The Research, Development and Support Center was established in 2011 to support and promote research activities of NIRS. It consists of one unit and three departments: the Planning and Promotion Unit, Department of Technical Support and Development, Department of Safety and Facility Management, and Department of Information Technology. The unit and each department are briefly introduced as follows.

The Planning and Promotion Unit functions as the secretariat of the center and is the hub linking the departments to the administrative sections of NIRS’s overall Department of Planning and Management and Department of General Affairs. The unit has an Education Section which offers many courses of education and training for human resource development. The section has had more than 10,000 attendees since its establishment in 1960.

The Department of Technical Support and Development has three sections: Radiation Engineering Section, Radiation Measurement Research Section and Laboratory Animal and Genome Sciences Section. The Radiation Engineering Section maintains the facilities for radiation generators and many devices which are used for experiments. There are seven gamma-ray generators, six X-ray generators and two Cockcroft-Walton accelerator systems which consist of proton accelerators and beamlines. One of the Cockcroft-Walton accelerator systems is used to generate neutron fluxes for research experiments on the biological effects of low dose radiation. The other Cockcroft-Walton accelerator system has three beamlines; two beamlines are used as atomic element analyzers and the third beamline is used to deliver focused proton beams as a few microbeamsto individual cells. Both systems were damaged in the 2011 Great East Japan Earthquake. The latter system experienced more serious damage; the vacuum condition of the accelerator itself broke, the magnets for steering and focusing proton beams moved from their original positions and some beamlines were badly bent. The members of the Radiation Engineering Section fixed them which took about 10 months. These radiation generators are used not only by the researchers of NIRS but also by the researchers from outside NIRS.

The synchrotron accelerator HIMAC is used for carbon ion radiotherapy for cancer and there are also three cyclotron accelerators used for radio-pharmacy development related to molecular imaging. HIMAC and these cyclotrons are managed and maintained by the Department of Accelerator and Medical Physics of the Research Center for Charged Particle Therapy.

The Radiation Measurement Research Section develops various radiation detectors. After the Fukushima Daiichi Nuclear Power Plant accident occurred, we began developing some detectors for surveying high level radiation areas in Fukushima Prefecture: these are a gamma-camera which can selectively detect the radiation from $^{137}$Cs radioisotope and a detector system which can find out hot-spots were very high levels of radiation are located. We are aiming at commercializing these items.

The Laboratory Animal and Genome Sciences Section supports researchers in conducting animal experiments of the highest quality. Seven species of animals for animal experiments are available. In this section, we breed more than 15,000 mice and 2,000 rats a year and have developed genetically modified mice in order that researchers can conduct even more advanced experiments. Since some mice and rats are bred in SPF conditions, it is very important to sterilize the area periodically and keep it clean all the time. We control the SPF areas very strictly.

The Department of Safety and Facility Management has four sections: Safety and Risk Management Section, Safety Control Section, Radiation Safety Section, and Facility Management Section. Only the last two sections are introduced here. In NIRS, about 1,600 persons including NIRS’s direct employees, researchers from outside NIRS, and contracted workers are registered as radiation workers who can work in the 20 radiation-controlled areas in NIRS. NIRS must instruct them regarding radiation safety and security before entering a radiation-controlled area for the first time. There are more than 400 kinds of radioisotopes used for experiments on radiobiology, radiation medicine and so forth. And NIRS also has many radiation generators as...
mentioned above. All items concerned with radiation have to be controlled strictly by rules. The Radiation Safety Section is charged with controlling all of them in accordance with the rules. There are about 50 buildings on the NIRS campus. The Facility Management Section maintains the buildings and their equipment such as elevators, air conditioners, etc., and the campus infrastructures such as electric power lines, telephone systems, gas lines, water supply lines, and so on. NIRS was established in 1957, so some buildings are very old and a few were damaged considerably in the March 2011 earthquake. Some of them have had to have seismic strengthening. This section has also been managing construction of a new building which will be used for human resource development. The Radiation Emergency Medicine Cooperative Research Facility has one building in which the use of actinide nuclei is allowed for research on radiation emergency medicine. This facility is the only one of its kind in Japan in which researchers can use, for instance, plutonium in animal experiments. Therefore, this building has to be strictly controlled to keep the inside of the building at a negative pressure according to the radiation safety law. In this case, the ventilation system of the building is maintained by the Radiation Safety Section in cooperation with the Promotion Section for Radiation Emergency Medicine Cooperative Research Facility of the Research Center for Radiation Emergency Medicine instead of the Facility Management Section because of existence of the strict rules.

The computer network system is one of the main infrastructures of NIRS. This network system has more than 1,100 users daily and about 4,000 computers are connected to it. The Department of Information Technology is responsible for maintenance and development of the computer network system. The administrative sections have many computer-aided service systems, for instance, personnel management, accounting procedures, patent database, etc. These service systems are maintained by the relevant section in principle, but Department of Information Technology has undertaken various jobs such as improving the systems or adding new functions to them. Now an institutional repository is being developed to replace the conventional database system used for registration of achievements of NIRS research activities. We plan to release it in December 2013. This department is also managing the library of NIRS and publications such as a research reports, proceedings and so on. One of the most important missions of this department is to secure information security. We instructed users on to keeping security in an e-learning exercise in the summer of 2012.
There is continuing interest in the use of microbeam irradiation systems designed to deliver a defined number of charged particles on a single cell with a resolution of a few micrometers. Irradiation of an exact number of charged particles on a single cell means that the limitations of the Poisson distribution of the number of charged particles can be overcome. This is especially important in low-dose regions because a small number of charged particles per cell will inevitably lead to large fluctuations in the cell population in a broad-beam irradiation field. Moreover, microbeams are particularly useful in the field of radiation-induced non-targeted effects, so called bystander effects that are considered to be one of the major effects in the low-dose region. In addition, microbeams with beam sizes of less than a few micrometers enable irradiation of a desired site within the cell.

Our microbeam irradiation system, the Single Particle Irradiation system to CEll (SPICE) provides a 3.4 MeV proton microbeam focused with a quadrupole magnetic lens on an upward vertical beam line. The construction of the prototype of SPICE began in 2003 with the primary goal of targeting 2,000 cells per hour with a 2-μm diameter proton microbeam. After improving the vertical beam line structures and accelerator stability, a beam size of 10 μm was obtained in 2006. Further optimization of the beam focusing system and improvements on the stability of the bending magnets led to the beam size being reduced to approximately 5 μm. In 2008, an automated cell recognition system for targeting cell nuclei in a 2.5 mm × 2.5 mm area of the cell dish was also completed[1]. Now, after additional improvements, SPICE provides a beam size of approximately 2 μm in diameter, and its irradiation procedures are fully automated with high-throughput irradiation of 3,000 cells in a 5 mm × 5 mm area in a single dish within 15 min after placing the cell dish on the micro-positioning stage.

SPICE was severely damaged by the Tohoku-oki Earthquake on March 11th 2011, and was out of operation for about a year and a half. We have successfully reconstructed the facility and it is now operational with system refinements. At present, SPICE is the only proton microbeam facility at which a single-ion single-cell irradiation can be performed on mammalian cells with stability and high throughput using an upward vertical beam of 2-μm diameter, focused with a magnetic quadrupole triplet lens. Fig. 1 is a microscopic image of the plastic track detector that indicates the beam size of the microbeam. The 2-μm diameter beam size enables us to irradiate the nucleus or cytoplasm of a single mammalian cell; and the number of protons irradiating a single nucleus can be controlled to be one to several thousand with a precision of 99%. SPICE is convenient and stable and all procedures are controlled automatically by the operation system except for setting the preset number of protons during the standard microbeam irradiation targeting monolayer cells. This is good for radiation biologists who are not familiar with microbeam experiments, but is also very time consuming.

A variety of irradiation modes have been established for radiation-induced bystander effects, cytoplasm irradiation, and so on. The default targeting pattern mode is single position irradiation at the center of the cell nucleus for all nuclei with the same preset number of protons or for each nucleus to be irradiated with a different number of protons. In addition to the default mode, three types of optional targeting modes are provided for a variety of radiobiological studies: a fractional population targeting mode, a multi-position targeting mode for nucleus irradiation, and a cytoplasm targeting mode. In the fractional population targeting mode, which is useful for bystander-effect studies, the percentage of irradiated cells among all cells is a set value. With a multi-position targeting mode, we can change the dose distribution in the targeted cell nuclei, and with cytoplasm targeting mode, we can target only the cytoplasm of the targeted cells. A schematic drawing is shown in Fig. 2. In addition, a time-controlled irradiation mode for targeting thick biomaterials has also been established, and this mode has been demonstrated with zebrafish embryos[2]. Representative images when targeting zebrafish embryos are shown in Fig. 3.

SPICE provides a stable microbeam for 3 h, and under the stan-
standard irradiation protocol conditions, 3,000 cells in a cell dish can be irradiated within 15 min, meaning that 12 dishes can be irradiated in 3 h. Overall specifications of SPICE have been reported in the literature. Since 2009, SPICE has been administrated as a “Joint-use Facility for Collaborative Research,” and thus researchers outside NIRS can apply for beam time of SPICE after their research proposals are approved.

References

The secondary particles produced by the nuclear interactions of high energy photons, protons and heavy charged particles play significant roles for extra radiation exposure to not only patients during the medical treatment but also astronauts during space missions at lower-Earth orbits and beyond. For example, proton beams can deposit a dose to surrounding healthy tissue through nuclear reactions with the production of secondary short range, high-LET (high-linear energy transfer) target fragments. The LET of such particles extends from about 20 keV/μm up to several thousand keV/μm, meaning that their biological effectiveness is relatively high compared to primary protons. To fully understand the possible risks from the secondary target fragment component, including the induction of secondary cancers, the experimental verification of the dose contribution from the secondary target fragments is necessary.

For the precise measurement of secondary high-LET particles, we have developed two technologies with CR-39 plastic nuclear track detectors. CR-39 detectors are commonly used as heavy ion detectors with a detection threshold of ~5 keV/μm; this means that they do not register tracks from primary protons with energy greater than ~12 MeV and thus are insensitive to primary protons in the radiotherapy beam. To cover the very high-LET region of a proton beam around its Bragg peak and of a carbon ion beam, we have developed a two-step chemical etching method for CR-39 plates with PEW-x solution [17wt% KOH + xwt% C2H5OH + (83-x) wt% H2O] as the pre-etching solution and 7N NaOH solution as the post-etching one. This method allows us to control the LET detection threshold of CR-39, further enabling selective measurement of particles as a function of LET as shown in Fig. 1.

In the conventional method for the analysis of CR-39 detectors using an optical microscope, it is difficult to measure secondary high-LET tracks due to the short range (< 10 μm) of such tracks, because those tracks are mostly lost when chemical etching removes the surface layer to a thickness of several tens of micrometers. We have established a precise LET spectrum measurement method for short range tracks by controlling the chemical etching to an extremely shallow layer of ~ 1μm. The produced minute nuclear tracks are precisely measured with an AFM (atomic force microscope) replacing the conventional optical microscope as shown in Fig. 2. Under the AFM measurement conditions, CR-39 detectors were calibrated using low energy (< 6 MeV/n) and high energy (>100 MeV/n) heavy ion beams at HIMAC. The exposed CR-39 plates were etched in a 7 N sodium hydroxide solution at a temperature of 70°C for 1 h. The AFM (Dimension V; Veeco) equipped with a 125 μm cantilever having a typical tip length of 10 μm was operated in the tapping mode. The cantilever was oscillated near its resonant frequency (~300 kHz) which allowed the whole detector surface to be scanned. The AFM images for
the track diameter measurement, which gives CR-39 response \( (S) \), were scanned as 25 \( \mu \)m \^2 sizes with 1024 \( \times \) 1024 pixels. The scan rate was 1.5 Hz. Fig. 3 shows typical AFM images of nuclear tracks from (a) 415.8 MeV/n Fe, (b) 2.49 MeV/n Ar, (c) 4.18 MeV/n N and (d) 5.62 MeV/n He. The accuracy of measurement was 24.4 nm/pixel. The response curve for the conversion from track response \( (S) \) to restricted energy loss (REL) with the \( \delta \)-ray cut off energy of 200 eV, which can be converted to LET in water, was obtained as shown in Fig. 4. We found that the track response in CR-39 can be scaled with a universal function over a wide energy range from low energy (a few MeV/n) to high energy (~500 MeV/n) by the AFM measurement method\(^3\). The reported results will be applied to the evaluation of the secondary short range particle tracks produced by target fragmentation reactions in the radiation field used in not only radiation cancer therapy but also space radiation fields.

References


Lysosomes were discovered more than half a century ago by Dr. Christian de Duve, a professor at Rockefeller University and a 1974 Nobel Prize recipient in Physiology or Medicine. Lysosomes are now recognized as the ubiquitous and acidic organelles responsible for the turnover of cellular constituents. One of the main functions of lysosomes is to degrade cellular constituents. Therefore, they contain more than 50 hydrolases (phosphatases, nucleases, glycosidases, proteases, peptidases, sulphatases and lipases) which function only in an acidic environment. It is known that many materials are delivered to lysosomes for digestion via several pathways—phagocytosis, endocytosis, and autophagy. Lysosomal function is critical for cellular homeostasis, since lysosomal defects can be linked to several diseases leading to cellular damage, such as Danon disease and Niemann-Pick disease, which is characterized by the accumulation of undigested materials. In addition, current research suggests that lysosomal activity decreases during aging, which can result in the accumulation of toxic materials, such as damaged organelles, protein aggregates, and lipofuscin, indicating that lysosomal activity is essential for maintaining cellular integrity.

Infertility has become a medical issue recognized worldwide. It has long been believed that oocyte/embryo quality decreases with maternal aging (after an age of 35 years in humans) and that the resulting low quality could be one of the major reasons for female infertility. However, little is known on the molecular mechanisms involved in oocyte/embryo quality control. Preimplantation development is a developmental process where a fertilized oocyte develops into a blastocyst (Fig. 1). Once fertilized, the embryo rapidly develops into the blastocyst through several mitotic events, a process taking about 4-5 days in mice and 5-6 days in humans. Considering the rapid development, bulk degradation via lysosomes could be critical for eliminating residual materials in the oocyte and recycling them for synthesis of new products that are essential for transition from differentiated oocytes to totipotent embryos. Recently, we showed that autophagy, in which the cytoplasmic contents are sequestered by the autophagosomes and fused with the lysosomes, followed by the degradation of those materials is critical for maintaining oocyte-embryo quality.
contents, was highly activated shortly after fertilization\(^2\). These observations shed light on the importance of lysosome-mediated degradation in early embryo development.

Our laboratory has focused on the lysosomal function during preimplantation development. We recently found that the size and number of lysosomes changes dramatically after fertilization\(^3\) (Fig. 2). Consistent with this observation, the level of mature cathepsin, which is one of the major lysosomal hydrolases, was high during early embryo development. We also showed that lysosomal dysfunction caused an accumulation of lipofuscin (Fig. 3), which is a toxic material and a hallmark of ageing, and that these embryos were not able to develop further. These observations indicate that lysosomal activity and its function are critical for preimplantation embryo development. Based on our observation, we are developing a method for monitoring the lysosomal activity in developing embryos. If this technique is established, we might be able to determine which oocytes and embryos have relatively high (good) or low (poor) development potential, since we speculate that the lysosomal activity will correlate with embryo viability. Because lysosomal function is conserved in different species, our developing technique will be applicable to not only laboratory mice but also other animal species, including humans.

**Figure 2** Distribution of lysosomes in mouse oocyte and embryos.

Lysosomes in unfertilized oocyte and 1-cell and 2-cell embryos were labeled with LysoTracker Red, specifically for staining of the lysosomes, and observed under a confocal laser fluorescence microscope. Note that the size and number of lysosomes changes after fertilization. (h) represents time after fertilization. The scale bar is 10 μm.

**Figure 3** Lipofuscin accumulation in lysosome-defective embryos.

Early embryos were co-cultured with both E64d and Pepstatin A, which inhibit lysosomal proteases, and were analyzed by electron microscopy. Large numbers of lipofuscins, indicated by arrows, were observed in the co-cultured embryos, while no visible lipofuscins were observed in the non-treated (control) embryos. The scale bar is 2 μm.

**References**


Technology using induced pluripotent stem cells (iPSCs) holds great promise in regenerative medicine. Because iPSC technology allows researchers to obtain embryonic stem (ES)-like cells from patients directly, no immune rejection is expected when the tissues derived from iPSCs are transplanted. However, recently, immunogenicity of iPSCs was claimed, while similar immunogenicity was not observed in ES cells (Zhao et al., Nature 474, 212-215, 2011). This is quite an important study, since it directly affects the future of regenerative medicine. Heated arguments have arisen about the study (Okita et al., Circulation Research 109, 720-721, 2011; Yamanaka, ISSCR 2011), because the report involves several big concerns: only one line of ES cells (ESCs) was examined; there was no assessment of the developmental ability, of which partiality elicits immune responses; and immunogenicity was evaluated by using the iPSCs themselves.

Here we established many lines of integration-free iPSCs and ESCs from an inbred mouse strain C57BL/6 to obtain a conclusion on this issue\(^1, \^2\). The fully reprogrammed state and their developmental ability were verified by the germline transmission test through chimeric mouse formation for most of the lines.

First, we conducted a teratoma formation test for seven iPSC lines and five ESC lines; full developmental ability was observed for the five out of the seven iPSC cell lines and four of the five lines of ES cells. Although slightly efficient formation was observed in iPSCs, little difference in incidence was observed basically between iPSCs and ESCs (Fig. 1).

![Fig.1 Teratoma formation](image1.png)

**Fig.1** Teratoma formation
Arrow heads indicate teratomas. Seven iPSC and five ESC lines were analyzed. SEs are shown.

![Fig.2 Expressions of Zg16 and Hormad1 genes in teratomas](image2.png)

**Fig.2** Expressions of Zg16 and Hormad1 genes in teratomas\(^3\).
Three germ layers were observed in the teratomas. In addition, although we investigated T cells for detecting immune responses, we could not detect meaningful T-cell-infiltration not only in the teratomas derived from iPSCs but also from ESCs. We also examined the expressions of Zg16 and Hormad1 that were demonstrated as the causative genes for their immunogenicity but their expressions in the teratomas derived from iPSCs were lower than those in ESCs (Fig. 2). Thus, contrary to the previous report, even using a large number of ESCs and iPSCs, we could not detect any differences between these two types of pluripotent stem cells.

Because iPSCs or ESCs would be converted into specific tissues and transplanted into a recipient body, not transplanted pluripotent stem cells themselves, evaluation of immunogenicity must be performed on the differentiated tissues, not iPSCs or ESCs themselves. Therefore, second, we assessed the immunogenicity of the terminally differentiated cells derived from iPSCs and ESCs, skin and bone marrow. In our study, donor tissues were prepared from chimera mice developed from either iPSCs or ESCs; we used 100% chimeric mice only that were generated by aggregation with GFP-mice embryos to completely exclude the recipient derived cells from donor tissues. Tissues that were confirmed to be GFP-negative were used for subsequent transplantations. Consequently, even in the cases focusing on these differentiated cells, we also observed little difference between the tissues derived from iPSCs and those from ESCs not only in incidence but also in T-cell response. Almost all transplantations were successful and very few T-cells were observed within the transplanted tissues in both cases using the two types of pluripotent stem cells. Skin transplantation was successful for iPSCs and ESCs derived tissues and the engraftment was maintained for more than 6 months. Transplantation of bone marrow cells into recipient mice without X-ray irradiation was also successful and hematopoietic reconstitution was achieved four months later, indicating an engraftment of long-term hematopoietic stem cells.

Thus, in the present study we could not observe or distinguish the immunogenicity of iPSC-derived tissues from those derived from ESCs.

**Fig. 3** Transplantation experiments of skin and bone marrow. (A) Schematic diagram of the method. (B) Grafted skin by GFP iPSCs. (C) Long-term reconstitution of bone marrow by GFP positive iPSCs.

**References**

