ION BEAMS AND ACCELERATORS FOR BIOLOGY-ORIENTATED APPLICATIONS AND RESEARCH – CMU PRACTICES

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Abstract

Applications and researcr of ion beams and accelerators for the biology-orientated issues have been rigorously developed at Chiang Mai University (CMU), Thailand, the national ion beam and accelerator research center, for two decades. The work is highly multiple, touching equipment development, genetic engineering, materials science, analytical technology, cell and molecular biology, biomedicine, and life science. CMU has more than ten various types of ion accelerators and implanters with ion energy in the range of < keV – 20 MeV for the work in this direction. Relevant applications involved ion-beam-assisted gene/DNA transfer in bacterial, plant and mammalian cells, ion beam induced mutations in rice, horticultural plants, vegetables and bacteria, ion-bombardment altered cell adhesion on material surfaces, ion beam analysis of biological samples, ion beam lithography of biological microfluidic devices/patterns, and cyclotron-production of radiopharmaceuticals for diagnostic services. Research concerned covered physical mechanisms of ion-beam-assisted gene/DNA transfer into cells and ion-beam-induced mutations of cells, low-energy ion bombardment effect on DNA strand breaks including both experimental ultra-low energy ion bombardment of naked DNA and computer program simulations of ultra-low energy ion impact on DNA, single-ion irradiation of cells for hyper low dose effect on cancer cell death, and cyclotron manufacture of radiopharmaceuticals for multi-purpose diagnostics.

1. INTRODUCTION

Ion beam is a branch of physics. The objects or targets of ion beams can be every type of materials in principle, and hence certainly biological living matter and materials served for biology and medicine are included. Here, word ‘biology’ has a very broad range of contents, including not only pure biology (including plants, animals, and microorganisms, from body to cell to DNA) but also agriculture (including horticulture and food), medicine, life and health, and materials and devices for biology uses. Table 1 depicts the scope of ion beam biology. Being the unique ion beam center in Thailand, Chiang Mai University (CMU) has rigorously developed ion beam technology with the particular focus on biological applications. Based on the technical capabilities of our ion beam facilities which are low-energy (mostly, < 200 keV) ion implanters, our research has been following the low-energy-ion-beam train of thoughts. In comparison with high-energy ion beams which can normally only do mutation induction, the low-energy ion beams served as a special ion beam biotechnology tool [1] provide us with advantageous opportunities by the capabilities of performing multi-topics investigations, including not only mutation induction, but also ion-beam-assisted gene/DNA transfer, fundamental studies on ion-DNA interaction, as well as ion beam modification of biomaterials for medicine uses. In addition, CMU has a 1.7-MV accelerator applied for ion beam analysis of biosamples and ion beam lithography of micro-biodevices and a 20-MeV cyclotron working on pharmaceutical manufacture for medical diagnosis and therapy.

2. FACILITIES

The ion-beam/accelerator facilities at CMU for biology-oriented applications and research include 150-kV mass-analyzed (CMU1) [2], non-mass-analyzed horizontal (CMU2) [3] and vertical compact ion implanters [4],

TABLE 1. ION BEAM BIOLOGY SCOPE FRAME. *: CMU work implemented. ^: CMU work in plan.

Overall umbrella	Secondary category	Tertiary category	Objects/contents	Objectives
Ion Beam Biology	Ion beam genetics	Ion beam assisted gene transfer	Bacteria*	For genetic modification
			Plants*	For genetic modification
			Mammals*	Study stem cell therapy
		Ion beam mutation induction	Food crops*	Rice, wheat, corn, etc. for yield and quality improvements
			Horticultural plants & vegetables*	Flowers and various vegetable species for quality improvements
			Other economic crops and environmental plants^	Rubber, cotton, etc. for yield and quality improvements; water weeds to control the growth, etc.
			Bacteria*	For gene identification
			Insects^	Mosquito sterilization
	Ion beam life science	Ion beam biomedicine	Ion beam therapy	Cancers
			Isotopes for diagnosis and treatment*	Cyclotron ion beam production of the isotopes
			Ion beam modification of biomaterials*	Enhancement of cell attachment
				Improve mechanical and chemical properties of artificial components for lifetime extension
			Ion beam formation of nanoparticles & patterns	For drug or stem cell delivery
				For neuro cell patterning to study brain neurology
			Ion beam lithography of biomedicine micro-devices*	Fabrications of microfluidic devices, and lab-on-chips, etc.
			Low-energy ion/plasma skin treatment*	For skin beauty and wound recovery
			Ion beam sterilization of hospital items^	Contaminated clothes and bed sheets, and disposals
		Ion beam health safety	Ion beam irradiation health effects^	Study accelerator safety and nuclear accident dangers
		Space travel irradiation risk assessment	Simulation of cosmic particle irradiation onto astronauts	Study the effect at high energy but low dose
		Ion-cell interaction fundamentals	Ion irradiation of cells^	Study cell response to irradiation
			Single ion irradiation of cells^	Study bystander effect, hyper low dose effect on cell death
		Ion-DNA interaction fundamentals	Low-energy ion bombardment of DNA*	Study DNA strand breaks, e.g. single strand break (SSB) and double strand break (DSB) and mutation patterns
			Computer simulation*	
	Ion beam biology analysis	Tissue or cells elemental determination	Concentrations*	Normal beam
			Mapping/imaging*	Microbeam
		Environmental monitoring analysis	Plant and animal cell element analysis^	For monitoring environmental changes
		Biological archaeology analysis	Analysis of archeological biological items*	Determine ages and study ancient climate, geological and environmental changes
		Analysis for food science & technology	Food quality and food container/package materials analyses^	For food safety monitoring
	Ion beam treatments	Ion beam/plasma food sterilization	Slowing decay of foods and liquids^	Benefit food industry
		Ion beam nano-structuring of materials	Ion beam induction of nanostructure for candidate material agents	Fabrication of loss control fertilizers which are normal fertilizers mixed with the agents*

respectively, 220-kV Varian ion implanter, 30-kV bioengineering-specialized vertical ion implanter (CMU3) [5], 25-kV plasma immersion ion implanter (PIII) [6], 10-kV neutralized ion beam implanter [7], low-energy single ion implanter, low-energy Mark II broad-beam high-output ion source-based ion implanter, 1.7-MV Tandetron tandem accelerator, and 20-MeV (H^-) cyclotron. Except the Varian ion implanter, the Tandetron accelerator and the cyclotron which are commercial machines, most of the facilities have been in-house developed. CMU1 has been applied to ion beam assisted DNA transfer and ion beam induced mutation, CMU2 and the compact ion implanter have been the main work horse for ion beam induction of mutations, the Varian implanter applied to ion beam modification of bio-membranes for cell adhesion modification, CMU3 applied to ion beam assisted gene transfection in mammalian cells and studies of low-energy ion bombardment effect on naked DNA and physical mechanisms involved in gene transfer and mutation inductions as well as the single ion implantation, PIII applied to low-energy ion bombardment effect on DNA, the ion beam neutralizer applied in studying ion charge effect on ion-beam-assisted DNA transfer through cell envelope materials, the Mark-II source-based ion implanter for high-output and broad beam application to mutation induction, the Tandetron accelerator with its beamline [8] applied in ion beam analysis of biological samples as well as microbeam application in lithography of biological micro-apparatus, and the cyclotron applied in production of pharmaceuticals for medical diagnosis.

3. APPLICATIONS AND RESEARCH

3.1. Ion-beam-assisted gene/DNA transfer

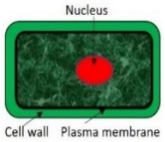
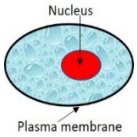
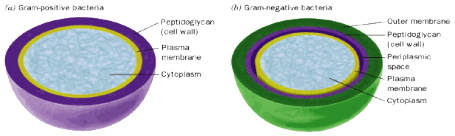
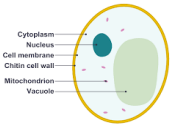
Ion-beam-assisted gene transfer was implemented, as the first application of ion beam biology at CMU, in the plant, bacterium, yeast and animal cells. These four types of cells have different cell envelope structures, as shown in Table 2, and the cell envelope is critical for ion beam assisted gene transfer. For different types of cells, ion beam conditions were adjusted to adapt the different cell envelopes, particularly in terms of the thickness, to make the gene transfer available. The general protocol of ion-beam-assisted DNA transfer includes steps of (1) specimen preparation, (2) specimen exposure to vacuum, (3) ion beam bombardment, (4) post-bombardment treatment, (5) transferring or transfection, and (6) observation and confirmation or analysis of the character expression of the transferred gene. Table 2 summarizes our experiments on ion-beam-assisted gene/DNA transfer or transfection in various types of cells. We also carried out studies on mechanisms involved in the process. We found that only when the cell envelope was appropriately tailored or modified by the ion beam to such a degree that the envelope permeability was enhanced but not severely damaged so that the cell could still be alive, DNA transfer could be successful. The cell envelope material is represented by cellulose, $C_6H_{10}O_5$. Manmade cellulose membranes were used to mimic the plant cell envelope for investigate the ion-beam induced materials modification. We found formations of bubbles, cracks, micro-craters and chain-scission on/in the membranes. These modified structures were speculated to play roles in enhancing the membrane permeability, which was then confirmed by measured increase in the diffusion coefficient of plasmid DNA through the membrane.

3.2. Ion beam induction of mutation

There is not a commonly applicable protocol of ion beam induction of mutation, while the protocol depends on the biological target, including the target type, plant or bacterium or yeast or even animal, and the target part, seed or tissue or cell. Nevertheless, in principle, the steps may include

- Preparation of the samples and specimens of seeds or tissues or cells
- Ion beam bombardment of the specimens
- Culture and growing of the bombarded material
- Observation and screening of the favorable variations
- Development of the selected variation to M2 and M3 generation to obtain mutants
- Stabilization of the mutants up to many generations
- Genetic analysis of the stabilized mutants
- Registration of the mutant lines to the government for official approval
- Distribution of the registered and approved mutant seeds to the production society for commercialization.

TABLE 2. CELL ENVELOPE STRUCTURES AND ROUGH THICKNESSES OF PLANT, BACTERIUM, YEAST AND ANIMAL CELLS[#] (not in scale).

Cell types	Plant	Animal	Bacteria	Yeast
Schematic of the cell				
Cell envelope thickness	0.1 – 10 µm	10 nm	(a) Gram-positive: 20 – 80 nm, (b) Gram-negative: 20 nm (bacterial cell as prokaryote has no nucleus)	10 – 100 nm
Cell example	*Curcuma* embryo	HEp-2 human laryngeal epitheloid cancer cell	*Escherichia coli* (*E. coli*) strain DH5α	*Saccharomyces cerevisiae* strain W303C
Ion beam	Ar^+, N^+; 15-30 keV; $5 \times 10^{14} – 3 \times 10^{16}$ ions/cm^2	N^+; 7-28 keV; $10^{15} – 10^{16}$ ions/cm^2	Ar^+, N^+; 26 keV; 0.5, 1, 2, and 4×10^{15} ions/cm^2	N^+; 50- and 60-keV; $1 – 100 \times 10^{15}$ ions/cm^2
Exogen-ous molecule	Neutral Red (NR) vital dye	Plasmid pEGFPN2	DNA plasmids: pGEM2, pGEM-T easy, pGFP, pUC18, p35s and pBI221	pYGFP and pYlip plasmids
Key results [ref]	NR molecule penetration depends on beam condition and cell type [9,10]	Only 14 keV, 3×10^{15} ions/cm^2 achieved transfection [11]	At fixed ion energy, the successful gene transfer, indicated by gene color expression and antibiotic survival, assisted by ion beam bombardment depends on the ion species, fluence, and the DNA size [12,13]	50-keV with 5, 10 and 20×10^{15} ions/cm^2 achieved successful gene transfer [14]

[#]The information can be easily found from many textbooks and websites.

As rice is the staple of Thailand, induction of rice mutation breeding has naturally become the first choice of the ion beam mutation research. Table 3 summarizes the experiments that have been carried out at CMU in this aspect. In all of the experiments, special technical cares were generally taken. Prior to the ion beam treatment, to break the rice dormancy, the rough seeds were first incubated at 49°C for 5 days. The incubated seeds were manually dehusked to expose the embryos and individually placed in holes of special rice seed sample holders in such a way that the embryos were faced to the ion beam incident direction. From our experiments, the mutation induction frequency was found around orders of 0.1-1‰. Up to now we have had 15 Thai Jasmine rice mutants induced by ion beams, namely, HyKOS3, HyKOS3-1, HyKOS16, HyKOS21, HyKOS22, FRK-1, MSY-1-2, MSY-1-3, MSY-4, OSSY-22, OSSY-23, MRD6-11 MRD6-12, OSSY-8 and OSSY-25 already registered and approved following the Plant Variety Act, B.E. 2518 and the Plant Variety Protection Act, B.E. 2542, Department of Agriculture, Royal Thai Government, Thailand.

We have also worked on other plants and organisms including flowers, vegetables and bacteria for mutation induction. On flowers, some information regarding the experiments is summarized in Table 4. Mutations at the DNA level was also confirmed [26]. On vegetables, mutation induction in the purple yard long bean (*Vigna unguiculata* ssp. *sesquipedalis*) cv. Nan1 was conducted using 80-keV N-ion beam to a fluence of 3×10^{17} ion/cm^2 to implant the bean seeds [28]. After planting to M3, mutants showed dark purple pods, earlier flowering, higher yield with more pod numbers per plant, longer pods, and higher pod weight [28]. On bacteria, cells of *Bacillus licheniformis*, which was shown to suppress conidia germination of the fungus and reduce symptoms caused by the disease *in planta*, were bombarded by N-ion beam at energy of 28–50 keV to a fluence range of $1–10 \times 10^{15}$ ions/cm^2. After this treatment, one mutant was found which had lost its antagonistic property. Molecular biology analysis identified the gene involved in the property expression [29]. The gene was transferred into yeast cells which were then conveniently applied in protection of the flowers from fungal infection [30,31].

Why low-energy ion beam bombardment of seeds can induce mutation has been always curious, as the seed coat is thicker than the ion range. We thoroughly studied the mechanisms and found the possibilities. We can now depict the entire process of low-energy heavy ion irradiation of crop seeds for mutation induction, as schematically shown in Fig. 1.

3.3. Low and ultra-low energy ion bombardment of DNA

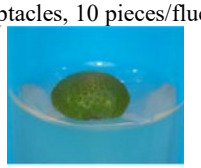
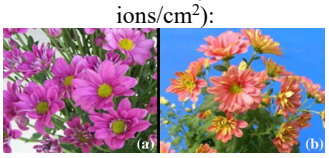
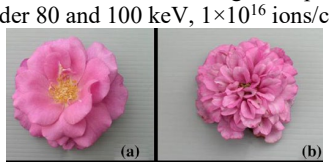
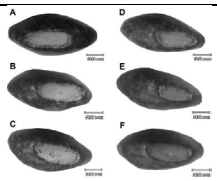
The ion energy which is originally low loses in the traveling paths through the materials of the cell envelope and substance inside the cell to a further low level and finally the ion energy deposition onto DNA to change DNA for mutation induction dominantly occurs around the Bragg peak and hence the ion energy becomes very low at this stage. To understand what happens at this final stage of ion implantation to DNA, studies on very low energy ion bombardment effects on DNA changes are necessary. We carried out investigations on the calculation of the low-energy ion range in DNA [32], ion bombardment using ions at low energy, around keV (Table 5), and ultra-low energy, down to 10 eV (Table 6), using various ion species and looking for ion beam condition thresholds of inducing double strand breaks (DSBs), and computer simulations.

TABLE 3. A SUMMARY OF THE EXPERIMENTS ON LOW-ENERGY ION-BEAM-INDUCED RICE MUTATIONS.

Rice	Ion beam	Phenotypic variation	Genetic variation
Thai purple rice (*Oryza sativa* indica strain Purple); 400 seeds/fluence. [15]	60-kV accelerated mixed N-ions; Fluences: 1, 4, and 8 × 10^{16} ions/cm^2; Facility: CMU2	2 mutants obtained from 1 × 10^{16} ions/cm^2: green pigment appearing in the leaf blade and stem sheath	Additional DNA fragment encoding partial sequence of protein belonging to members of P450 protein family
Thai jasmine rice (Oryza sativa L. cv. KDML 105); 800 seeds/fluence. [16]	Mixed N-ions at 60–125 keV; Fluences: 1×10^{16}–5×10^{17} ions/cm^2; Facility: CMU2	Mutants obtained: PKOS1 (60 kV, 2×10^{17} ions/cm^2): photoperiod insensitive and short in stature. TKOS4 (80 kV, 8×10^{16} ions/cm^2): photoperiod insensitive, tall, and early-flowering. BKOS6 (60 kV, 2×10^{16} ions/cm^2): photoperiod insensitive, short, early maturing HyKOS1: photoperiod-insensitive, short, and late-flowering [17].	Additional fragments, BKPK10450 and BKPH15400, detected by PCR reaction in BKOS6, belonging to members of anthocyanin and cytochrome P450, respectively. Expressions of transcripts of genes in the semidwarf and spindly mutants correlated with genes *OsSPY* and *14-3-3*, respectively [18].
Thai jasmine rice (Oryza sativa L. cv. KDML 105); 4,800 seeds. [19]	Mixed N-ions accelerated by 60 kV; Fluence: 4×10^{16} ions/cm^2; Facility: CMU2	Mutant: BKOS: strikingly short, photoperiod-insensitive, deep purple color in multiple tissues	Anthocyanin synthase enzyme expressed (but not in wild type) [19]; the highest total phenol content and improved antioxidant activities [20]
Primary ion-beam-induced mutant BKOS6 of Thai jasmine rice (Oryza sativa L. cv. KDML 105); 6,000 seeds. [21]	Mixed N-ions accelerated by 60 or 80 kV; Fluence: 1, 2×10^{16} ions/cm^2; Facility: CMU2	Mutant HyKOS21: photoperiod insensitivity, semi-dwarfness, high yield potential, the highest seed storability among 7 tested varieties with a seed germination percentage nearly two times as that of the original KDML 105	The lipid peroxidation level of the mutant seeds the lowest; lacked expression of lipoxygenase isoenzyme *lox1* and *lox2* and expressed only *lox3* in seeds
Thai jasmine rice (Oryza sativa L. cv. KDML 105); 22,800 seeds. 6,000 BKOS6 seeds. [22,23]	Mixed N-ions accelerated by 60, 80, 100 kV; Fluence: 1, 2, 4, 8×10^{16} ions/cm^2; Facility: CMU2	23 mutants from KDML 105 and 6 mutants from BKOS6 (for details in phenotypes, refer to [22])	Polymorphisms distinguished from that of KDML105; four additional fragments in mutant profiles encoding proteins involved in the high yield characteristics [23]
		HyKOS22 (bred up to M8 generation, obtained under the beam condition of 60 kV and 2×10^{16} ions/cm^2): drought tolerance + high yield, in contrast to original KDML 105 and drought tolerant check variety, CT9993 [24]	Genetic changes confirmed by established microsatellite DNA markers, suggesting both insertions and deletions induced by ion bombardment [24]
Sangyod Phatthalung (SYP) rice variety (*Oryza sativa* L. cv. Sangyod Phatthalung); 7,000 seeds. [25]	Mixed N-ions accelerated by 50 kV; Fluence: 4×10^{16} ions/cm^2; Facility: non-mass-analyzing vertical compact ion implanter	7 mutants: photoperiod insensitive, shorter, higher number of panicles, better water absorption index and pasting properties, resulted in an increased rice vermicelli production yield	Point mutations and deletion present in the examined locus; genetic changes were polymorphic, leading to possible additional genetic changes and differences among the mutants

In order to obtain ultra-low-energy ion beam, we designed and constructed a deceleration lens [48]. The results experimentally confirmed that ion irradiation of naked DNA at energy as low as the order of 10 eV could definitely induce DNA strand breaks in terms of SSBs and DSBs, depending on ion energy, fluence, ion mass and inertia, generally speaking, the higher the energy and fluence, the heavier the mass and the more active the ion, the easier the induction, with correlated thresholds discovered [41]. Computer simulations were also performed to touch the molecular levels. Monte Carlo methods [43], molecular dynamics [44,45] and Geant4-DNA software [46] were applied, respectively. An important result obtained is that ion irradiation effect on DNA changes is not random but preferential.

TABLE 4. A SUMMARY OF INFORMATION ON FLOWER EXPERIMENTS.

Flower species (Ref.)	Samples	Ion beam condition	Variations/Mutations
Dendranthema morifolium (Chrysanthe-mum), the variety Reagan Dark [26]	Receptacles, 10 pieces/fluence  diameter: several mm	60-keV N-ions, fluences of 1, 2, 4, 6 and 8×10^{16} ions/cm^2 (150-kV mass-analyzed heavy ion implanter)	14 plants with steak on petal; 1 plant with color change (under 4×10^{16} ions/cm^2):  (a) Control. (b) Mutant
Rosa hybrids [26]	Internode sections well wound by parafilm 	60, 80 and 100-keV N-ions, fluences of 1, 5 and 10×10^{16} ions/cm^2 (150-kV mass-analyzed heavy ion implanter)	3 plants with flowers having more petals (under 80 and 100 keV, 1×10^{16} ions/cm^2)  (a) Control. (b) Mutant
Petunia, the variety Dream Midnight Blue [26]	F_1 hybrid seeds  Seed size: ≤ 0.5 mm	60-keV N-ions, fluences of 1, 2, 4, 6, 8 and 10×10^{16} ions/cm^2 (150-kV mass-analyzed heavy ion implanter)	On average 10% plants malformed  (a) Control. (b) Mutant (under 1×10^{16} ions/cm^2)
Clitoria ternatea L., Butterfly pea [27]	 Seed size: 0.1 mm (scale bar: 20 μm) A: control, B – F: 1, 2, 4, 8, and 12×10^{16} ions/cm^2, respectively	50-keV N-ions, fluences of 1, 2, 4, 8, and 12×10^{16} ions/cm^2 (220-kV Varian ion implanter)	In M1, 1/150 mutated in dwarf character (at 12×10^{16} ions/cm^2), and in M2 and M3 (photo below), mutants drastically reduced the climbing habit with smaller leaves and flowers. 

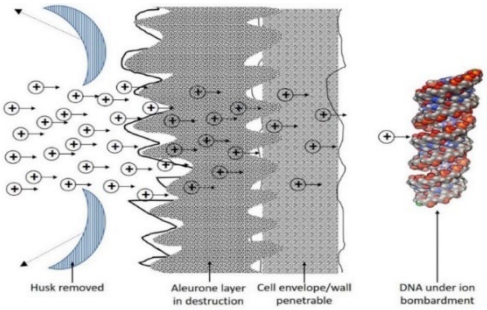


Fig. 1. Schematic (the cross section of a plant cell, including, from left to right, outside of the cell, the cell envelope and the nuclear DNA, not in scale) of the process and probability of low-energy heavy ion beam irradiation induction of mutation. ⊕→ represents an ion with its incident direction. The number of ions and the ion energy are decreasing from left to right, and down to DNA, both become ultra-small and low, due to the ion loss in the interaction with the materials in the path and the energy loss.

3.4. Ion bombardment modification of biomaterial surface for human cell attachment

One of the biological properties on the material surface is human cell attachment which is sometimes required such as in cases of artificial components but sometimes avoided such as in cases of wound dressings. Ion bombardment treatment of the biomaterial surface has been developed as an effective method for the biomaterials modification. In our experiment [47,48], the ion bombardment was operated using the PIII machine with nitrogen or argon as the working gas. The biomaterial was artificially fabricated chitosan membranes, $C_6H_{11}O_4N$. The cell lines were human skin (or dermal) fibroblasts F1544 and amelanotic melanoma cell line C32, skin cancer cells. The results showed that Ar-PIII had an enhancement effect on the cell attachment while N-PIII had an inhibition effect. The cell filopodia more liked to extend and spread on the Ar-treated surface while the filopodia had footknobs to anchor their development on the N-treated surface.

TABLE 5. A SUMMARY OF EXPERIMENTS ON LOW-ENERGY ION BOMBARDMENT OF NAKED DNA (unless *-marked that is pUC19 which is similar to pUC18 but the MCS region is reversed, all are pGFP).

Particle species	Energy (keV)	Fluence (ions/cm^2)	Facility	Main results	Ref.
(Vacuum only)	0	0	PIII chamber	Low pressures only induced SSBs but no DSBs, mainly depending on the pressure change rate but not the pressure itself; low pressures had no effect on mutation induction in DNA-transferred *E. coli*	[33]
N and Ar ions	2.5 and 5, respectively	3, 6, 9 × 10^{13}	CMU3	SSBs and DSBs increased with increasing of the fluence; lighter and active N-ions more effective than heavier and inert Ar-ions in inducing DNA changes; mutation induced in the changed DNA transferred *E. coli*	[34]
N and Ar ions	0, 1.25, 2.5 keV (bias: 0 and -2.5 kV)	0, 10^{11}, 10^{12}, 10^{13}	PIII	SSBs increased with increasing of the fluence, while no DSBs observed; in the DNA transferred *E. coli*, mutation induction only observed for biased (1.25 and 2.5 keV) PIII bombarded DNA but not for un-biased PIII bombarded DNA; DNA sequences differed in some fragments between original and bombarded DNAs	[35]
N and Ar ions and neutrals	2.5 keV	10^{11}, 10^{12}, 10^{13}	Ion beam neutralizer	Charged and neutralized ion beams produced similar amounts of DNA changes in SSBs and DSBs, but ion bombardment had more probability to induce mutation in the DNA transferred *E. coli*; heavier Ar-ions induced the bacterial mutation more than lighter N-ions did, increased with increasing of the fluence	[36]
N ions	Negative bias: 2.5, 3.5, 5, 7, 9 kV	0.5, 1, 2, 4, 6 × 10^{15}	PIII	The bombarded DNA- and *lacZ*-gene-fragment-transferred *E. coli* mutation was induced with the frequency linearly proportional to the ion energy and fluence; ion bombardment induced damage to the *lacZ* gene was identified as the dominant mutation source; the DNA mutation types were dominated by the base substitution and cytosine had the highest radiation-sensitivity	[37]*

3.5. Ion beam lithography for microfluidic biochips

Two economic MeV-ion microbeam techniques, programmable L-shape blade micro-aperture collimated microbeam and tapered capillary microbeam, have been developed for application in microfluidic biomedical device fabrications [49]. In the L-shape blade micro-aperture system [50], the combination of the movements of the pair of the blades in X- and Y-directions realizes a micro-aperture which controls the microbeam in a μm-rectangular/square shape with a size down to 1 μm in minimum. The materials irradiated included positive or negative tone poly(methyl methacrylate) (PMMA), poly(dimethylsiloxane) (PDMS) and amorphous silica (SiO_2). Some examples of applications in MeV-microbeam lithography of microfluidic devices using the L-shaped micro-aperture have been reported [50,51,52]. In the tapered glass microcapillary system [49,53,54], the tapered glass capillary tube is mounted on a holder plate whose position is adjustable by using a pair of micro-linear translation stages for alignment. In MeV-proton lithography, normally the capillary microbeam had a relatively higher beam current or intensity due to a focusing effect, while the aperture microbeam had a more homogeneous distribution of the beam intensity in the beam spot.

TABLE 6. A SUMMARY OF EXPERIMENTS ON ULTRA-LOW ENERGY ION BOMBARDMENT OF NAKED DNA, pGFP, APPLYING THE DECELERATION LENS.

Ion species	Ion energy (eV)	Fluence (ions/cm^2)	Main results	Ref.
N_2^+	64, thus 32 for final N^+	10^{15}	DSBs induced	[38]
Ar^+	242, 304, 407, 510	10^{15}	SSBs induced only, but no DSBs observed	[39]
Ar^+	50, 100, 300, 750, 1000	1, 2, 4 × 10^{15}	SSBs and DSBs induced. The induction of the DNA form changes was ion energy and fluence dependent: the ion energy and fluence thresholds for DNA change from the supercoiled and relaxed forms to the linear form were 750 eV for 2×10^{15} ions/cm^2, or 1 keV for 1×10^{15} ions/cm^2, respectively	[40]
He^+ N^+	100, 300, 1,000, 1,500; 26, 52, 100, 400, 600, 2,000	1, 2, 4 × 10^{15}	The ion energy and fluence thresholds, in terms of the areal energy density, to induce DNA DSBs were found to be 300 eV/Å^2 and 100-150 eV/Å^2 for He- and Ar-ion (from the result obtained from the study shown in the row above) bombardments, respectively, and lower for active N-ions	[41]
C^+	50, 100, 300	1, 2, 4 × 10^{15}	DSBs induced, depending on the ion energy and fluence. The threshold of C-ion beam conditions for DSB occurrence was found to be a minimum energy of 50 eV with fluence 4×10^{15} ions/cm^2, or a minimum fluence of 1×10^{15} ions/cm^2 with ion energy 100 eV or higher	[42]

3.6. Ion beam analysis of biological living materials

We mainly applied particle induced X-ray emission (PIXE) to analyze various biological samples. We used macro beam for measurements of the elemental concentrations of rice grains including ion-beam-induced mutants, human blood, medicinal plant tissues including roots, stems and leaves, and human cardiac muscle, etc. [55], while using capillary microbeam for elemental concentration mapping of plant leaves, particularly focusing on the crossing area between the veins and laminas [56]. Cooperated with Kyoto University, Japan, we also carried out secondary ion mass spectrometry (SIMS) analysis of bio-macromolecules (see next part).

3.7. SIMS (Secondary Ion Mass Spectrometry)

Preliminary work on conceptual designing and construction of SIMS systems was carried out for analysis of biomolecules at CMU. There were two versions of the ion mass separation of the spectrometer proposed based on the time-of-flight (ToF) technique [57] and the quadrupole mass analyzer [58]. In SIMS analysis of biomolecules, we cooperated with Kyoto University utilizing their home-developed combined gas-cluster ion beam (GCIB) + quadrupole-ToF (Q-ToF) tandem mass spectrometer (MS/MS) SIMS system, or GCIB-Q-ToF (MS/MS) SIMS [59]. In the experiment, GCIB bombarded large biomolecules and MS/MS SIMS analyzed produced molecular fragments [60]. The biomolecules were DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine, C44H88NO8P, monoisotopic molecular weight 789.6 Da), angiotensin II (C50H71N13O12, monoisotopic molecular weight 1045.5 Da) and leucine enkephalin. The secondary ion products from these biomolecules under Ar-cluster ion beam (Ar_{2000}^+) bombardment at energy of 10 keV were investigated. The protonated molecular ions $[M+H]^+$ at high masses and fragment species were observed. The secondary ion yield (SIY) tendencies of the dissociated DSPC, angiotensin II, and leucine enkephalin ions as a function of the collision energy were studied.

3.8. Cyclotron for medicine

The objective of the PET/CT & Cyclotron Center, CMU (PCCCMU) is to provide high standard healthcare technology to patients in Thailand's northernmost regions, and to deliver improved diagnoses and therapeutic treatments for cancer and other chronic diseases like cardiac and dementia. In addition to capacity building, we have established a new 200-m^2 PET radiopharmaceutical laboratory, complete with a 20-MeV cyclotron, five hot cells for PET radiopharmaceutical synthesis, a solid target for Cu-64, I-123 and I-124. The cyclotron contributes in two main directions to radiopharmaceuticals for diagnostic services and research. Table 7 lists the isotopic radionuclides and radiopharmaceuticals produced at PCCCMU with their relevant properties and applications [61].

3.9. Single ion irradiation of cells

In cooperation with the Surrey Ion Beam Centre (SIBC), we used the SIBC nanobeam line to carry out single ion irradiation of cells [62]. Chinese hamster V79 cell line was used as a human cancer cell substitute. The ion was 3.8-MeV proton and both single ion and broad beam irradiations were adopted for comparison. After irradiation and cell incubation, the number of surviving colonies as a function of the number of the irradiating ions was measured for the cell survival fraction curve. A lower survival for the single ion beam irradiation than that of the broad beam case was observed, implying the hypersensitivity for the cell to respond to ultra-low dose and bystander effect, which was lacked in the broad beam irradiation.

TABLE 7. ISOTOPIC RADIONUCLIDES AND THEIR RADIOPHARMACEUTICALS PRODUCED AT PCCCMU AND RELEVANT PROPERTIES AND BIOMEDICAL APPLICATIONS. β^+: positron emission. EC: electron capture.

Isotopic radio-nuclide	Target material	Nuclear reaction	Ion energy (MeV)	Half-life	Decay mode (% proportion)	Radio-Pharmaceu-tical	Examples of biomedical applications
^{18}F	O-18 enriched water (^{18}O-H_2O)	$^{18}O(p,n)^{18}F$	3-16	110 min	β^+(97), EC(3)	^{18}F-FDG	Glucose metabolism
						^{18}F-PSMA-1007	Prostate cancer
						^{18}F-NaF	Bone scintigraphy
^{13}N	H_2O+5mM ethanol	$^{16}O(p,\alpha)^{13}N$	7-16	9.97 min	β^+(100)	^{13}N-Ammonia	Myocardial perfusion imaging (MPI)
^{11}C	N_2 + 0.5%O_2	$^{14}N(p,\alpha)^{11}C$	3-13	20.4 min	β^+(100)	^{11}C-Methionine	Amino acids metabolism
						^{11}C-Choline	Biosynthesis of phospholipid
						^{11}C-Acetate	Cell oxidative metabolism, prostate and liver tumors
^{15}O	N_2	$^{14}N(d,n)^{15}O$	0-8	122 sec	β^+(100)	^{15}O-Water	Myocardial perfusion imaging (MPI)
^{124}I	$^{124}TeO_2$ + 5%Al_2O_3	$^{124}Te(p,n)^{124}I$	9-14	4.18 days	β^+(22), EC(78)	^{124}I-NaI	Thyroid diagnostics

4. SUMMARY

Ion beams and accelerators at CMU have been vigorously applied in biology-oriented fields, covering genetics, biomaterials, biomedicine devices, biological analysis, and medicine, etc. Our experiments and research have demonstrated significant potentials of ion beams and accelerators in service of something more related to human lives and thus socioeconomics.

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