In vitro irradiation of colorectal cancer cells by pulsed radiation emitted from a hundred joules plasma focus device and its comparison with continuous irradiation.

J. Jain¹,²,³, J. Moreno²,³,⁴, R. Andaur⁵, R. Armisen⁶,⁷, G. Avaria²,³,⁴, B. Bora²,³,⁴, S. Davis²,³,⁴, C. Pavez²,³,⁴, K. Marcelain⁵, and L. Soto²,³,⁴*  

¹Comisión Chilena de Energía Nuclear, Casilla 188-D, Santiago Chile  
²Center for Research and Applications in Plasma Physics and Pulsed Power, P4  
³Universidad de Talca, Chile  
⁴Universidad Andres Bello, Departamento de Ciencias Física, Republica 220, Santiago, Chile  
⁵Deparatamento de Oncología Básico-clínica, Facultad de Medicina, Universidad de Chile, Independencia 1027, Independencia, Santiago, Chile  
⁶Centro de Investigación y Tratamiento del Cáncer, Facultad de Medicina, Universidad de Chile, Independencia 1027, Independencia, Santiago, Chile  
⁷Current affiliation: Center for Excellence in Precision Medicine, Pfizer Chile, Santiago, Chile  

*E-mail: jalajjain83@gmail.com, lsoto@cchen.cl  

Abstract. In the last years, pulsed reduced low dose radiation has been proposed as an alternative for treatment of recurrent cancer. Nonetheless, distinction between the effects of low dose pulsed and continuous radiation is barely known at cellular level. In order to study the effects of low dose pulsed radiation at cellular level, in vitro experiments are important to further advance the basic understanding in this area. In the present work we demonstrate the usefulness of a low-energy plasma focus device PF-400J as a potential source of low-dose pulsed radiation for in vitro cancer cell experiments. Colorectal cancer cell line, DLD-1, were irradiated by pulsed x-rays. Fifty pulses of x-rays provide ~0.12 Gy dosis, which were measured using thermoluminescence detectors (TLD-100 dosimeters). Irradiation-induced DNA damage was assessed at different time points after irradiation. A statistically significant double strand break (DSB) DNA damage was observed at 30 minutes after irradiation. A comparison of DSB induced by continuous source in the same type cancer cells and pulsed irradiation is made at 30 minutes post-irradiation. In the case of pulsed irradiation, DSB per unit dose found higher. Our findings suggest that low-energy plasma focus devices could have potential application as pulsed radiation source in the area of in vitro cancer cell experiments.
1. Introduction

Dense plasma focus (DPF) devices produce short-lived plasma in various phases. This short-lived plasma produces various kinds of radiations such as pulsed x-rays [1-5], charged particles [6-10] and neutrons if working gas is deuterium [11-14, 22].

Normally a DPF consists in a central electrode (usually anode) that is partially covered by an insulator. Cathode bars symmetrically surround the anode. Plasma in DPF is categorized in various phases that are described in the following. On the application of pulsed-high voltage, at first, the discharge takes place over the insulator and form a plasma current sheet (PCS). This stage is known as breakdown phase. Later, due to Lorentz force the PCS starts to expand and moves along the length of the anode. This phase is called rundown phase. At the top of the anode, the PCS starts to move in radial inward direction and compress the neutral gas there. This is known as compression phase. The fast compression of neutral gas produce radial shock waves that ionize neutrals at the top of the anode. These shock waves travel to the axis of the anode and reflect back. At the time, when reflection and compression balance, a plasma column forms. This is known as pinch phase. Various physical phenomena take place during compression and pinch phases and cause emission of electromagnetic and particle radiation. Indeed, induction of electromagnetic fields cause acceleration of charged particles. Electrons accelerate toward the anode and produce x-rays via bremsstrahlung upon impinging the anode. On the other hand, ions accelerate away from the anode and produce neutrons via beam-target fusion mechanism, if the discharges are produced with deuterium. Disruption of the pinch phase provides axial shock and plasma jet [15-16]. These later phases have been recently characterized and found applications in plasma wall interaction in fusion reactor and astrophysical jet phenomena. Electromagnetic radiation, emitted from DPF, covers a wide range of its spectrum from visible to hard x-rays that can be used for in vitro experiments on cancer cells.

Radiation therapy is often used for cancer treatment, which includes the exposure of cancerous tumors at high dose of radiation. However, high doses are fractionated over a long period. Still, the fractionated doses have certain side effects. Due to this reason, studies in the area of low dose effects on cancer cells have been carried out [17-18].

Pulsed reduced low dose radiation (PLDR) is useful in the treatment of recurrent cancerous tumors [19]. In order to study the effects of low dose pulsed radiation effects at cellular level (in vitro), DPF will be a good choice. DPF emits neutrons as well as x-rays. X-rays are known to provide low linear energy transfer (LET). On the other hand, particles (neutrons, heavy ions) are high LET. The peculiarity of DPF that it emits pulsed x-rays and neutrons; can be explored further in order to study the effects of pulsed radiation (x-rays and neutron) on cancer cell lines in in vitro experiments that is the focus of present paper.

In section 2, experimental setup and cancer cell culture preparation is presented. The results and discussion are presented in section 3. The work is concluded in section 4.

2. Experimental setup

PF-400J (880 nF, 38 nH, 20-30 kV, 176-539J, ~300 ns time to achieve peak current) [1, 6, 11, 15] consists in a stainless steel (SS) hollow anode of diameter 12 mm, symmetrically surrounded by eight stainless steel cylindrical cathode bars. The effective length, was 7.0 mm in this case. A schematic of PF-400J with electrical signals and various phases is shown in figure 1. During pinch phase, the impedance, mainly the inductance, increases and current decreases. This signature of pinching action (that is important to have radiation emission from DPF), can be seen in current derivative and voltage signals (dI/dt), please see figure 1. In order to irradiate cancer cells it is mandatory to characterize radiation with respect to dose. Thermoluminiscence detectors (TLD-100) have been used in order to measure the dosis for x-rays. TLD-100 can be used to measure the dosis for wide range of x-rays (5 keV – tens of MeV). In addition, TLD-100 are tissue equivalent, therefore, are suitable for present study. Here it is worth to mention that PF-400J emits x-rays in low (SXR) and high-energy (HXR) range. HXR are the one that are detected outside the plasma focus vacuum chamber and SXR are detected inside and cannot penetrate the vacuum chamber. In order to confirm the detection and distinction, P-I-N diode
(BPX65, 0.5 < E < 30 keV) was mounted inside and photomultiplier tube with scintillator (BC408, 0.1 < E < 5 MeV) was mounted outside the vacuum chamber along the same line of sight. It was observed that when there was signal in P-I-N diode no signal was observed in photomultiplier tube. With this observation, dosis outside and inside the vacuum chamber were measured simultaneously. The outside dosimeter array that was placed over the aluminum vacuum window of width 1mm provides hundred times lower dosis that were obtained inside the vacuum chamber. It was realized that most of the low-energy x-rays would have been attenuated while passing through the aluminum window. With this observation, the aluminum vacuum window was replaced by plastic vacuum window (polyethylene) of width ~ 1 mm, and with this arrangement the dosis outside the vacuum chamber were about 0.12 Gy for fifty pulses of x-rays, while placing a lead piece inside the hollow anode.

Based on the abovementioned observations cell cultures were irradiated by fifty pulses of x-rays, outside the PF-400J vacuum chamber by keeping the cell culture over vacuum window that was made of plastic material, along the PF axis. Petri dishes of diameter ~ 3.5 cm with four partitions were used to culture cancer cells and dosimeter measurements. Three partitions were used to culture cells and one to keep dosimeters. In this way, it was possible to monitor dosis provided by pulsed x-rays in real time experiment. The distance between the top of the anode and cell culture petri dish was ~ 7 cm. In addition, a photomultiplier tube was kept at ~ 80 cm from top of the anode along the PF-axis. In this study, photomultiplier was used as a referential device in order to count x-ray pulses. Hydrogen gas at nine mbar was used to produce discharges. Figure 2 shows the experimental setup for cell culture irradiation by pulsed x-rays.
2.1 Cell culture and viability assays

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% CO₂ humidified atmosphere at 37°C. Cells were seeded in 4-chamber 35 mm dish (In vitro Scientific, Sunnyvale, CA, USA) at a density of 9.6 x 10⁵ 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 an equal volume of 0.4% trypan blue solution (Logos Biosystems, Gyunggi-Do, Korea) and counted using a LUNAM Åutomated Cell Counter (Logos Biosystem). All assays were performed at least three independent times.

2.2 Immunofluorescence Assay

For immunofluorescence assays, 2.4 x 10⁵ 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.

3. Results and discussion

3.1 X-rays dose measurement

Pulsed x-ray dosis were characterized using TLD-100 dosimeters. PMT was used as referential device. Average dose in 50 x-ray pulses were found ~ 0.12 Gy with the insertion of lead piece inside the hollow anode.
3.2 Double strand break (DSB)

DLD-1 cells cultured in a petri dish, were irradiated with fifty pulses of x-rays. DNA damage was 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. 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 double strand break (DSB), a focus of γ-H2AX protein is detected in the vicinity of DSB. Hence, DSB can be quantified by counting γ-H2AX foci. Figure 3 shows the results of DSB quantification. An increase in DSB induction is evident at 30 minutes after pulsed x-rays irradiation.

Three independent experiments (n=3) for DNA damage study were performed. Increase in the γ-H2AX foci was significant only at 30 minute post irradiation. At later time points, statistical test showed no significance difference between pulsed x-rays irradiation and mock control. It is highly possible that DNA damage is been repaired later. Moreover, if DNA damage cannot be repaired cell death process take place [20]. However, in these cells, pulsed x-rays irradiation with 0.12 Gy, did not induce cell death, as analyzed using trypan blue dye assay. Since we did not find any positive results of the cell death, in the case of 50 pulses of x-rays, cell death measurement was limited to n=1.

![Figure 3 DNA damage was evaluated by detection of γ-H2AX foci in mock and irradiated cells at 30, 60, 120 minutes post irradiation quantification of γ-H2AX foci fold change. * Difference was statistically significant (* P<0.05); Student’s T-test.](image)

3.3 Comparison of DSB induced by pulsed and continuous irradiation

Figure 4 shows a comparison between DSB induced per unit dose (DSB/dose) by continuous high (12 Gy) and low dose (0.6 Gy) irradiation [21] and pulsed low dose irradiation (~0.12 Gy, emitted from PF-4001). It can be seen from figure 4 that DSB per unit dose is higher in the case of pulsed radiation. It can be say that pulsed radiation is more effective in DSB induction than continuous radiation. The number of DSB in the case of continuous low dose (0.6 Gy) x-ray irradiation was found about 3.7-fold at 30 minutes [21]. In the case of pulsed x-rays, this data is ~ 1.7-fold. The ratio of dosis ~ 0.6/0.12 = 5 and the ratio of the fold change of the DSB will be 3.7/1.7 = 2.18 at 30 minute. With these estimations, it can be concluded that the effect of DSB induction in the case of low dose pulsed x-rays irradiation cannot be linearly extrapolated from low dose continuous x-rays DSB induction. In addition, a similar comparison between high dose (12 Gy), obtained from continuous x-rays [21] and low dose pulsed x-rays (~0.12 Gy) irradiation is made. The ratio of dosis is 12/0.12 = 100 and the ratio of fold change in DSB is 8/1.7 = 4.7, at 30 minutes. This comparison reveals that doses are 100 times higher in the case of continuous x-rays irradiation but the induced fold change in DSB ~ 4 – 5 times.
4. Conclusion

Radiation emitted from DPF device PF-400J was applied to irradiate colorectal cancer cell lines DLD-1 in in vitro experiments. Dosis for 50 x-ray pulses were ~ 0.12 Gy, while placing a lead piece inside the hollow anode. Double strand break (DSB) in DNA was found at 30 minutes post-irradiation with statistical significance. In this case, cell death was not observed. A comparison between high and low dose irradiation, provided by continuous x-ray source and low dose pulsed radiation, provided by PF-400J DPF device was made. It was found that pulsed radiation is more effective in inducing DSB in DNA than continuous radiation.

Acknowledgement
The work is supported by grant ACT-1115, CONICYT, Chile.

References
[1] Zambra M, Silva P, Moreno M, Pavez C and Soto L 2009 Plasma Phys. Control. Fusion 51 125003
[2] Barbaglia M, Bruzzone H, Acuña H, Soto L and Clausse 2009 Plasma Phys. Control. Fusion 51 045001
[3] Barbaglia M, Soto L and Clausse A 2012 Journal of Fusion Energy 31 105
[4] Pavez C, Pedreros J, Zambra M, Veloso F, Moreno J, Tarifeño-Saldívar A and Soto L 2012 Plasma Phys. Control. Fusion 54 105018
[5] Neog N K, Mohanty S R and Borthakur T K 2008 Physics Letters A 372 2294
[6] Jain J, Moreno J, Pavez C, Bora B, Inestrosa-Izurieta M J, Avaria G and Soto L 2016 J. Phys.: Conf. Ser. 720 012042
[7] Bhuyan H, Chuaqui H, Favre M, Mitchell I and Wyndham E 2005 J. Phys. D: Appl. Phys. 38 1164
[8] Mohanty S R, Bhuyan H, Neog N K, Rout R K and Hotta 2005 Jpn. J. Appl. Phys. 44 5199
[9] Jakubowski L, Sadowski M and Zebrowski J 2001 Nucl. Fusion 41 755
[10] Neog N K and Mohanty S R 2007 Physics Letter A 361 377
[11] Silva P, Moreno J, Soto L, Birstein L, Mayer R and Kies W 2003 Appl. Phys. Lett. 83 3269
[12] Verma R, Rawat R S, Lee P, Krishnan M, Springham S V and Tan T L 2009 Plasma Phys.
Control. Fusion 51 075008

[13] Soto L, Silva P, Moreno J, Zambra M, Kies W, Mayer R E, Clausse A, Altamirano L, Pavez C and Huerta L 2008 J. Phys. D: Appl. Phys. 41 205215

[14] Ellsworth J L, Falabella S, Tang V, Schmidt A, Guethlein G, Hawkins S and Rusnak B 2014 Rev. Sci. Instrum. 85 013504

[15] Soto L, Pavez C, Moreno J, Inestrosa-Izurieta M J, Veloso F, Gutierrez G, Vergara J, Castillo F, Clausse A, Bruzzone H and Delgado-Aparicio L 2014 Phys. Plasmas 21 122703

[16] Pavez C, Pedreros J, Tarifeño-Saldívar A and Soto L 2015 Phys. Plasmas 22 040705

[17] Cuttler J M and Pollycove M 2003 J. American Physicians and Surgeons 8 108

[18] Litvyakov N V, Rostov V V, Bol'shakov M A, Buldakov M A, Afanas'ev K V, Astapenko A N, Kutenkov O P and Cherdyntseva N V 2005 50 S51-S58

[19] Charlie Ma C M, Luxton G and Orton C G 2011 Med. Phys. 38 4909

[20] Roos W P, and Kaina B 2006 Trends in Molecular Medicine 12, 440

[21] Private communication with Faculty of Medicine, University of Chile group

[22] Soto L, Pavez C, Moreno J, Altamirano L, Huerta L, Barbaglia M, Clausse A, and Mayer R E 2017 Physics of Plasmas 24 082703