RECENT TECHNICAL PROGRESS IN APPLICATION OF ION ACCELERATOR BEAMS TO BIOLOGICAL STUDIES IN TIARA

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Abstract

High-energy heavy ions cause severe local damage, injury, or inactivation to living organisms through high- density local energy delivery. The JAERI AVF cyclotron and electrostatic accelerators in TIARA have been applied to biological studies covering cell surgery technique for plant breeding, high-LET specific mutation induced by ion beam, and the repair of DNA damage on radiation resistant bacteria. A study on cell surgery technique suggests the importance of atomic displacement effect of low-energy ions. Ion beam are now becoming a new experimental tool for local inactivation of organisms and also for analytical probe. The paper summarizes recent technical and research progress for application of ion beams to biological studies in TIARA.

1 INTRODUCTION

Energetic heavy charged particle transfer their energy to living organisms with high-density ionization and excitation along the particle track even by uniform irradiation. This characteristic results in microscopically nonuniform dose delivery, which is expected to induce unique mutation and local inactivation in living organisms. This is in contrast to relatively uniform dose delivery in gamma-ray or electron beam irradiation.

Another important characteristic is that ion beams can make localized irradiation to the target organisms. The small energy-loss struggling allows high-resolution control of the penetration depth for relatively low energy ions, which may induce local structural damage resulting from atomic displacement.

Ion beam handling such as microbeam irradiation and single ion hit also makes the localized irradiation. Limitation of target area or localized ionization can be applied to inactivation of microscopic region, cell surgery technique, and simulation of radiation risk for human space flight.

In these applications, the small cross-section of elastic scattering in relatively high energy range is also useful to define the local target volume in deep region of the irradiated medium.

Ion beams are also becoming useful for analysis of living organisms by using secondarily produced radiation such as X-rays, gamma-rays and positrons, which are applied to PIXE and nuclear reaction analyses (NRA) and positron emission tomography (PET), respectively.

High-energy ion beams have been applied to radiation therapy development in world-wide scale. However, there is no ion accelerator facility adapted for biological or botanical research because of the lack of the strong incentive to the research using accelerator beams. The ion beam irradiation research facility, TIARA, may be the first facility to promote the research in a large scale. The facility was completed in 1993, and extensive applications of ion beams have been conducted mainly for R&D in materials science and biotechnology under full-scale operation of the AVF cyclotron and three electrostatic accelerators [1]. A wide range of LET giving different biological effectiveness and relatively deep penetration for heavy ions with very high LET are covered by various ion beams from the four accelerators.

The paper describes recent technical and research progress in new application of the ion accelerator beams to biological studies.

2 FACILITIES FOR BIOLOGICAL APPLICATIONS

2.1 Cyclotron Facility

To meet various research requirement in the fields of materials science and biotechnology, many kinds of ion species from proton to xenon can be accelerated by the JAERI AVF cyclotron in a wide range of beam energies from 10 to several hundreds MeV[2]. Light and heavy ions are axially injected into cyclotron from two external ion sources, a multi-cusp and an ECR ion sources, respectively.

Ion beams has been mainly used for evaluation study of radiation risk on mammalian cells using proton and helium ions with energies of a few and several MeV from Van de Graaff accelerators [3]. However, heavy ion beams from the accelerators cannot be applied to the study because of the poor penetration. The relatively deep penetration in heavy ions from the cyclotron allows to analyze the fundamental effect of high-LET radiation to living organisms in a wider LET range.

The cyclotron is equipped with nine horizontal beam courses and four vertical branch beam courses which are used mainly for irradiation experiments in biological

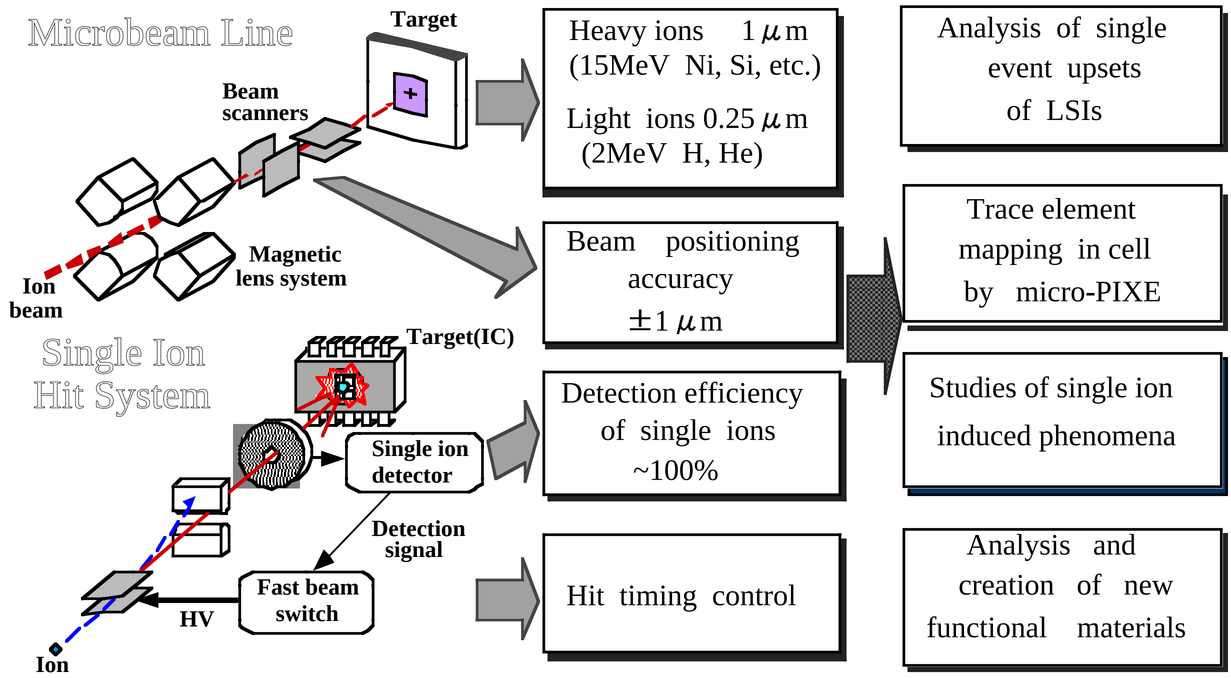

Fig. 1 Ion microbeam technique and its appliocations using electrostatic accelerators in TIARA.

studies. In a vertical beam course, the cyclotron beam is two-dimensionally scanned over an area up to 50 x 50 mm^2 for uniform high-energy beam irradiation to biological samples. A number of different samples can be irradiated continuously and by passing through the radiation field using a conveyer system.

At the end of another vertical beam course, we installed an apparatus for local irradiation of a single living cell. Very low current high-energy heavy-ion microbeam is formed by a collimation system with three steps and finally extracted into air through a bottom micro-aperture. The position of the cell is adjusted just under the aperture by using an optical microscope. Each particle extracted is detected for hit verification after sample penetration [4].

Another research activity in biological application is dynamical study of physiological function of plants *in vivo* using positron emission tomography (PET). A positron emitting tracer imaging system has been developed together with the production methods of labeled compounds as short-lived positron emitters [5].

2.2 Eectrostatic Accelerator Facility

Three electrostatic accelerators, a tandem and a single-ended machines with a maximum voltage of 3MV and an ion implanter with 0.4 MV, make a combined beamline network [6]. The feature of the facility is that the two or three machines can be operated simulta-neously in triple or dual beam mode.

The facility has 10 beam courses, two of which are equipped with apparatus exclusively for biological studies. One is an apparatus for heavy ion irradiation to cells in air with penetration depth control installed at a beam course end of the tandem machine. The other is a light ion microbeam apparatus for biological study, installed at a course end of the single-ended machine.

3 MICROBEAM TECHNIQUE FOR BIOLOGICAL APPLICATION

For application of the ion accelerators to R&D of materials science and biotechnology, an emphasis is put on microbeam technique. A couple of microbeam apparatus was developed by using the 3MV electrostatic machines; one is the light ion microbeam apparatus and the other a heavy ion one connected with the tandem machine.

Figure 1 shows technical achievements which has been accumulated in our microbeam development using electrostatic machines so far and their application fields. To achieve submicron beam easily, the high-voltage generator was designed to have a high-stability terminal voltage of 10^{-5}. It was satisfied by installing a balanced Schenkel type DC power supply. We already evaluated a beam size of a quarter-micron meter with a beam current of more than 10 pA [7]. As an application of the submicron beam, we are now developing a high-sensitivity micro-PIXE camera for trace element mapping in cells [8].

In energetic heavy-ion beams, only a single particle causes severe local damage or injury to living organisms. We can control it microscopically by a single ion hit or a small number of ion hits to an aimed local area in the organisms. We developed a single ion hit system for heavy ion microbeams within a precision of hit positioning of 1μ m [9].

4 COCKTAIL BEAM ACCELERATION TECHNIQUE FOR CYCLOTRON

Linear energy transfer (LET) is the most important parameter which characterizes the radiation effect in application of energetic ion beams to biology. In study on LET dependence in biological application, it is required that we quickly switch beam parameters with different LET values to obtain biologically consistent results and also to operate the cyclotron efficiently. However, the switching time is usually 1 to 2 hours in the cyclotron, mainly because of time-consuming magnet excitation process.

To reduce the switching time, cocktail beam accel-eration (CBA) technique [10] has been developed based on the principle that the ions with the same mass to charge (M/Q) ratio can be accelerated using the same isochronous magnetic field and acceleration frequency. Different ions with an almost identical M/Q ratio are injected into cyclotron without separation of the ion species, and accelerated simultaneously just like cocktail ions.

All the cyclotron parameters are optimized for one of the cocktail ions and a set of parameters determines a standard condition for the CBA. Small difference of M/Q ratios from the setting value gives rise to deviation from the isochronous condition. The deviation causes a drift of a beam phase, which results in the gradual decrease of energy gains. If the ions lead or lag behind the ions under the tuning condition by 90 degrees in an RF phase, the ions fall into a decceleration phase region and disappear some-where in a center region. Only the ions under the tuning condition are fully accelerated and extracted from the cyclotron.

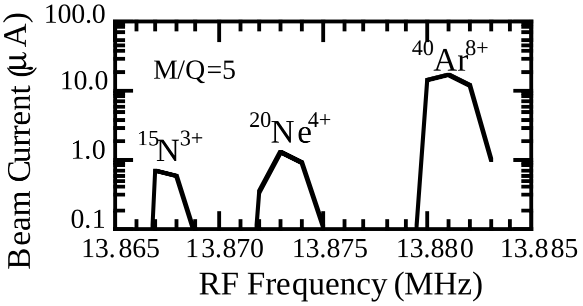

Fig.2 The dependence of beam intensity on the frequency for M/Q=5 cocktail ions.

Other cocktail ions can be also fully accelerated by slight change of the acceleration frequency relative to the difference of the M/Q ratios. The dependence of beam intensity on the frequency for M/Q=5 cocktail ions is shown in Fig. 2 as an example. It takes only several minutes or less for switching ion species in CBA operation mode.

5 RECENT PROGRESS IN BIOLOGICAL STUDIES

Several application studies using ion beams to biology are going on in TIARA by making the most of the ion beam characteristics described above. Recent research and technical progress in the field are outlined below.

5.1 Repair of DNA Damage in Deinococcus Radiodurance

Deinococcus radiodurance (DR) is an extraordi-nary radiation resistant bacterium, of which the radiation sensitivity is more than a thousand times that of typical microorganisms and mammalian cells. However, the reason of the highest resistance has not been made clear. From ion beam irradiation experiments, it was found that the relative biological effectiveness (RBE) of DR de-creases monotonically with increase of LET [11], as shown in Fig. 3. This is quite different from a well known LET dependence on RBE, having a maximum around 100keV/μm, which has been observed for mammalian and typical microorganisms. The low RBE values of DR show high repairability of severe DNA damage such as clusters of damage due to the high LET. In the study of LET dependence of the DNA rejoining, it is found by using a pulsed-field gel electrophoresis technique that the total repair time necessary for the rejoining after irradiation increases with LET. We are analyzing the molecular mechanism of DNA repair by cloning several genes responsible for the repair. Subsequently transposon mutagenesis of uvrA gene [12] and a novel repair gene have been discovered.

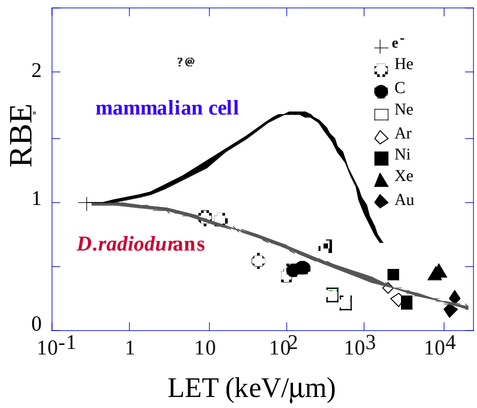

Fig. 3 Relationship between LET and RBE on *D.radiodurans*

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5.2 Application to Plant Breeding

Low LET radiation such as X and gamma rays, as well as chemicals, has been applied to expand genetic resources in plants so far. Recent diversification of accelerator beams promotes the applicability of high-LET radiation to improvement of plant varieties. The R&D for appli-cation of ion beams for varietal improvement in plants has been conducted by various collaboration research works.

As a typical result, callus proliferation of leaf and floral petals of chrysanthemum irradiated with 220 MeV carbon ion beam resulted in the mutants with huge variety of flower colors, while the variety of colors induced by gamma-ray was limited. This characteristic of ion beam was observed in other plants as well. Ion beam irradiation of *Arabidopsis* allows the isolation of hitherto unobserved mutants resistant to ultraviolet radiation.

The above findings show that ion beam irradiation allows to create varieties with new genetic traits from specific seeds, and is effective as a new source for mutation breeding. A DNA analysis of the *Arabidopsis* mutants also shows that ion beam induced mutants are a new material for isolating specific genes.

Another new application is the production of inter-specific hybrid by the cross with ion beam irradiated pollen. It is often impossible to produce hybrids between distantly related plants. For example, normal cross tech-nique has not succeeded in introduction of disease- or insect-resistant gene from wild tobacco plants to cultivar. Ion beam irradiation to pollen was found to be effective to produce interspecific hybrid with insect-resistance near that of wild tobacco, and its production efficiency is much higher than that of gamma-ray induced cross [13].

Table 1 lists typical examples of new and improved plant varieties induced by ion beam radiation in TIARA. The observed difference between ion beam induced mutations and gamma-ray induced ones can be explained by the LET dependence of the characteristics of microscopic energy delivery. Though uniform dose delivery from gamma-rays may cause various mutations including undesirable ones in a large number of genes, nonuniform dose delivery from ion particles may allow to produce a sufficient number of desirable ones which are little influenced by undesirable ones.

Table 1 Plant Breeding Using Ion Beams

Mutation Induced by Ion Beams

1. Ultraviolet (UV-B) resistant mutants of Arabidopsis
2. Bacterial leaf blight (BLB) resistant mutants of rice
3. Disease resistant mutants of barley
4. Flower color mutants of Chrysanthemum

Interspecific Hybrid Produced by the Cross with Ion Beam-Irradiated Pollen

1. Insect resistant hybrids of Tobacco plant

5.3 Gene Transfer Technique Using Low-Energy Ion Beam

Direct gene transfer to plant pollens is one of cell surgery techniques to create new, useful plant varieties. However, a major difficulty is pollen-specific envelope which is hard to pass for exogenous DNA. The irradiation of low-energy ion beam into shallow depth of the envelope is expected as an effective method to solve the difficulty because of the high resolution control of the penetration depth.

We succeeded in giving rise to effective physical lesion in tobacco envelope by irradiation of He ions within a depth of 4 μm, which made the pollen leaky, and in introducing exogenous DNA into the pollen.

To examine the effect of penetration depth on the induction of leaky pollens, the frequency of the produc-tion was measured with changing the air distance from a Kapton-film beam window to the leaky pollen. The result is shown in Fig. 4. A peak observed around the end of the penetration range suggests that the physical lesion in the envelope is the most effective in very low energy region where high-density atomic displacement effect is dominant resulting from stopping by screened nuclei [14].

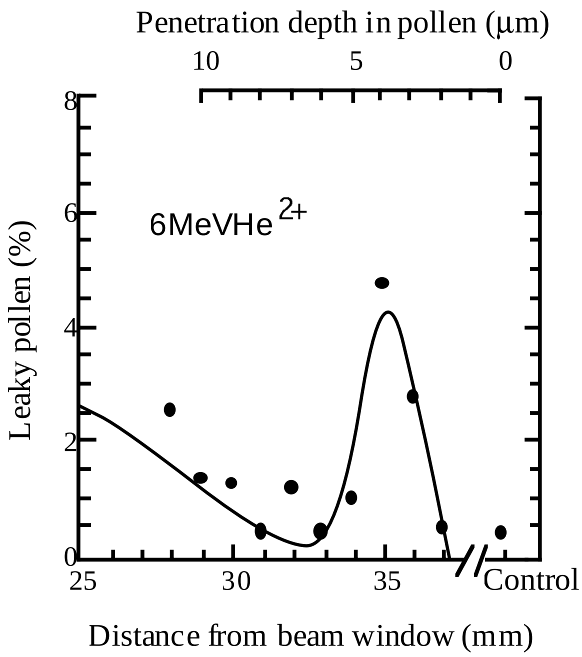

Fig.4 Relation between formation of leaky pollen and penetration depth in pollen

5.4 Application of High-Energy Heavy Ion Microbeam

High-energy heavy-ion microbeam irradiation is also useful for development of cell surgery technique. We are developing irradiation technique to a local region of living organism using extremely week collimated beams from the cyclotron. The final goal of the beam size is a few μm. We already achieved a beam size less than 10 μm.

The performance of cyclotron beams is basically limited because of their relatively poor beam quality. Beam focusing or cooling is required to extend their application to new fields such as asymmetric cell fusion. The beam quality is also influenced mainly by edge scattering at the final micro-aperture, giving rise to beam halo around the beam spot.

High-energy single ion hit or a predetermined number of the ion hits with timing control will be useful for basic study for estimation of radiation risk in human space flight, since the frequency of ion particle incidence to a single cell is extremely low. We are planning to apply the single ion hit technique developed using the tandem accelerator to the high-energy cyclotron micro-beam.

From a recent study of local irradiation effects of heavy ion microbeam on the embryogeneisis in silkworm, it is considered that the microbeam is useful as a new surgical tool to analyze cell determination and differentiation mechanism in various living organisms, because it can inactivate them effectively and reliably without destroying the structure [15].

5.5 Application of Micro-PIXE Camera

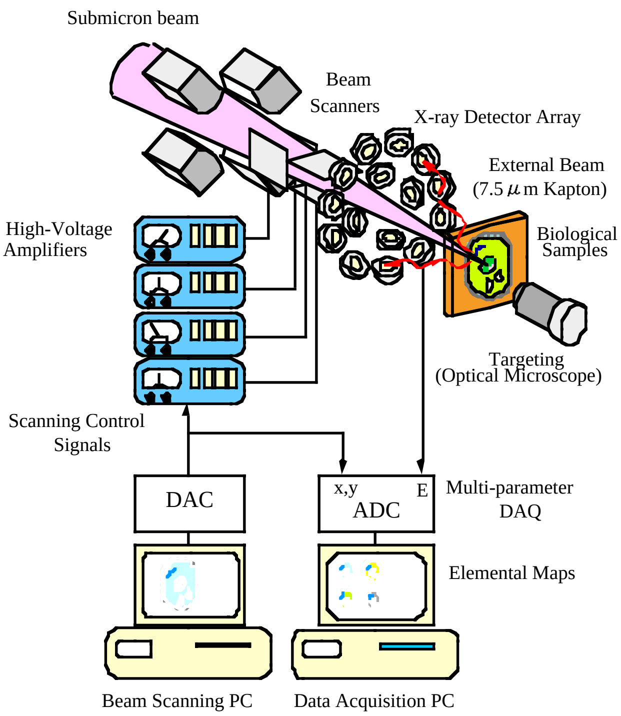

Fig. 5 Schematic view of the micro-PIXE camera.

In micro-PIXE analysis for biological or medical samples, there are technical difficulties that the sample are easily destroyed or deformed and the volatile elements are evaporated by the irradiation of the locally high-fluence rate beam. To minimize the difficulty without increasing the time for analysis, we are develop-ing a high-sensitivity X-ray multi-detector system, receiving X-ray emitted from the target with a large solid angle. We are also developing a high-speed analyzing system which acquires X-ray signals and a large number of spectral information to obtain element distribution data. The schematic view of the micro-PIXE camera under development is shown in Fig. 5. The microbeam can be extracted into air for analysis of biological samples with keeping them intact.

We are planning to apply the system mainly to trace element mapping in cells, which will contribute for example to the study of environmental responsiveness on the physiological functions of plants.

6 SUMMARY

From above technical and research progress on biological application, it is becoming clear that ion accelerator beam is useful as a new experimental tool for wide fields of biological and biotechnological studies. The tool has two functions, that is an analytical probe and a surgical tool for microscopic inactivation. It is expected for biological research and development that the two functions will be combined effectively to make ion beam a more elaborate tool for microscopic radiation processing.

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