Post-focus expansion of ion beams for low fluence and large area MeV ion irradiation: scaling from the single-event to the system level in human brain tissue and electronics devices.

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Abstract

Irradiation with $\sim$ 3 MeV proton fluences of $10^6 - 10^9$ protons cm$^{-2}$ have been applied to study the effects on human brain tissue corresponding to single-cell irradiation doses and doses received by electronic components in low-Earth orbit. The low fluence irradiations were carried out using a proton microbeam with the post-focus expansion of the beam; a method developed by the group of Breese [1]. It was found from electrophysiological measurements that the mean neuronal frequency of human brain tissue decreased to zero as the dose increased to 0 to 1050 Gy. Enhancement-mode MOSFET transistors exhibited a 10\% reduction in threshold voltage for 2.7 MeV proton doses of 10 Gy while a NPN bipolar transistor required $\sim$800 Gy to reduce the $I_{FE}$ by 10\%, which is consistent with the expected values.

Keywords: MeV broad beam ion irradiation, Neurospheres, MOS-devices, Bipolar-devices

1. Introduction

Complex systems such as vertebrates, automobiles, computers, satellites, mobile phones etc can be thought of as being made up of a large number of smaller communicating functional units. For example, different cell types (basic biological units [2]) are organized into different tissue types which in turn make up the organs of vertebrates [2]. MeV ion and nanobeams are very powerful tools for study of single events effects in individual units [3, 4]. Table 1 presents the fluences for impingement of an average of one ion per unit for different size units. (Here ”units” is taken to mean the basic building blocks of the system, e.g. biological cells or devices in a microcircuit.) The fluences in Table 1 should be considered as an approximate guideline because the average fluence to disrupt a system may be higher or lower depending on if the units are packed in 2D (one ion penetrates one unit) or 3D (where one ion can penetrate a number of units), the cross-section for disrupting a single unit and the system sensitivity due to disruption of a single unit. There is no \textit{a priori} reason why scaling from a single disturbance in a single unit to disturbances in an entire system is linear. This is because processes such as DNA repair mechanisms and error-correcting algorithms may come into play. It should also be borne in mind that in a complex system, the ion induced damage may only be detrimental after a considerable period of time. (e.g. The evolution of a cancer tumour, or when corrupted data stored in disrupted memory cell is read-out and used.) Hence, it is not a reliable approach to determine the frequencies of different disruption modes of a complex system directly from single event studies, instead tests on either the whole, or a representative part of a complex system must be made.

MeV ion micro/nanobeams are serial scanning instruments. This means they are too slow to deliver fluences corresponding to single events in a unit over of an entire system consisting of $10^6-10^{12}$ units in a reasonable time ($\sim$1000 s). The conventional approach for system-level studies in both electronic and radiobiological studies is to use scanned or broad high energy ion beams of 10-150 MeV which can penetrate overlying biological tissue or encapsulation and circuit boards. The cost of operating these facilities is very high and generally ac-
cess for testing is low due to competing experiments. Lower energy scanned broad beam Mev ion irradiation systems such as used for ion implantation typically deliver current fluxes of 1 μA cm² s⁻¹. This would require just 1.6 ms to deliver a fluence of 10⁸ ions cm². It is difficult to reduce the ions source current and accelerator system transmission by the sufficiently large factors which are needed to perform the irradiation within a reasonable time (1-1000 s). Furthermore, slow (magnetic) scanning speeds may result in only part of the system being irradiated at such short exposure times. These cannot then be used to test disruption mechanisms due to correlated impacts. Such time-correlated disruptions may originate from particle showers.

The group of Breese[1] have developed a broad-beam MeV ion irradiation technique for line and area irradiations based on expanding the ion beam after the conjugate focus in a MeV micro/nanobeam. Focused beam spots of 1 pA to 1 nA beam current can easily be produced in a micro/nanobeam. The ion fluxes after allowing a 1 pA beam to diverge behind the conjugate focus to 1 cm² area are on average 6.3×10⁶ ions cm⁻² s⁻¹ which are well suited to irradiation with fluences at the 10⁸ ions cm² level. A special feature of the low MeV-energy ions, such as protons available with this technique is that they have closely similar energies to the energy with which the higher energy ions used in conventional radiation damage studies arrive at the Bragg peak. The excess energy in conventional experiments is dissipated in traversing overlying material such as overlying tissue or circuit boards and encapsulation.

Here we report work using the post-focus expansion method for study of proton irradiation of neurospheres (3D cohorts of neural cells) and developing qualification of highly integrated components-of-the-shelf (COTS) electronics for near-Earth orbit nanosatellite missions we have developed a system based on the beam expansion optics.

2. Experimental Method

The Oxford Microbeam system attached to the 1.7 MV Tandetron accelerator at Haute Ecole Arc Ingénierie, La Chaux-de-Fonds, Switzerland was used for this study. 2.7 and 3 MeV proton beams were used. A drift section consisting of an extension tube with a KF-32 connection on one end, allowed an end-piece with a either a fluorescent screen or a 1 mm ×1 mm and 200 nm thick Si₃N₄ radiation window to be mounted after the drift length. A microfluidic cassette (Fig. 1) for neurosphere irradiation or a circuit board for testing electronic devices could be mounted behind the radiation window. (The energy loss in the radiation window and a mm or so of air between the exit window and sample was negligible compared to stopping in the sample.) The compact size of the set-up allowed the irradiation to be performed in a normal laboratory environment with the external beam enclosed by a PMMA box with door and i.r. motion interlocks that shut down the accelerator to prevent exposure to the external beam. In addition precautions were taken to limit n- and y-ray personnel exposure from the 2.7 and 3 MeV proton beam striking the apertures [5].

The fluence Θ was determined by measuring the beam current before and after each exposure from a metal sample moved into the beam focus and calculating the exposure time using a factor for the beam expansion determined from the WINTRAX code [6] and verified using an internal fluorescent screen. The use of this approach allowed the different objective and collimator sizes to be taken into account. The average dose D in a sample of density ρ and thickness t is then:

\[ D = \frac{\Theta}{\rho} \int_0^t \frac{dE}{dx} dx \]  

(1)

In the limit of small t, this reduces to:

\[ D = \frac{\Theta}{\rho} \frac{dE}{dx} \]  

(2)

The stopping forces in equations (1) and (2) were obtained from the SRIM code [7, 8].

2.1. Ion irradiation of neurospheres

The neurospheres were neural progenitors derived from induced pluripotent HIP stem cells, (from MTI-Globalstem Inc. USA). These were cultivated at an air-liquid interface on porous growth substrate (PTFE membrane) to generate a 3D brain tissue-like structure containing a few thousand cells. Human cells were used for the study because animal models may exhibit significant physiological differences to humans. Moreover, in related work (not reported here) it was found glioblastoma cells (GE904) could be co-cultivated with HIP to create neurospheres with normal and glioblastomer cells representative of cancerous tissue.

The microfluidic cassettes used for irradiation are shown in Fig. 1. The permeable growth substrate was mounted in the plexiglass cassette so that growth media and drugs such as oxygen permeation promoters could be flowed to feed the neurosphere using standard disposable syringes. The cassette fits over the end of the
tube end with radiation radiation window so the neurosphere was separated by a 1 mm air gap from the radiation window. The neurospheres were cultured and analysed in Geneva and irradiations performed in La Chaux-de-Fonds. During the 2 h journey the cassettes were kept in an insulated food box at 37 °C. It was found that careful pH control using a buffer was critical to maintain viability during the travel and irradiation steps. To characterise the viability using fluorescence markers, insults of different doses of 3 MeV protons were delivered to a series of neurospheres. The neurospheres were cultivated for 5 days before staining with Hoechst, Propidium Iodide and Calceine-AM to mark DNA, dead cells and living cells, respectively. Longitudinal studies were carried out by delivering insults of different radiation doses to a second batch of neurospheres. These were cultured for 30 days and subsequently transferred to multi-electrode array substrates attached to USB wireless chips to measure the electrophysiological activity[10, 11].

2.2. Irradiation of MOS and bipolar transistors

In this preliminary study, two types of transistor were investigated to validate the method: a Si enhancement mode n-channel MOSFET (2N6660) and a bipolar Si NPN transistor (BC107). The MOSFET and NPN devices were chosen as the die could be easily exposed by removing the metal can and they required relatively low voltages to operate that were compatible with our measurement system. A programmable microcontroller was used to generate test voltages and digitise the currents and voltages. A Matlab program running on a host computer allowed programmable measurement and registration of the output characteristics of the devices via the USB interface. In this way $I_d$ vs $V_{sd}$ curves were measured for a series of $V_{gs}$ voltages of the MOSFET and $I_c$ vs $V_{ce}$ curves for different $I_{be}$ currents for the NPN transistor. The time to measure a complete set of characteristics was about 250 s. Irradiations of each device were carried out in a series of dose steps. The output characteristics were measured before and after each dose step.

3. Results and discussion

3.1. Ion irradiation of neurospheres

In a first set of experiments a series of neurospheres were irradiated to different doses, cultured for 5 days and stained with Hoersch for DNA (blue), Propidium Iodide (red) for dead cells and Calceine-AM (green) for live cells. Fluorescence microscopy images (not shown) revealed that the cells survived for many times the acute dose (<50 Gy) required to produce deterministic effects in the cerebrovascular system (CVS) in humans [12]. Moreover, no systematic increase in cell death was observed with increasing dose. The underlying cause was most probably that the neurosphere was coated with a thin film of cell growth media of uncontrolled thickness which was sufficiently thick to stop the ions delivering the dose to the whole neurosphere.

In a second set of experiments the electrophysiological response of the neurospheres was investigated. To minimise dose absorption by excess growth media, the neurospheres were swabbed free of excess media prior to irradiation in the cassette. After the irradiation the neurospheres were transferred to multi-electrode array (MEA) substrates with WiFi readout and cultured after irradiation. The WiFi chip enabled the electrophysiological signals to be logged to a computer without the need for troublesome electrode connections. Fig. 2(a) presents the electrical signals from the MEA electrodes with time for an unirradiated neurosphere. The electrophysiological activity of the neurospheres shows up as spikes of a few ms duration that are characteristic of human brain activity[13]. Some representative neural spikes are shown in detail in Fig. 2(b). Using spike recognition software a raster spike plot (Fig. 2(c)) was produced which shows the time of each spike occurred. The advantage of using electrophysiological monitoring is that longitudinal study of individual neurospheres exposed to different radiation dose insults could be followed in time. This is not possible with fluorescent imaging because the stains used are cytotoxic and kill the neurosphere. Fig. 2(d) presents the mean neuronal frequency with radiation dose. It is seen from Fig. 2(d) that considerable reduction of the neuronal activity by ∼50% occurred for an acute dose of 300 Gy. Complete loss of electrophysiological activity was achieved for an acute dose insult of 800-1050 Gy. For humans, acute radiation doses > 15 Gy to the CVS leads to rapid unconsciousness [12]. The current results show the method can be used to investigate the neuronal response of neural tissue and how this relates to clinical radiation exposures. This type of study, which used standard human cell lines has the advantages that it overcomes problems associated with the difference in physiology between animal and human models. Moreover, because it does not require the use of animals or human subjects and allows longitudinal studies, it permits questions to be studied experimentally that would otherwise be impossible on ethical grounds. and/or need a large number of subjects. These include how does the presence of normal and cancerous tissue affect the acute radiation
3.2. Irradiation testing of electronic devices

Fig. 3(a) and (b) show the radiation response of the two transistor types. In the case of the MOS transistor (Fig. 3(a)) a 10% reduction in threshold voltage, (defined as the fitted corner voltage in the $I_d$ vs $V_{ds}$ output characteristic curve) occurs for an exposure at 10 Gy. A rule of thumb is this dose corresponds to two years of exposure in low Earth orbit. Although solar activity can increase this by a factor of up to ~50x. The threshold shift is most probably due to irradiation induced formation of charges in the gate-oxide which makes MOS devices mainly sensitive to the total ionizing dose (TID).

A 10% reduction in $h_{fe}$ for the bipolar transistor required a much higher dose of ~800 Gy (Fig. 3(b)). Radiation damage in bipolar devices is mostly caused by formation of electrically active defect centres in the depletion regions by atomic displacement (AD) damage. Since the latter depends on nuclear energy deposition from Fig. 3(a) and (b) that the method described is able to probe both TID and AD induced component damage. The ability to work with proton beams of a few MeV is very significant because the the cross section for AD production for these low energy protons is much higher than for the commonly used 30-100 MeV proton beams. The much higher operation costs (about 5-15 times) of the large (30-150 MeV) accelerators compared to the low MeV energy machines used with MeV ion microbeams as well as much greater access gives major cost and time advantages for the present method.

3.3. Potential improvements

The dose is critically dependent on the beam current passing through the Si$_2$N$_4$ aperture. Here it is based on measurement of the current from a metal sample in the beam focus combined with WINTRAX simulations and the degree of beam expansion checked with a fluorescent screen. The uncertainty is estimated to be ~±35% and mostly due to the uncertainty in secondary electron yield. This could be improved by directly measuring the ions using a charged particle detector behind a small aperture that can be positioned in place of the electronic device or tissue irradiation cassette.

Irradiation of large areas (1–50 cm$^2$) that may release volatile compounds that would contaminate the microbeam (e.g. circuit boards) could be achieved by mounting them in a chamber placed behind the microbeam focus and using the Si$_2$N$_4$ window placed close behind the conjugate focus to separate the clean microbeam vacuum from the dirty irradiation vacuum.

4. Conclusions

A technique based on post-focus beam expansion in a microbeam for irradiating biological tissue and electronics components with MeV proton fluxes in the range $10^6$ – $10^9$ ions cm$^{-2}$ s$^{-1}$ has been investigated. Proton irradiation induced changes in the electrophysiological activity of neurospheres has been demonstrated. The method has also been shown to be applicable to study proton irradiation of MOSFET transistors where a change in threshold voltage was observed as well as a reduction of the $h_{fe}$ of a NPN bipolar transistor. The method expands the range of application of microbeams from single-ion studies to study of entire systems of biological cells and electronic microcircuits. Furthermore, the use of low-MeV energy ions gives the method described major cost and time advantages compared to conventional broad-beam approaches. The accuracy of the delivered radiation dose could be improved by directly counting the ion flux using a charged particle detector and the method can be simply adapted to irradiate large areas of material that out-gas without degrading the microbeam vacuum.

Acknowledgements

The work reported was financed in part by the HESO RadioBeam project ref. 38396.

[3] M. Iakšič; Single ion microprobe techniques, current status and perspectives. (This conference).


Figure 1: Schematic illustration of the neurosphere microfluidic irradiation cassette. (a) Schematic section. (b) General view.
Table 1: Fluences for irradiation of the basic building units of typical complex biomedical and electronic systems with an average of one ion hit per unit.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Typical area</th>
<th>Fluence (ions cm(^{-2}))</th>
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<tbody>
<tr>
<td>Small transistor</td>
<td>1000 nm(^2)</td>
<td>(1 \times 10^{11})</td>
</tr>
<tr>
<td>Large transistor</td>
<td>3 µm(^2)</td>
<td>(3.3 \times 10^{7})</td>
</tr>
<tr>
<td>Cell nucleus</td>
<td>25 µm(^2)</td>
<td>(4 \times 10^{6})</td>
</tr>
<tr>
<td>Eukaryote cell</td>
<td>100 µm(^2)</td>
<td>(1 \times 10^{6})</td>
</tr>
</tbody>
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Figure 2: Electrophysiological data (a,b,c) Spontaneous activity in neurons from a control 3D culture. (d) Decrease in mean neuronal frequency with average dose \(D\).
Figure 3: Effect of 2.7 MeV proton irradiation on: (a) enhancement mode n-channel MOSFET, (b) NPN bipolar transistor.