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DESIGN AND MODELLING OF CELL IRRADIATION WITH LASER-DRIVEN PROTONS

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ABSTRACT

The biological effectiveness of laser driven protons on cells in a single exposure has been studied. In this experiment, V79 cell lines were irradiated with laser driven protons whereas comparison with the effectiveness of X-ray exposures was studied. Here design of the experiment and irradiation setup are explained in details as it is a first and foremost experiment in this field.

Keywords: *Radiobiological studies; Laser driven protons*

1. INTRODUCTION

The study of intense laser-plasma interaction has evolved over a number of decades into many inter-related sub-fields of research. Acceleration of ions by laser is a highly focused area of research and this area is encouraged by the outstanding development in laser technology. The observed accelerated proton beam shows interesting properties in terms of ion energy, divergence and energy scaling, and offer high promise for progress in the future. High power laser systems providing pulse rates on target of a few pulses per minute in the field of laser ion acceleration, has brought medical applications such as ion therapy of cancer closer into reach. Even though these compact laser systems cannot provide the proton energies needed in radiation therapy yet, they display the tool for preparatory radiobiological studies with laser-accelerated protons.

Over the recent years, novel field of laser-based particle acceleration has made a tremendous progress. Due to these developments, a large number of authors have proposed different type of application of the laser-based particle acceleration as a potential alternative accelerator technology in ion radiotherapy. A number of potential applications have been proposed for modern laser-driven ion accelerators [1-2]. They are often cited as a possible replacement for, or at least, as a complementary technology to, conventional particle accelerators, with obvious advantages including their reduced size and cost. Bulanov et al [3-4] discussed the feasibility of laser-ion accelerators in proton therapy. Several advantages for using lasers in terms of cost and compactness were discussed. Martin [5] also discussed the potential of laser accelerator as most promising technique in future for proton beam therapy. He termed laser accelerated proton beam as clinically and financially safe. A large number of authors [2, 6-10] have shown the laser acceleration promises innovation in particle beam therapy of cancer. Studies dealing with simulations and proposals to

improve the characteristics of proton acceleration have been published over the last decade[3, 11-13]. Beam transport system for medical application [14, 15-18] have also been discussed. Even simulations with laser-accelerated protons [19-21] have been carried out which shown treatment planning. Besides the improvements proposals of proton acceleration and simulations, for practical purposes, the design clinically-applied laser particle accelerators [22] have been studied. In spite of the great keenness evoked in this field and in the medical physics community, significant future progress needs to be achieved before clinical application of laser accelerated particle can be considered. Some publications [2,8, 23-24] have been focusing on the development of irradiation equipment, the stable and reliable irradiation of patients with prescribed doses while addressing patient safety.

The real-time physical and dosimetric characterization and the investigation of the biological effectiveness of laser accelerated particle beams are necessary. To date there have only been very few experimental studies [25-28] focusing on these issues. All of the experimental studies [25-28] to date, with laser accelerated protons, cell samples were irradiated with different doses, by applying several numbers of laser-accelerated proton pulses i.e., by accumulating shots on the cells. There was no quantitative study of the biological effect of acute proton dose on cells in a single exposure before [29]. In this paper, the method, design of the experiment of irradiating cells with laser driven proton in a single exposure is presented along with the cell handling method.

2. EXPERIMENTAL METHOD AND DESIGN OF IRRADIATION SET UP

The cell irradiation experiment was carried out at Queen's University of Belfast (QUB) by using the multi-Terawatt chirped pulse amplification (CPA) laser system. This system named TARANIS is a Ti:Sapphire-Nd: glass laser working at a wavelength of 1053nm with a pulse duration of 700fs and beam energy up to 20J. The laser is focused by a f/3, f=300mm off-axis parabola (OAP) leading to an intensity on target ~2×10^{19} W/cm^2. The system performance in producing laser-driven proton beams was explored by using thin metal foils while focal spot was around 10 μm. In this scenario, the protons are created by Target Normal Sheath Acceleration mechanism (TNSA) as described below.

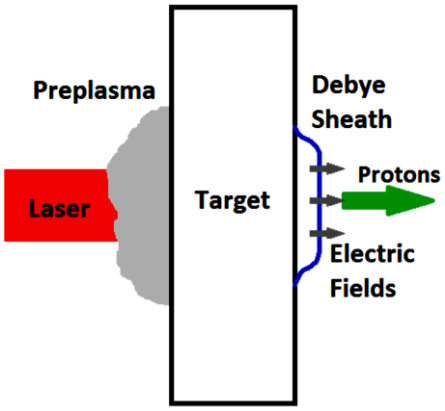

Fig. 1. Target Normal Sheath Acceleration mechanism.

In this process (Figure 1), the energy of the laser is transferred to the target when the target strike by an intense laser (10^{18} W/cm^2). In the target the electrons accelerate faster than the protons due to its lighter mass and achieve greater speeds. Some of the electrons escape off the back of the target. So, the target becomes positively charged and the electrons that escape will stay on the back target, creating a layer named as Debye Sheath. The electric force attracts protons and accelerates the protons off of the back of the target. As the electric force is created by the electrons, his accelerated protons are collimated [31].

2.1 Setup for Irradiation

A setup for radiobiological experiments with these accelerated protons must have a beam filter that suppresses background radiation as well as controls the spectral selections of the proton beam, which means the low energy tail of the proton spectrum must be filtered out. Keeping all these points in mind, Figure 2 shows a simple sketch of the setup of the magnet that has been used to angularly disperse the laser driven protons of different energy into different angles. The position of the Mylar window and the placement of cells outside the chamber have also been shown in this figure. The values of different parameters in the setup are as follows: distance between the target and slit (proton source) = 10 mm; diameter of the aperture (slit size) = 500 μm; slit and magnet distance, 7 mm; the magnetic field is 0.9 Tesla and uniform; length of magnets = 100 mm; separation between magnets = 5 mm.

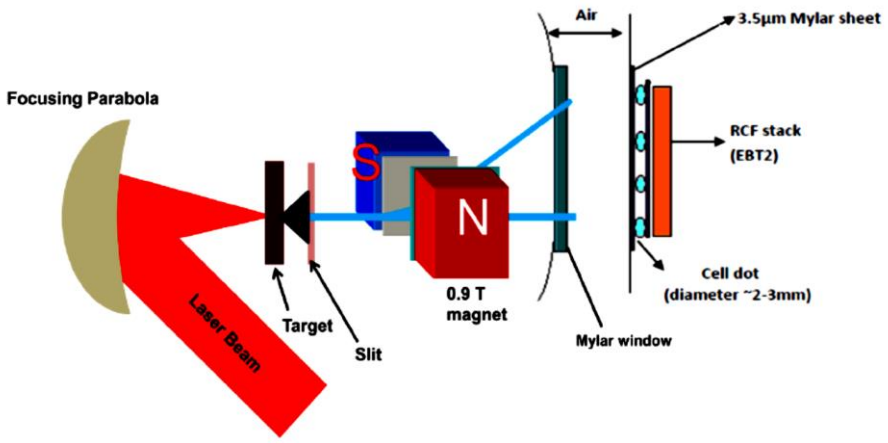

Fig. 2. A view of basic set up inside and outside of the chamber.

A dipole magnet was placed 10 mm behind the slit to deflect the protons. The magnet disperses the protons according to their energies prior to the cell irradiation. A 50 μm thick Mylar window of vacuum chamber stopped protons lower than 2 MeV in energies from exiting the chamber. The window to the magnet distance was 150 mm while cell dish was placed 1 cm from the window. The deflection angle of 10 MeV protons with respect to the direct source-aperture line-of-sight is

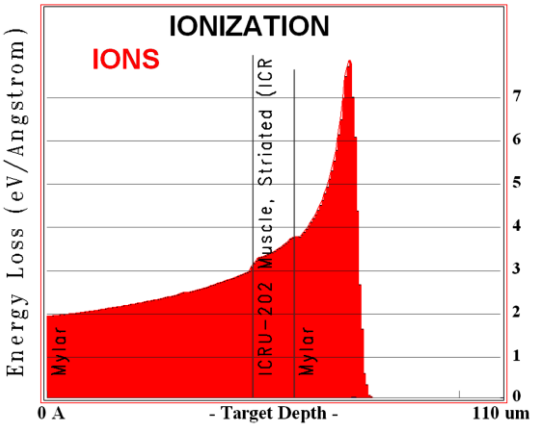

Fig. 3. SRIM simulation for 2.3 MeV protons with Mylar sheet of thickness 50 μm. After passing through the Mylar proton energy on the cells is 1 MeV.

about 14° which helped to avoid cell irradiation by unwanted X-ray radiation from the target. Before starting the experiment in this set up, different settings have been used in the TRIM (Transport of Ions in Matter) [32] simulations depending on the energy resolution and some other constraints such as the desirable dose released by proton on a single cell. From the simulation result (Fig. 3), we know that the cells can be irradiated by 1 MeV protons, when 2.3 MeV protons leave the vacuum chamber through a 50 μm thick Mylar window. Simulation results help us to avoid the Bragg peak (to have a better control over the dose on the cell) being on the cells during the experiment. The magnet deviated the beam from the right (high energy protons) to the left (low energy protons), so the deposited dose on the cells increases from the right to the left. Due to this energy and dose difference, the cells were placed on a cell dish at six different locations; each location was described as cell dot. The diameter of each cell dot was 2- 3 mm. The cell dish was placed vertically behind the mylar window. A thin Mylar film of thickness 3.5 μm covered all the cell dots. EBT2 (Radiochromic film) RCF stack with the active layer facing the proton beam radiating the cells was placed after cell dish.

In the experiment a stack of 5 films has been placed at about 1 cm behind the cell dish along the beam line, for each shot. The degree of darkness is related with the amount of energy deposited in that layer. Irradiated stacks will always be darker on the first film with large number of protons of low energy depositing all their energy on the film and lighter on the last film with few protons with high energy (the dose response of RCF film has already discussed in another research paper, ref. [30]). As already mentioned, that the magnet deviated the beam from the right to the left, so the deposited dose on the first film of the stack increases from the right to the left (see Fig 4), we have the maximum dose for the lowest energy protons that reach the first film and are stopped inside it and the minimum dose for the highest energy protons that lose energy crossing the stack and are stopped in the last film. The 29 MeV monoenergetic cyclotron beam from the University of Birmingham [30], had been used to calibrate the radiochromic film. In addition, simulations with monoenergetic proton beams were also performed by the University of Birmingham to discover the energy edges (the lowest energy of protons that can deposit dose in a given layer) for the EBT2

stack. The experiment has been simulated with Fluka simulation, which is a Monte Carlo integral simulation package for the interaction and transport of particles and nuclei in matter [33]. To obtain the minimum initial proton energy needed to darken a film, the mylar window with thickness 50 μm and the cell dish with a 3.5 μm mylar film were also simulated because the protons lose energy while passing through them.

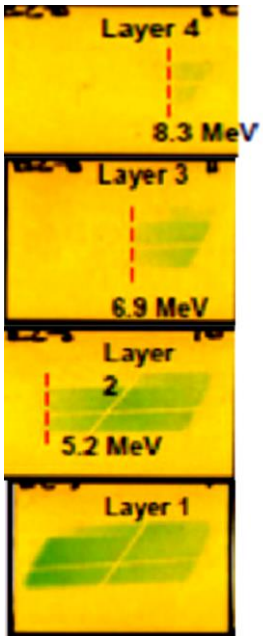

Fig. 4. Traces left on different layers of EBT2 by proton beam.

2.2 Procedures Associated with Cells

In the 19th century, the concept of separating the living cell lines from their original tissue source and maintaining them for a long time was discovered [34]. It became possible to place cells in tissue culture dishes or flasks for growth in an appropriate complex growth medium by the application of modern techniques of tissue culture. Culturing a particular cell line is a complex process where cells are grown under controlled conditions. With temperature maintained at 37 °C and CO_2 level at 5% in an incubator, the cells grow and divide by multiplication method. At this point the cells are attached to the surface of a tissue culture flask and form a rod like structure. The conditions for culturing vary widely for different cell types. Temperature, the growth medium with different pH and the essential nutrients are the factors which differ in culture systems.

Cell culture can be done in two steps: suspension and adherent cultures. Some cells require surfaces for growth and differentiation (adherent cultures) while others naturally live without being attached to a surface (suspension). A method of transferring a small number of cells into a new flask is called

Splitting (also called passaging). It is easy to passage the suspension culture by diluting a small number of cultured cells in a bigger volume of fresh media. On the other hand, the cells are detached from the surface for adherent cultures using trypsin-EDTA mixture (an enzyme that detaches cells from surfaces). Then a small number of detached cells are diluted in fresh media for a new culture. In order to avoid contamination with yeast and bacteria, sterile techniques are used. A biosafety hood was used to be safe side from contamination. The cell line used for this experiment is V79 (Chinese Hamster lung cancer cells) with the width of the cells about 10 μm diameter and thickness about 4-10 μm. This is a widely used cell line for irradiations as it is easy to culture (i.e., to grow in the laboratory) and handle and is highly resistive to contamination. V79 cell has been used for different kind of biophysical experiments, using a conventional accelerator [34-38]. The media used for the cells was EMEM/DMEM(Dulbecco's minimum essential medium) (500 ml) containing Fetal Calf Serum (10%), Penicillin/Streptocyllin (1%) and L-glutamine (1%) added in it. A buffer solution DPBS (Dulbecco's phosphate buffer solution) containing 10% versane, was used to wash the cells. The steps of cell culturing are given below:

The flask was taken out from incubator and media from the flask was poured off and the cells were washed with DPBS. 1% trypsin (2 ml trypsin in 18 ml versene) was added to it (trypsin was used to detach the cells from the surface of flask) and the flask was left over for 3-4 minutes in the incubator. Afterwards 5 ml of media was added and this solution was transferred to a universal tube. Now the solution was centrifuged for 5 min at 1300 RPM, as a result a cell pellet was formed at the bottom of tube. Media was poured off and the cell pellet was re-suspended in 4 ml of fresh media. The cells from this suspension were taken out and placed inside the culture flask. This culture flask was then placed in the incubator so that cell can divide (to grow or to multiply the cells) (for e.g., 150 μL of cells in 8 ml of media for 3 days growth and 2 ml of cells in 8 ml media for next day growth). The cells left over suspension in the universal tube were used to prepare the cell dishes for the experiment. The cell dish used for placing the cells is shown in Fig. 6. The details of how the cell dish was prepared are as follows:

2.2. a. Preparing a cell blanket

A thick Mylar sheet of thickness ~200 μm was used to prepare a cell blanket. The transparent sheet was placed tightly between the metal plates of the cell dish to avoid any leakage of media. 2 ml of the cell solution (cell after culturing) was placed on the sheet and flooded with media. This was left overnight (8-10 hours) in the incubator to grow and attach on the thick Mylar sheet. This sheet is referred as the blanket of cells. The next day, a uniform layer (uniformity is checked by confocal microscope) of attached cells was observed over the Mylar sheet. The uniformity can be also checked with the naked eye. Cells were counted by using a hemocytometer (Fig. 5).

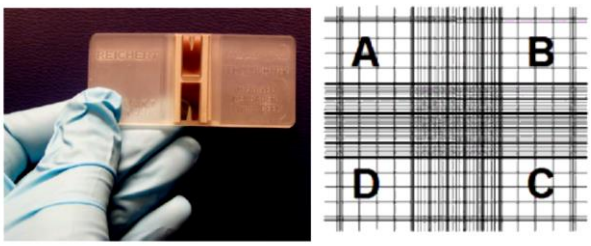

Fig. 5. View of hemocytometer (Left) and view of its section under the microscope (Right).

A haemocytometer is a device carefully crafted with 16 squares in which the depth of the chamber and the area bounded by the lines is known. The number of cells or particles per ml can be found by simply multiplying the total number, found in the haemocytometer grid, by 104 and thus it is possible to calculate the overall concentration of cells in the fluid. The blanket was then removed from the cell dish and the outer edges were cut carefully. The blanket was then placed carefully inside a container full of media. The cell dish used for placing the cells is shown in Figure 6. The details of how the cell dish was prepared are as follows:

2. 2. b. Placing cell dots on dish

A thin strip (~4 mm wide) was cut from the blanket using sterile scissor. A cell dot of diameter ~2.5 mm was cut from the thin stripe using a single hole hand puncher. It should be noted that the flathead of the puncher must be pressed against the side with no cell layer. Puncher was always sterilized to avoid contamination. It takes 8-10 minutes for the cells in the cell dot, to be dry. The cell has therefore been cut from a wet stripe of blanket and processed further immediately. In this way six cell dots were cut, among them two were used as a control dot (which were not irradiated by protons). After cutting the cell dots, the remaining stripe of the blanket was stored in the same container with the rest of the blanket. The piece of blanket was separated just enough from the stripe to avoid scratching the cell layers with one another's edges. In this way, about 20 dots can be taken from one blanket. Before cell irradiation the first dot taken from the blanket was kept as a reference and the cells were counted from it with the procedure explained below.

2. 2. c. Preparing for the shot

After cutting from the blanket the cell dot was immediately placed in 50 µl of 1% trypsin and left for 5-7 minutes so that cells can be detached from the blanket sheet. Afterwards 100 µl of media was added to it. In order to avoid any clumps, the cells solution was properly mixed with a pipette. The cells in this 150 µl solution are counted using the haemocytometer over four different squares of 1 mm^2 area (as shown in fig 5). An average of four counting was taken as a final figure. Thus, the dilution figures were calculated for making the wells for colony growth after irradiation. For example, 10 µl solution taken out from this solution gives average counts to be 65. This means there are $65x10^4$ cells/ml in 150 µl solution which gives ~9.75×10^4 cells in one dot. If 500 µl is the final solution with a dot, then for placing 1500 cells, ~7.6 µl of cell solution should be diluted in 10 ml of the media.

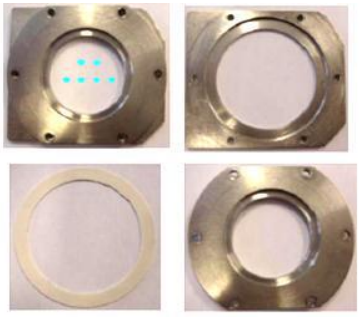

Fig. 6. The metal plates of the cell dish used, in sequence (clockwise): lower plate of the dish, upper plate of the dish, gasket in between the two parts and all the parts together, blue circles show the positions of cells.

Hence, the dilution procedures after irradiation, using cell dots from same blanket were: The counting has done for every sample individually. A cell dot was placed in 50 µl of Trypsin in Eppendorf (a small plastic tube, can contain 2 ml liquid), and 450 µl of media was added after 5-7 min. For well plates: ~ 8 µl of solution has been taken from the Eppendorf into 10 ml of media in a universal tube and then 2 ml of solution from this into a well (giving ~ 300 cells per well).

Cell dots were placed on the dish carefully, in a way so that the side with the cells is in contact with the 3.5 µm Mylar film. 6 dots are placed in total: 2 for control and 4 for irradiation. The wet dots stuck perfectly to the Mylar by surface tension. Both the cell dish and 6 eppendorfs (filled with 50 trypsin) were then taken to the target area for irradiation. The shot was taken, placing the dish vertically within 3-4 min.

2.2.d. After the shot

The cell dots were carefully cut by sharp knife from the dish and placed in eppendorfs tubes. Each eppendorf contains a single cell dot. The time of placing the 1st dot in trypsin was noted. The eppendorfs are then transported back to the bio lab. The cells were left in trypsin for about 5-7 minutes so that the cells are detached from the blanket sheet. About 450 µl of media was added to the eppendorfs containing trypsin and the cells. The solution was mixed gently by the pipette. According to the calculated dilution figures, the appropriate amount of solution from the eppendorfs was diluted in 10 ml of media in the universal tube so that each universal tube contains 1500 cells. 2 ml of cells (300 cells) from the universal tube was placed in each well plate. Thus 3-well plates were prepared from 10 ml solution of one universal tube. All the well–plates were left over in the incubator for the growth of cell colonies.

2. 2. e. Colonogenic Assay

After 4-5 days, colonies (i. e. A gathering of few hundred cells called a cell colony) were easily observed with the naked eye in the well plates. The media was then removed and the wells were stained with crystal violet mixed with methanol. After 5 min, well plates were washed with water and the colonies (see figure 7) were counted. The plating efficiency (PE)is the percentage of the cells which grow into colonies. For example, if 100 cells are seeded into the dish after irradiation and there are 70 colonies formed, then the PE is 70% [38]. The surviving fraction is calculated as, Surviving Fraction= Colonies Counted/ (Cells seeded * (PE/100)). Thus, a survival curve was obtained from the data. A cell survival curve describes how the radiation dose is related with the proportion of cells that survive. The irradiation of the cells makes them unable to divide and cease further growth. The loss of this ability as a function of the radiation dose is described by the dose survival curve.

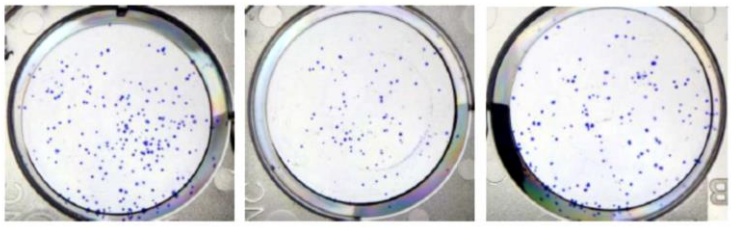

Fig. 7. Example of colony growth (from left to right), (A) for control dot, (B) low energy-high dose dot and then (C) high energy-low dose dot.

These processes were repeated for a number of shots to have an estimation of survival for a range of doses [39]. The counting of colonies was done using ImageJ software. After staining all the well plates, pictures of each well were taken using a digital camera. The counting was then done digitally. The numbers of counts were then compared and survival fractions were thus obtained corresponding to all the shots taken.

3. RESULTS AND DISCUSSION

After 4/5 days of laser shots, cell colonies (Figure 7) were found in the well plates where the cells were kept after irradiation. In fig. 7, it is clear that the number of colonies in the control dot is larger than that of other dots as the control dot was not irradiated. Also, the high dose cell dot shows a lower number of colonies than the low dose cell dot, which is reasonable. The array of high dose and low dose cell dots is shown in fig. 7, whereas doses increase from right to left. Colonies were counted to get the survival fraction as stated before. It was found that the dose measurement for all the shots ranges from 0.1 Gy (minimum) to 5 Gy (maximum). After completing the spectral analysis and the dose calculations, the survival fraction was plotted for the V79 irradiated cells. The survival results for cells are shown in Fig. 8. The survival results from 'low energy-high dose cell dot' and 'high energy-low dose cell dot' are shown here whereas colony formation for these cases is shown already in Figure 7. The term 'low energy-high dose cell dot' refers the cell dots which were irradiated by low energetic protons (0.5- 3 MeV which had released doses up to 5 Gy) and 'high energy-low dose cell dot' refers the cell dots which were irradiated by comparatively higher energetic protons (3- 5 MeV which had released doses up to 2.5 Gy). At a certain distance/ depth of cell here comparatively higher energetic protons released lower dose due to its Bragg peak [25, 28]. Fitting equations for different fitting lines are given in the side caption of figure. All these fitting lines are used here to have a summarized look of the graph and get a clearer picture of survival curves.

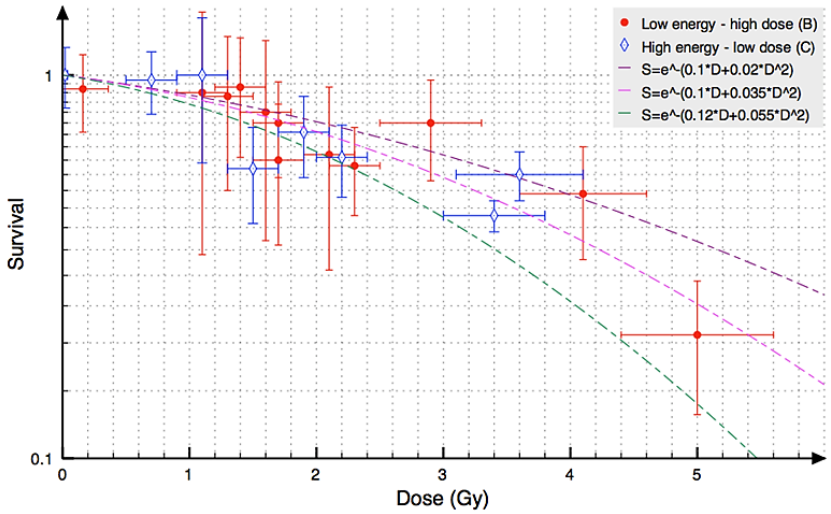

Fig. 8. Survival results for the irradiations of V79 cells. Vertical and horizontal error bars are due to the plating efficiency and overlapping of different proton energies.

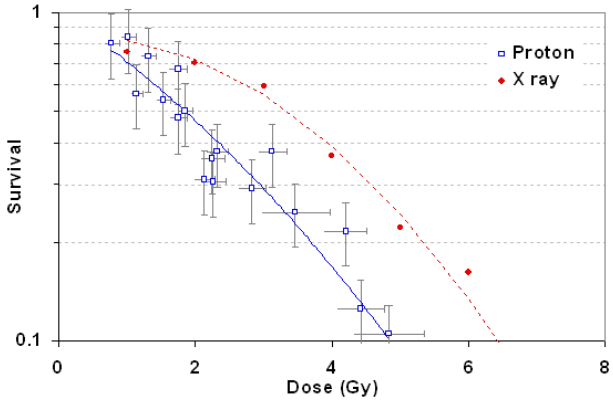

Fig. 9. Survival curves of the V79 cancer cells obtained by proton and X-ray irradiation with different doses. The fittings are usual fittings of radiobiology which is accepted by radiologist worldwide [40].

Again, the same cell samples were prepared and irradiated with an X-ray source at centre for cancer research and Cell Biology (CCRCB) in QUB. A calibrated 'XRAD225' 225 kVp X-ray cabinet (Precision X-ray Inc. N. Branford, CT, USA) fitted with a 2 mm copper filter was used as an X-ray source to irradiate the cell samples. A calibrated ISOVOLT Titan E (GE Inspection Technologies) was used to control the X ray delivery electronically. Doses of 0 to 5 Gy were delivered to cell samples with a dose rate of 0.54 Gy / min and at a distance of 50 cm from the source at room temperature. Before the experiment, all the calibrations of instruments were done in the cancer centre of Belfast City Hospital by a secondary standard Farmer Chamber. Figure 9 shows the resulted survival curve. The finite size (2.5 mm) of the cell dots gave the uncertainty in doses. Cancer cells which were irradiated by laser driven protons show lower survival rate than X ray, that means laser driven protons has higher killing efficiency / biological efficiency. Cancer cells which were irradiated by X rays show higher survival rate than laser driven protons, means X ray has lower killing efficiency / biological efficiency. So, we can say that Figure 9 clearly depicts the higher biological efficiency of protons with respect to X-rays.

This is a first and foremost experiment in the field laser driven protons [41] where dose is delivered by a single laser shot whereas in other cases dose is accumulated by several laser shots and conventional accelerators were used to produce protons [42-43]. All other works so far done, dealing with theory, simulation, and conventional proton sources [25, 28 and 30]. Some works were done, dealing with the laser driven protons but they focused on dosimetry, LET (Linear energy transfer), comparison with conventional proton sources, DNA double strand break [29-30, 44-46]. A study is currently focusing on laser driven carbon ions as well [45]. So, this is the first ever experiment which dealt with laser driven protons and shows a comparison with X ray in killing the cancer cells in practical.

4. CONCLUSIONS

Apart from dosimetry and LET calculation, experimental design and irradiation setup is very important for the successful completion of this type of experiment. Moreover, same experimental design can be used for any radiobiological studies of laser driven protons. This study has a detailed

explanation on cell handling procedures with laser driven protons as well as contains all the parameters for irradiation setup. Hence this study of biological effectiveness of laser driven protons is very interesting and give us a new light for a compact, cost-effective clinical approach of laser-based accelerator in future.

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