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# Hundred joules plasma focus device as a potential pulsed source for *in vitro* cancer cell irradiation

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Plasma focus devices may arise as useful source to perform experiments aimed to study the effects of pulsed radiation on human cells *in vitro*. In the present work, a table top hundred joules plasma focus device, namely "PF-400J", was adapted to irradiate colorectal cancer cell line, DLD-1. For pulsed x-rays, the doses (energy absorbed per unit mass, measured in Gy) were measured using thermoluminescence detectors (TLD-100 dosimeters). The neutron fluence and the average energy were used to estimate the pulsed neutron doses. Fifty pulses of x-rays (0.12 Gy) and fifty pulses of neutrons (3.5 $\mu$Gy) were used to irradiate the cancer cells. Irradiation-induced DNA damage and cell death were assessed at different time points after irradiation. Cell death was observed using pulsed neutron irradiation, at ultralow doses. Our results indicate that the PF-400J can be used for *in vitro* assessment of the effect of pulsed radiation in cancer cell research. © *2017 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).* [http://dx.doi.org/10.1063/1.4994655]

The plasma focus (PF) is a pulsed source of radiation and particles as x-rays, ion and electron beams, neutron bursts, plasma shocks and plasma jets. Normally, a PF device consists in a central electrode (anode) that is partially covered by an insulator. Cathode bars symmetrically surround the anode. The plasma dynamics in the PF devices is categorized in various phases. On the application of the high voltage at the anode, at first, the discharge takes place over the insulator and form a plasma current sheet. This stage is known as the breakdown phase. After, the plasma current sheet starts to expand and moves along the length of the anode. This phase is called the rundown phase. At the top of the anode, the plasma current sheet moves in the radial inward direction and compresses the neutral gas there. This is known as the compression phase. The fast compression of the neutral gas produces the shock waves that ionize the neutrals at the top of the anode. These shock waves travel to the axis of the anode and reflect back. At the time, when the reflection and the compression balance, a plasma column forms. This is known as the pinch phase. Various physical phenomena take place during the pinch phase and cause the emission of pulsed x-rays,[1–5] charged particles,[6–10] and neutrons[11–14,26] if working gas is deuterium. After pinch an axial shock[15] and plasma jets are produced.[16] These later phases have been recently characterized and found applications in

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the plasma wall interaction in fusion reactor[15,17] and possible application related to astrophysical jet phenomena.[15]

The radiation emitted from the PF devices have been applied widely in material science and nanotechnology[23] and recently in biology.[18,19,24] In this letter, the pulsed x-ray radiation and pulsed neutron bursts are used to irradiate the cancer cell lines during *in vitro* experiments. Indeed, the pulsed low dose radiation (PLDR) has been proposed as the treatment of the recurrent cancerous tumor.[20] In order to provide the low doses in the pulsed form the PF devices may be a good choice. PF devices emit neutrons as well as x-rays. The x-rays are known to provide low linear energy transfer (LET). On the other hand, the particles (neutrons, heavy ions) are the high LET. In the present study, both pulsed x-rays in low dose regime and pulsed neutrons in ultralow dose regime are used to irradiate the colorectal cancer cell DLD-1.

PF-400J[1,11,15–18] (880 nF, 38 nH, 20-30 kV, 176-539J, ~300 ns time to achieve peak current) consists of a stainless steel (SS) hollow anode of 12 mm external diameter, symmetrically surrounded by eight stainless steel cylindrical cathode bars. An insulator covers a partial length of the anode. The uncovered length, called effective length, was 7.0 mm in this case. In this study, PF-400J device is used to irradiate the colon cancer lines DLD-1 during *in vitro* experiment.

In order to irradiate the cancer cells, it is mandatory to characterize the doses. Thermoluminescence detectors (TLD-100) have been used in order to measure the doses for the pulsed x-rays. TLD-100 can be used to measure the doses for wide range of x-rays (tens of keV – tens of MeV). In addition, these TLDs are tissue equivalent, therefore, are suitable for the present study.

In plasma focus devices, during constriction, various physical phenomena take place. In particular, electrons accelerate towards the anode. At the bottom of the anode, electron beams produce x-rays via bremsstrahlung. The bremsstrahlung increases with higher atomic number materials. In addition, it has been shown that the effective x-rays energy increases if a lead material is placed inside the anode of plasma focus device.[27] In order to enhance the x-rays emission a lead piece was inserted inside the hollow anode of the PF-400J.

A photomultiplier tube (PMT) was kept at ~ 80 cm from the top of the anode. In this study, the PMT was used as a referential device in order to count the x-ray pulses. Hydrogen gas at nine mbar was used to produce discharges. A vacuum window made of a plastic material (1.2 mm thickness), over which the cell culture will be kept, was used. With our previous experience, it was realized that the metallic window (1 mm Al) provides about 1 mGy dose for 100 pulses of x-rays outside the vacuum chamber. Due to this, the plastic vacuum window was used in order to irradiate the cancer cells to have significant amount of doses. The cell cultures were seeded in 4-chamber 35 mm petri dish (In vitro Scientific, Sunnyvale, CA, USA). These dishes have four partitions. Three partitions were used for seeding the cells and one partition was used for the dosimeters. In this way, the doses can be measured in real time experiment and the doses measured for 50 pulses of x-rays were ~ 0.12 Gy. The duration of the x-rays pulses was measured using a PIN diode, ~10 ns at FWHM. Figure 1 shows the schematic of the experimental arrangement.

The neutron doses were obtained by estimating the neutron fluence at the petri dish and the neutron energy. The neutron yield for the PF-400J in $4\pi$ is reported ~ $10^6$.[11] Time of flight (ToF) methodology[25] was applied in order to measure the neutron energy. Deuterium gas was used to produce the discharges at 8 mbar pressure. Figure 2 shows the experimental arrangement for ToF, to estimate the neutron energy. Two PMTs, namely FM25 and FM26, were separated by 20 cm. The travel time between the PMTs considered as time of flight of neutrons. This time is used to measure the neutrons speed and energy. Average neutron energy was measured in 2.14 ± 1.0 MeV and the duration of the neutron pulses is in the order of ~10 to 20 ns at FWHM. The neutron fluence at the position of the petri dish that was kept at 6 cm from the top of the anode is ~ $2.2 \times 10^3$ n/cm$^2$. With this energy and fluence, the dose for tissue equivalent material, for 50 pulses of the neutron, was obtained ~ 3.5 $\mu$Gy. The dose conversion is described in the "Neutron dosimetry for biology and medicine, ICRU report **26**, pages 74, 85 (1977)"[21] where data for tissue equivalent material were used.

The electrical signals of the x-rays and the neutrons captured by the PMTs are shown in figure 2. The x-rays travel with speed of light. Therefore, the first pulse in the signals should corresponds
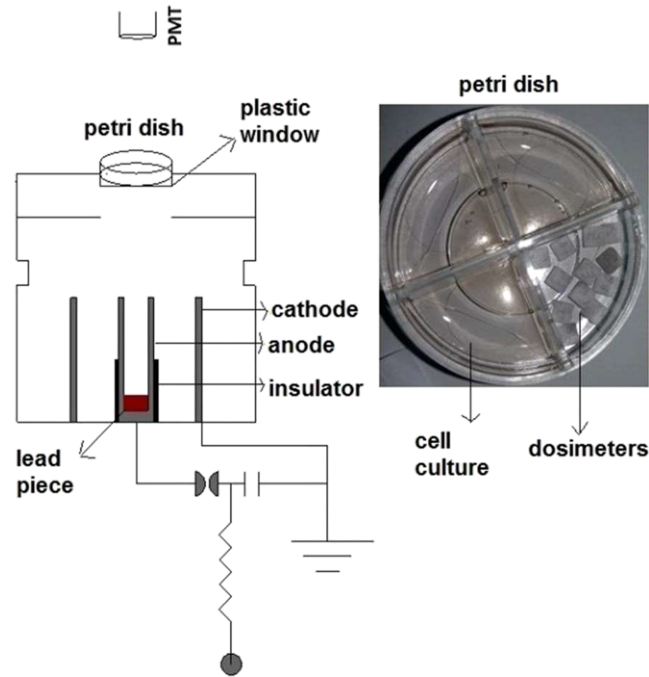
FIG. 1. Experimental arrangement for cancer cell irradiation and dose measurement in case of pulsed x-rays. PF-400J is used for this experiment.

to the x-rays and the neutron signal should appear later. Please note, in case of neutron irradiation Al vacuum window was used so that the effects of the x-rays on the cancer cells can be neglected.

DLD-1 human colorectal cancer cells were cultured in RPMI1640 (Mediatech, Herndon, VA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Mediatech), penicillin G (100 U/ml), and streptomycin (100 μg/ml) and incubated in a 5% $CO_2$ humidified atmosphere at 37°C. Cells were seeded in 4-chamber 35 mm dish (In vitro Scientific, Sunnyvale, CA, USA) at
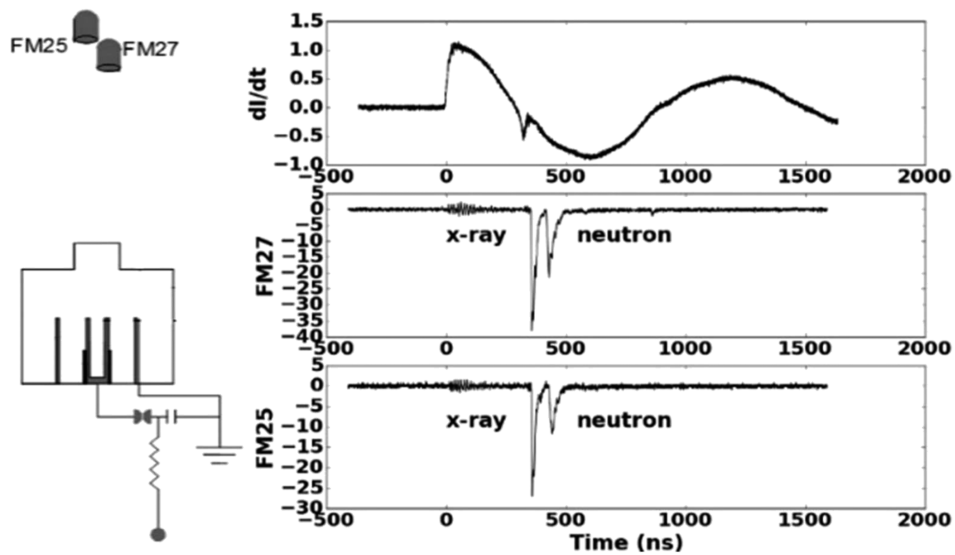


FIG. 2. Schematic experimental arrangement for the neutrons kinetic energy estimation using ToF and the x-rays and the neutron signals captured by the PMTs.

a density of 9.6 x $10^5$ cells/well a day before radiation. After irradiation, viability was evaluated by trypan blue exclusion assay. Briefly, a volume of represented cells were mixed with and equal volume of 0.4 % trypan blue solution (Logos Biosystems, Gyunggi-Do, Korea) and counted using a LUNA$^{TM}$ Automated Cell Counter (Logos Biosystem). All assays were performed at least three independent times.

For immunofluorescence assays, 2.4 x $10^5$ cells/well were grown on glass coverslips in 4-chamber 35 mm dish (In vitro Scientific, Sunnyvale, CA, USA). At 30, 60 and 120 minute after radiation, cells were fixed using 4% p-formaldehyde for 10 minutes, permeabilized with 0.25% Triton X-100 at room temperature and blocked with 3% BSA/PBS for 45 minutes. Cells were incubated overnight with an anti-γ-H2AX antibody (1:1000, Millipore, Temecula, CA, 05-636) diluted in 0.05% Triton X-100 and 1% BSA/PBS. After washing steps, cells were incubated with an Alexa Fluor-488 secondary antibody (1:500, Molecular Probes, A-21042). Slides were mounted with ProLong Gold Antifade Reagent with DAPI (Life Technologies, NY). Cells were observed under a fluorescence microscope (BX53, Olympus). For γ-H2AX quantification, foci were counted using a Find Maxima plugin and normalized by nuclei numbers using ImageJ software (Rasband, National Institutes of Health, USA). Cells were counted in 5 different fields and at least 100 cells were evaluated per sample.

DLD-1 cells cultured in a petri dish, were irradiated with fifty pulses of x-rays and neutrons. The DNA damage and cell death were assessed after irradiation. A mock condition (cell culture that was not irradiated but follow the same path as the irradiated cell culture) was used as control. The DNA damage was evaluated at 30, 60, and 120 minutes after irradiation by detecting the phosphorylation on Ser-139 in histone H2AX (named γ-H2AX). Whenever there is induction of the double strand break (DSB), a focus of γ-H2AX protein is detected in the vicinity of the DSB. Hence, the DSB can be quantified by counting the γ-H2AX foci. Figure 3 shows one of the representative images of the γ-H2AX detection in mock control (figure 3A) and the cells irradiated with 50 pulses of the x-rays (figure 3B) at 30, 60, 120 minutes post irradiation. Please note the formation of the foci (are the foci of γ-H2AX biomarker) that are the bright white dots in the green structures. The green structures are individual nuclei and the white spots are the foci that indicate the presence of DSB. A clearly increase in DSB induction was evident at 30 minutes after pulsed x-rays irradiation.

Three independent experiments for DNA damage study were performed and γ-H2AX foci were quantified. Mean ± Standard Deviation is shown in figure 3B. Increase in the γ-H2AX foci was significant only at 30 minutes post irradiation. At later time points, statistical test showed no significance difference between pulsed x-rays irradiation and mock control (i.e. cell culture that were not irradiated but followed the same path as irradiated cell culture). It is highly possible that DNA damage is been repaired later. Moreover, if DNA damage cannot be repaired the cell death process take place.[22] However, in these cells, pulsed x-rays irradiation with 0.12 Gy, did not induce
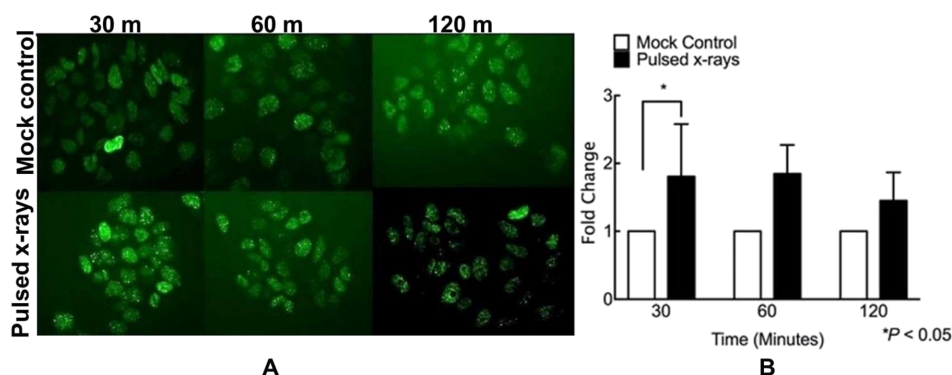


FIG. 3. DNA damage was evaluated by detection of γ-H2AX foci in mock and irradiated cells at 30, 60, 120 minutes post irradiation. A) Representative images of cell nuclei (green structure) stained for γ-H2AX (white spots). B) Shows the quantification of γ-H2AX foci fold change, i.e. DNA damage in the irradiate cells normalized with respect to the mock control cells. * Difference was statistically significant (* P<0.05); Student's T-test.
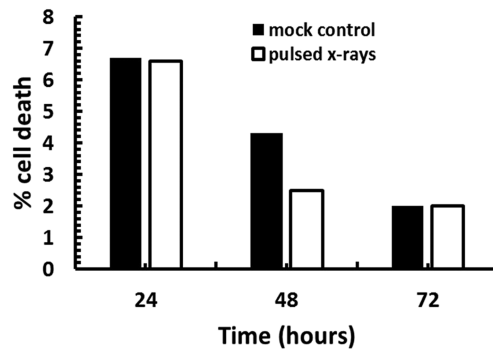
FIG. 4.  Cell death in DLD-1 cell exposed to 50 pulses of x-rays. N = 1.

cell death, as analyzed using trypan blue dye assay (Figure 4). Since we did not find any positive results of the cell death, in case of 50 pulses of x-rays, cell death measurement was limited to n=1. Result of the cell death in this case are shown in figure 4 at 24, 48 and 72 hours post irradiation.

In cells irradiated with pulsed neutrons, DSB were increased at 60 and 120 minutes after irradiation. Figure 5A shows the fold change in the DSB for the cells that were irradiated with pulsed neutron with respect to the mock control.

Figure 5B, shows the results of the cell death (n=3), in case of the pulsed neutron irradiation. The cell death in irradiated culture was normalized with respect to the mock control. It can be seen that the neutron irradiated cells show increment in the death at 48 and 72 hours after irradiation (p < 0.001). This increment is ~ 2 times the mock control. At 24 hours, the increase in cell death was not significant. The processes that are responsible to trigger the cell death take place after various checkpoints in the cell cycle. That may be the reason that the cell death is observed during 48 and 72 hours with significant statistical differences. Please note, the DNA damage analysis in the case of pulsed neutron irradiation do not provide trust worthy results. It is a common believe that the severe damage in the DNA is one of the important pathways of the cell death. Nonetheless, the neutron interaction with the cells at ultralow doses may provoke other pathways of the cell death. Identification of such pathways is out of scope of present work and will be considered in future studies. Please note that the neutrons irradiation is considered as high LET interaction. The damage produced by the neutrons during their interaction with cells and its medium may not necessarily be of the same type as the low LET x-rays.
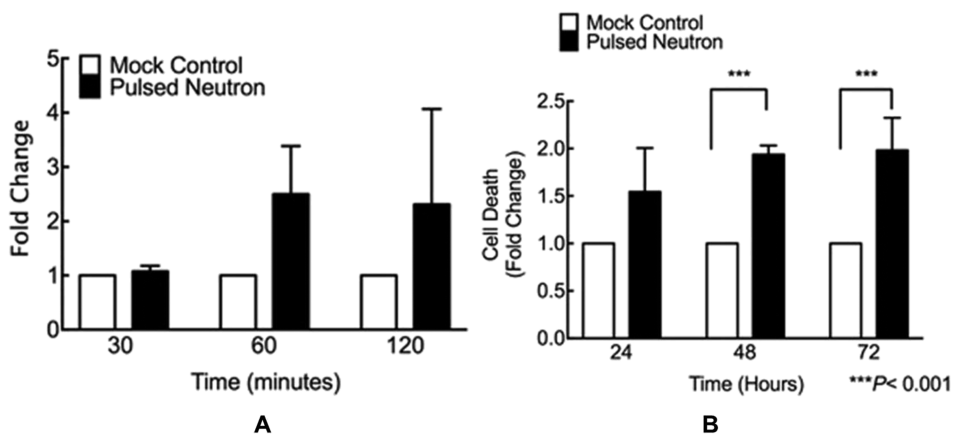


FIG. 5.  DLD-1 cells were irradiated with 50 pulses of neutrons. A) DNA damage was evaluated by detection of $\gamma$-H2AX foci in mock and irradiated cells at 30, 60, and 120 minutes post irradiation. Quantification of $\gamma$-H2AX foci (fold change relative to mock) is shown. B) Cell death was determined by trypan blue incorporation assay at 24, 48 and 72 hours after irradiation. Fold change relative to mock control is shown.

The present studies are carried out keeping in mind that the plasma focus device PF-400J could be useful in the area of the cancer research during *in vitro* experiments. With this aim preliminary experiments were carried out. An important observation of the cell death at ultralow doses in the case of the pulsed neutron irradiation was observed. Further studies are required to understand the interaction of neutron and living cells and its consequences. In conclusion, the possibility to use a hundred joules plasma focus device to study the effects of pulsed radiation in cancer cell has been shown.

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