Small-scale laser based electron accelerators for biology and medicine: a comparative study of the biological effectiveness

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ABSTRACT

Laser-driven electron accelerators based on the Laser Wakefield Acceleration process has entered a mature phase to be considered as alternative devices to conventional radiofrequency linear accelerators used in medical applications. Before entering the medical practice, however, deep studies of the radiobiological effects of such short bunches as the ones produced by laser-driven accelerators have to be performed. Here we report on the setup, characterization and first test of a small-scale laser accelerator for radiobiology experiments. A brief description of the experimental setup will be given at first, followed by an overview of the electron bunch characterization, in particular in terms of dose delivered to the samples. Finally, the first results from the irradiation of biological samples will be briefly discussed.

Keywords: Laser WakeField Acceleration, medical applications, radiotherapy

1. INTRODUCTION

Electron accelerators relying on the Laser Wakefield Acceleration (LWFA) process in plasmas are now entering a mature phase, allowing them to be considered as reliable alternatives to the RF LINACs used in medical practice such as, for instance, radiotherapy. The field of laser-driven electron acceleration, after the original proposal by Tajima and Dawson in 1979,[1] has experienced an outstanding development in the latter decade, mainly due to the increasing availability of table-top, multi-terawatt, ultrashort pulse duration CPA laser systems. As it is well known, LWFA takes advantage of the high longitudinal electric field supported by electron plasma waves in a plasma; electrons are trapped in the plasma wave that has a phase velocity close to the speed of light, and gain energy as long as they are in phase with the aceelerating region of the field (see Reference[2] and Refs. therein). Experiments reported starting from 2004[3–5] have been showing that quasi-monoenergetic electron bunches can be accelerated from the background plasma electron population up to hundreds MeV energy with increasing

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Laser Acceleration of Electrons, Protons, and Ions II; and Medical Applications of Laser-Generated Beams of Particles II; and Harnessing Relativistic Plasma Waves III, edited by Eric Esarey, et al., Proc. of SPIE Vol. 8779, 87790O

bunch quality.[6,7] Numerical simulations[8,9] showed that for sufficiently short and intense laser pulses, the so-called bubble regime of LWFA is activated, in which a single accelerating cavity is produced and effective electron trapping occurs, leading to interesting features of the accelerated bunch.

Beside to the frontier applications that laser-driven accelerators may open up (for instance in the field of particle physics, new concept photon sources and so on), which still require significant scientific steps forward (in terms, e.g., of stability and reproducibility, monochromaticity, transverse emittance and so on), applications in biology and medicine can now be foreseen within a decade, in particular in the field of radiotherapy.[10,11]

As a matter of fact, electron accelerators based upon ultrashort and ultraintense table-top lasers would exhibit a wealth of advantages when compared to the conventional accelerators currently used in the medical practice, in terms, for instance, of radioprotection requirements, operation reliability and flexibility and so on. Furthermore, electron bunches with energies up to a few tens or even hundreds of MeV would be easily achievable, thus representing a new option in radiotherapy and other medical practices.

It is worth stressing that this kind of studies and applications would not require 100 TW class laser systems, so that a) these studies are currently accessible to medium scale laboratories and b) the laser footprint would be suitable for an hospital environment. As of today, the figures of the electron bunches delivered by small-scale laser based accelerators are comparable to the ones produced by conventional LINACs as for the electron energy, total charge, average current and delivered dose, etc. However, due to the ultrashort bunch duration (of the order of a few up to a few tens of femtoseconds), the peak current is much higher (typically, up to six orders of magnitude) than in conventional LINACs, leading to a corresponding huge increase in the peak dose. On one side, this opens up a wealth of potential new studies and applications, involving possible unexplored biological responses to such high current and ultrashort beams.[12] On the other hands, this demand accurate studies at a pre-clinical stage before even considering laser-driven accelerators for an actual medical usage. The group operating at the Intense Laser Irradiation Laboratory (ILIL) of the CNR in Pisa is currently pursuing a project aimed at studying the biological response to electrons from laser-driven accelerators. The project gathers together people with multidisciplinary expertises (namely, physics, medicine and biology). Here we will briefly report on the first activities carried out within this project. In particular, results from the first studies on biological samples will be discussed.

2. DESCRIPTION OF THE ELECTRON SOURCE

2.1 The experimental setup

The studies reported here were carried out at the ILIL laboratory, using a 2TW laser system. Figure 1 *left)* shows a schematic layout of the experimental setup inside the vacuum chamber. The laser delivered up to 100 mJ energy, 40 fs duration pulses. The beam was focused using an $f/4.5$ OAP mirror down to a $\sim 10\,\mu$m diameter spot into a supersonic gas-jet produced using a rectangular nozzle with size 4×1.2 mm (the laser propagation being along the smallest direction); the corresponding maximum intensity was up to $\sim 2 \times 10^{18}$ W/cm^2. The laser-plasma interaction was diagnosed using standard techniques such as optical (Nomarski) interferometry and Thomson scattering imaging. The electron production was monitored using a LANEX scintillator screen imaged out using a CCD camera and NaI scintillators coupled to photomultipliers. The gas used was either He or N_2, resulting in different plasma electron densities and accelerated electron spectra. The electron spectrum was measured using a magnetic spectrometer (not shown in Figure). In general, the produced electron bunches featured a large spectrum (maxwellian in shape), with typical energy going from ~ 0.3 MeV up to a few MeV (the higher electron energy was obtained using the He gas). The electron spectrum when using the He gas also featured a small gaussian component centered at a few MeV. We omit here a deeper description of the diagnostics and a discussion of the regime of laser-plasma interaction and electron acceleration; we refer to Refs[13,14] for a general overview. We just give a brief account of the results of the electron bunch characterization useful for an assessment of the dose delivered to the biological samples.

In order to allow the irradiation of *in vitro* biological samples, the experimental setup was modified as shown in Figure 1 *right)*. In detail, a steel tube was inserted from one flange, within which the samples can be placed, at a distance of about 10 cm from the plasma. A thin ($50\,\mu$m) plastic (kapton) window was used as vacuum-air interface. The electron propagation in vacuum and across the window up to the sample position was simulated using the Monte Carlo GEANT4 toolkit.[15]

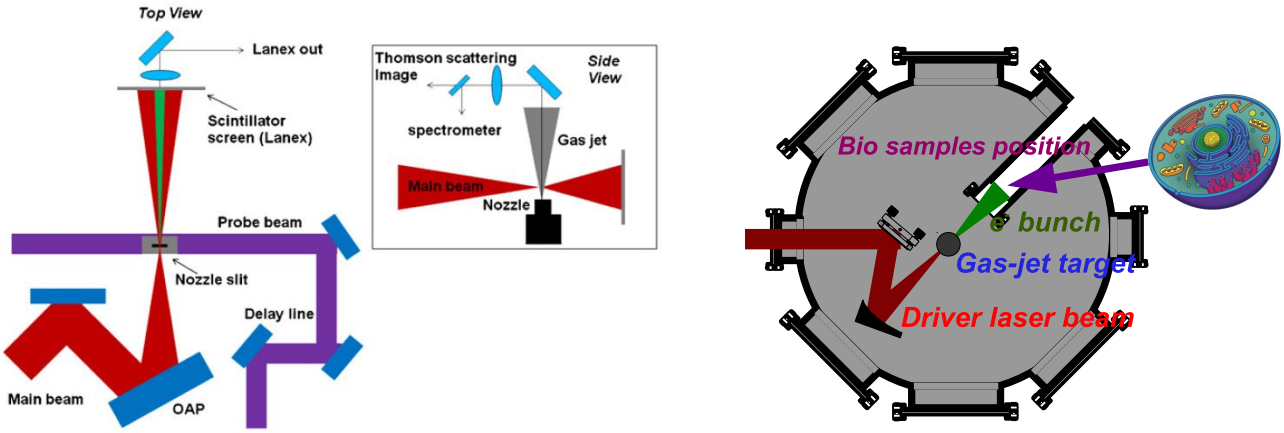

Figure 1. *left)* Schematic layout of ther experimental setup. The main laser-plasma interaction diagnostics are visible, namely interferometry and Thomson imaging (see the inset). The electron production was monitored by LANEX screen imaged out by CCD detectors and NaI scintillator coupled to photomultipliers. The electron bunch diagnostics are not shown in the Figure (see text). On the *right)*, the layout of the vacuum chamber as modified for the irradiation of biological samples is shown.

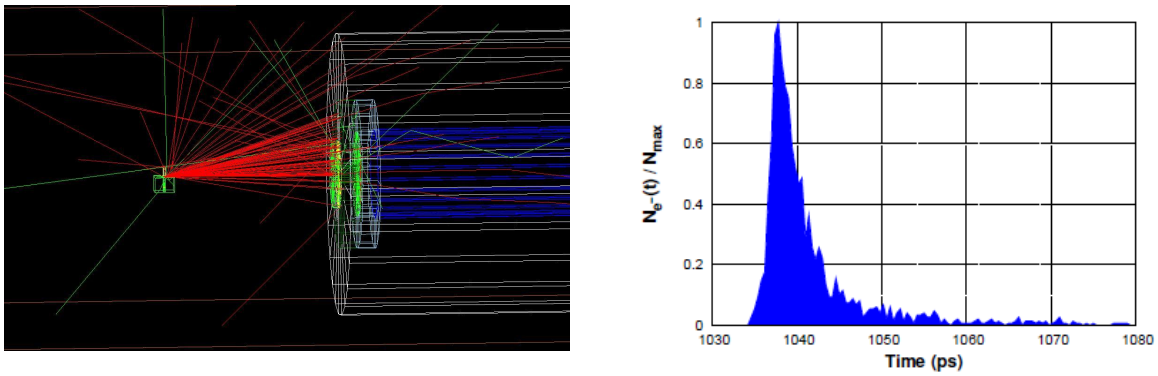

Figure 2. *top)* Scan image of three different layers of RCF. The distance from the electron source increases going from left to right. *bottom)* A pictorial view of the simulated setup (also showing a few particle tracks) (*left*) and the distribution of the arrival times of the electrons at the sample position as gathered from the GEANT4 simulations (*right*).

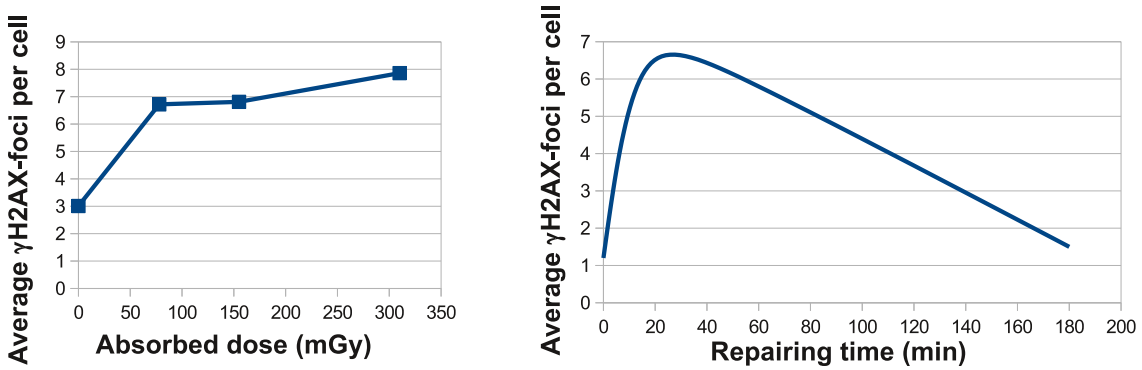

Figure 3. *left)* Average number of $\gamma - \mathrm{H2AX}$ foci as a function of the absorbed dose. *right)* Residual DNA DSBs at different times corresponding to an absorbed dose of 175 mGy.

The electron bunches were fully characterized, in terms of spectrum, divergence and total charge, using GAFChromic Films (GAF) packed in a stack and separated by solid water (see for instance[16] for an overview of the procedure). In particular, MD55-v2 GAF were used in our case, separated by RW3 slabs, water equivalent material (see www.ptw.de). The GEANT4 library was then used to retrieve the dose delivered to the biological samples. As an example, Figure 2 *top)* shows the scan of three GAF layers in the case of the He gas target (higher electron energy, as mentioned above), at increasing distances from the electron source as going from left to right. In particular, the first 2 layers were placed inside the vacuum chamber and the third one at the sample position. Figure 2 *bottom)* shows the simulated setup, with a small sample of the particle tracks. On the right, the distribution of the arrival times of the electrons at the sample position is shown, as gathered from the GEANT4 simulation, starting with an infinitely short bunch. The width of this curve, of the order of a few ps, just confirms that, even after the passage through the vacuum-air interface, electron bunches with duration 5-6 orders of magnitudes smaller than the ones produced by RF LINACs can reach the samples in air. The dose delivered to the samples as retrieved by the Monte Carlo simulations was estimated to be ~ 3.5 mGy/shot when using the N_2 gas (electron energy below 1 MeV) and ~ 5.7 mGy/shot when using He gas (electron energy up to a few MeV).

3. FIRST RADIOBIOLOGY TESTS

In what follows we briefly discuss, as an example, some of the biological tests we have carried out.

As it is known, DNA double-strand breaks (DSB) are a major form of DNA damage and a key mechanism through which radiotherapy and some chemotherapeutic agents kill cancer cells. Nuclear $\gamma - \mathrm{H2AX}$ foci represent a marker for DSB formation and the first detectable response of cells to DSBs. We used the $\gamma - \mathrm{H2AX}$ foci assay to measure the induced DSBs on human lymphocites irradiated with the ultrashort bunches described above. In particular, this test was carried out with the lowest energy bunches (obtained using the N_2 gas); the samples were irradiated using different numbers of cumulated laser shots (typically from a few tens up to a few hundreds of shots) in order to assess the DNA damage at different doses. Figure 3 *left)* shows the average number of $\gamma - \mathrm{H2AX}$ foci as a function of the absorbed dose. Given that on average $0.2 - 0.4$ foci are induced per 10 mGy per cell when using LINAC electrons,[17,18] this would suggest an absorbed dose comparable to the one actually delivered in our case. As a comparison, we report that an average of 0.3 foci per cell are induced per 10 mGy of absorbed dose from standard (LINAC accelerated) electron bunches (citazione). Figure 3 *right)* shows the residual DNA DSBs at different times corresponding to an absorbed dose of 175 mGy. Cells were fixed after 15min, 1hr and 3 hr; a maximum foci level was observed at 15min (5.9 foci/cell). Our first preliminary data showed a radiobiological response as mirrored by the induction and repair of DNA double-strand breaks assessed by $\gamma - \mathrm{H2AX}$ foci after irradiation in vitro of human cells with the ultrashort electron bunches.

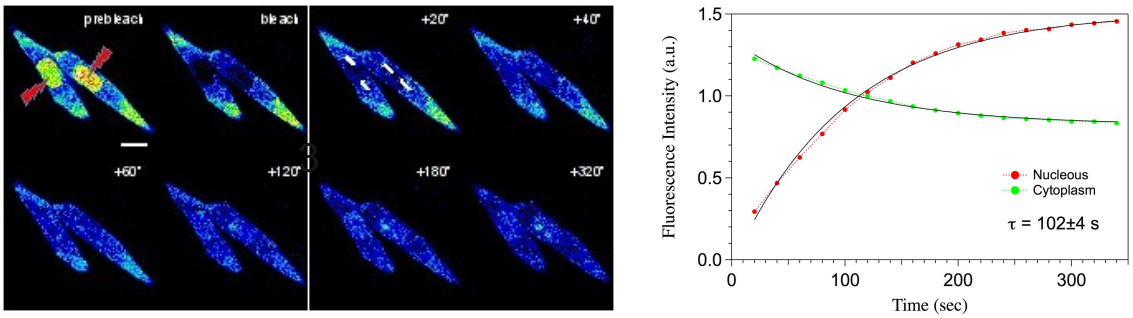

Figure 4. *left)* FRAP time-lapse collected on two NLS-GFP transfected CHO cells. *right)* Analysis (monoexponential fitting) of FRAP recovery curves.

A different test was carried out in order to evaluate the possible effects on the biochemical mechanisms that regulate the nuclocytoplasmic translocation in standard CHO cells and thereby on protein/RNA shuttling at intracellular level. The spatial separation of transcription and translation functions demands finely-tuned transport mechanisms between nucleus and cytoplasm in order to maintain the distinctive composition of each compartment. In this respect the nuclear envelope (NE) plays a crucial role since it is the barrier through which proteins and RNA must be transported in a regulated manner.[19–21] The mediators of this exchange are nuclear pore complexes (NPCs). These comprise multiple copies of about 30 distinct proteins collectively called nucleoporins. Transport across the NPC was reviewed in detail;[19,21,22] we refer to these Refs for more details.

In recent years a new approach to study the kinetic features of nucleocytoplasmic translocation has been developed (for a review, see[23]). More explicitly, it has been set up a method relying on combination of time-resolved microscopy imaging techniques probing molecular fluxes (FRAP: Fluorescence Recovery After Photobleaching) and steady-state microscopy imaging techniques probing protein binding (FRET: Forster Resonance Energy Transfer) and intracellular concentrations.[24,25] We refer to the given References for more details.

On account of the involvement of nucleocytoplasmic translocation in most regulative biochemical pathways of the cell, the aforementioned FRAP approach is particularly suitable to study alterations of the physiological state of cell samples. Indeed, changes in the cell capability to relocate protein and RNA cargoes are known to be strictly related to apoptotic mechanisms and the onset of several pathologies. In our case, we exposed CHO cells transiently expressing NLS-GFP to 175 mGy and we evaluated the modifications in the translocation time (τ) and permeability to passive diffusion (P) as compared to a set of non-exposed cells. As an example of the obtained results, Figure 4 *left)* shows a time-lapse within few milliseconds after bleaching. The model of nucleocytoplasmic exchange in the presence of both passive diffusion and active transport shows that the concentration of fluorescent species in the cytoplasm and nucleoplasm follows a first-order kinetics. Consequently the collected FRAP curves in both compartments were fitted to a single exponential, as the fluorescence signal is assumed proportional to the concentration (Figure 4 *right)*). Moreover, all images were normalized to the prebleaching signal to identify the presence of an immobile fraction. Data analysis[23] afforded $< \tau >= 135 \pm 69\,\mathrm{s}$ for control cells (#10 cells) and $< \tau >= 152 \pm 97\,\mathrm{s}$ (#14 cells) for irradiated cells; T-test indicated that the two data sets were not statistically significant ($p = 0.64$). As for the passive permeability, data analysis yielded $P = 7.5 \pm 5.7\,\mu\mathrm{m}^3/\mathrm{s}$ for control cells and $P = 5.7 \pm 3.3\,\mu\mathrm{m}^3/\mathrm{s}$ for irradiated cells, and the T-test afforded p=0.4, again showing the negligible differences between the two data sets. Thus, this preliminary analysis indicate that the radiobiological effect of the electron source is not acting on the biochemical mechanisms that regulate the nucleocytoplasmic translocation in standard CHO cells and thereby on protein/RNA shuttling at intracellular level. Although other effects cannot be ruled out, the comparison with other cell models could help elucidating whether the radiobiological exposure exerts some or no action on this fundamental biochemical system.

4. SUMMARY AND CONCLUSIONS

We have reported on the setup and characterization of a laser-driven electron accelerator for radiobiological studies. As a starting point, different human cells (namely, lymphocytes, fibroblasts and CHO cells) have been

irradiated using laser-accelerated electrons at low energy (up to a few MeV) and the induced biological effects in terms of cell alteration and survival have been studied by means of well-established biological techniques (scoring of $\gamma - H2AX$, FRAP). The absorbed dose was studied by combining experimental measurements and Monte Carlo (GEANT4) simulations. A preliminary discussion of the obtained results has been given; in particular, our experiments showed a DNA DSB damage similar to the one induced by conventional electron beams, while no appreciable alteration was found affecting the biochemical mechanisms that regulate the nuclocytoplasmic translocation.

ACKNOWLEDGMENTS

We acknowledge support from the italian Ministry of Health through the project GR-2009-1608935 "Study of Radiobiological and Radiotherapic Effects of a Novel Laser Driven Electron Accelerator" (bando Giovani Ricercatori 2009 - Destinatario Istituzionale Agenzia Nazionale per i Servizi Sanitari Regionali - AgeNaS). The High Field Photonics Unit (MD.P03.034) at INO-PI acknowledges financial support from CNR through the Project "ELI-Italy".

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