and the cell survival, the cells were exposed to 0, 3, and 10 Gy of X-ray at the distance of 320 mm and 960 mm. Dose rate was 3.56 Gy/min at 320 mm, and was 0.28 Gy/min at 960 mm. The results did not show clear correlation between the dose rate of X-ray and the cell survival, as shown in Table 2. On the control plates without X-ray exposure, the number of colony formed by using 10² times dilutions were 159, 151, and 142, respectively. It was calculated to be 151 on average with standard deviation of 4.9. At the dose of

### Table 1. Summary of the experimental results examining the correlation between the shielding material and cell survival. Pb represents lead and W represents tungsten

| dose (Gy) | dose rate (Gy/min) | shielding material | number of colony (Mean ± SE) | % survival |
|-----------|--------------------|--------------------|-------------------------------|------------|
| 0         | 0                  | no shield          | 677 ± 6.2                     | 100        |
| 10        | 3.56               | 1 mm Pb            | 40 ± 1.5                      | 6          |
| 10        | 0.29               | 1 mm W             | 87 ± 5.2                      | 13         |
| 10        | 0.19               | 1 mm Pb            | 148 ± 4.5                     | 22         |
3 Gy, the cells exposed to X-ray at the lower dose rate of 0.28 Gy/min formed 109, 81, and 87 colonies, respectively. It was calculated to be 92 colonies on average with standard error of the mean of 8.5 in 10 Gy of X-ray, 10 Gy exposure experiment showed slightly lower survival rate. The result, however, was opposite when 10 Gy of X-ray was exposed to the cells at two different dose rates. When the cells were exposed to 10 Gy of X-ray, 10^4 times dilutions of the higher dose rate group formed 166, 163, and 189 colonies, respectively. It was calculated to be 173 colonies on average with standard error of the mean of 8.2, whereas the same dilution of the lower dose rate group formed 130, 140, and 124 colonies, respectively. It was calculated to be 131 colonies on average with standard error of the mean of 4.7. The result of the 10 Gy exposure experiment showed slightly lower survival rate in the lower exposure rate group. We, therefore, concluded that the differences in number of colonies formed were not significant, and that the clear correlation between the dose rate of X-ray exposure and the cell survival was not observed. We also concluded that the results obtained in the previous experiments showed the significant differences in the biological effectiveness of the X-rays with or without filtration.

To examine the usefulness of bioassay system for evaluation of materials against toxic effect of ionizing radiation, we attempted to detect the changes in the biological effect of X-ray penetrated through a metal plate shield in this study. Cell culture experiment sometimes may not reflect the toxicological effects on individual animals, however, it is cost effective and especially suitable for primary screening purposes. Accumulation of experimental results using Chinese Hamster V79 cells enabled us to compare the biological effects caused by various substances, including ionizing radiations.

The results obtained in this study showed the biological effectiveness of X-ray was significantly reduced when X-ray was filtrated with a metal plate, as shown in Table 1. These results suggested that the bioassay system developed in this study would be useful for evaluating materials shielding toxic effect of ionizing radiations. Since perfect shielding of ionizing radiations with highly penetrative property, such as X-rays and gamma rays, is very difficult, our second best attempt would be useful for developing practical PPE materials reducing toxic effects caused by ionizing radiations. At this moment, we do not have proper explanation for the reduction of toxic effects of the filtrated X-ray. At least, the energy of the filtrated X-ray was sufficiently high to be detected as ionizing radiation, since it was detected by the ionization chamber. Further study examining the correlation of biological effectiveness between the X-rays at different energy levels may be informative for understanding the phenomenon observed in this study.

**Acknowledgements**

We would like to express my many thanks to Dr Yoshiya FURUSAWA in National Institute of Radiological Sciences for fruitful discussions. This research work was supported by JSPS KAKENHI Grant Number 15H01789.

**References**

1) Daniel J (1896) The X-rays. Science 3, 562–3. [Medline] [CrossRef]
2) Sources, Effects and Risks of Ionizing Radiation (2016) UNSCEAR 2016 Report, http://www.unscear.org/docs/publications/2016/UNSCEAR_2016_Report.pdf Accessed on 14th July 2017.
3) Grant EJ, Brenner A, Sugiyama H, Sakata R, Sadakane A, Utada M, Cahoon EK, Milder CM, Soda M, Cullings HM, Preston DL, Mabuchi K, Ozasa K (2017) Solid Cancer Incidence among the Life Span Study of Atomic Bomb Survivors: 1958–2009. Radiat Res 187, 513–37. [Medline] [CrossRef]
4) Cahoon EK, Preston DL, Pierce DA, Grant E, Brenner AV, Mabuchi K, Utada M, Ozasa K (2017) Lung, Laryngeal and Other Respiratory Cancer Incidence among Japanese Atomic Bomb Survivors: An Updated Analysis from 1958 through 2009. Radiat Res 187, 538–48. [Medline] [CrossRef]
5) Berrington de González A, Darby S (2004) Risk of cancer from diagnostic X-rays: estimates for the UK and 14 other countries. Lancet 363, 345–51. [Medline] [CrossRef]
6) Bradley MO, Bhuayan B, Francis MC, Langenbach R,
Peterson A, Huberman E (1981) Mutagenesis by chemical agents in V79 chinese hamster cells: a review and analysis of the literature. A report of the Gene-Tox Program. Mutat Res 87, 81–142. [Medline] [CrossRef]

7) Sakai A, Iwase Y, Nakamura Y, Sasaki K, Tanaka N, Umeda M (2002) Use of a cell transformation assay with established cell lines, and a metabolic cooperation assay with V79 cells for the detection of tumour promoters: a review. Altern Lab Anim 30, 33–59. [Medline]