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Pulsed radiobiology with laser-driven plasma accelerators

Antonio Giulietti, Maria Grazia Andreassi*, Carlo Greco^

Istituto Nazionale di Ottica del CNR - www.ino.it
Sezione "Adriano Gozzini", Area della Ricerca di Pisa
Via Moruzzi 1, 56124 Pisa, Italy

*Istituto di Fisiologia Clinica del CNR, Pisa, Italy
^Department of Oncology, University of Pisa, Italy

Abstract

Recently, a high efficiency regime of acceleration in laser plasmas has been discovered, allowing table top equipment to deliver doses of interest for radiotherapy with electron bunches of suitable kinetic energy. In view of an R&D program aimed to the realization of an innovative class of accelerators for medical uses, a radiobiological validation is needed. At the present time, the biological effects of electron bunches from the laser-driven electron accelerator are largely unknown. In radiobiology and radiotherapy, it is known that the early spatial distribution of energy deposition following ionizing radiation interactions with DNA molecule is crucial for the prediction of damages at cellular or tissue levels and during the clinical responses to this irradiation. The purpose of the present study is to evaluate the radio-biological effects obtained with electron bunches from a laser-driven electron accelerator compared with bunches coming from a IORT-dedicated medical Radio-frequency based linac's on human cells by the cytokinesis block micronucleus assay (CBMN). To this purpose a multidisciplinary team including radiotherapists, biologists, medical physicists, laser and plasma physicists is working at CNR Campus and University of Pisa. Dose on samples is delivered alternatively by the "laser-linac" operating at ILIL lab of Istituto Nazionale di Ottica and an RF-linac operating for IORT at Pisa S. Chiara Hospital. Experimental data are analyzed on the basis of suitable radiobiological models as well as with numerical simulation based on Monte Carlo codes. Possible collective effects are also considered in the case of ultrashort, ultradense bunches of ionizing radiation.

Introduction

CPA technology allowed laser systems to achieve unprecedented e.m. field intensities in focused femtosecond pulses. Powerful femtosecond lasers, coupled with suitable focusing optics, can shoot into matter optical "bullets" whose transversal size (the spot size) is comparable with the longitudinal size (the pulse length) and whose photon density is of the order of 10^{27} phot/cm^3. The oscillating electric field in the bullet volume can exceed 10^{12} V/cm, much higher that

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atomic fields. When interacting with matter, such pulses are able to ionize atoms in a time of the order of a single optical cycle. Free electrons are then moved by the oscillating electric field to relativistic quiver velocities. This is the new exciting scenario of high field photonics that can be investigated in dense plasmas produced by the laser itself. The interaction is obviously highly non-linear and a wide class of new phenomena can be studied, starting from the pulse propagation. One of the most striking results in this investigation has been the evidence of propagation of intense femtosecond pulses in plasmas whose density is well above the critical density (i.e. the density above which propagation is not allowed by the classical linear theory). Though the interpretation of this effect is still controversial, it can have important consequences and applications. On the other hand the propagation of intense femtosecond pulses in under- dense plasmas has to be carefully investigated in view of relevant applications. Besides the non linear aspects, a critical point is the presence of precursors of the fs pulse which can modify the medium before the arrival of the main pulse and modify consequently its propagation. These aspects are particularly sensible for the use of intense fs laser pulses to accelerate electrons in plasmas.

When focused in a suitable underdense plasma, the e.m. fields of such pulses are able to support electron waves of very large amplitude, whose longitudinal electric field can accelerate free electrons up to relativistic electron energies with local acceleration fields several orders of magnitude higher than in ordinary accelerators. A number of schemes for laser driven acceleration of electrons in plasmas have been proposed and studied, some of which suc-
cessfully tested. New experimental records have been reported in the recent literature, about the maximum electron energy achieved, the minimum energy spread, as well maximum collimation, stability, and so on. These records are in general obtained with lasers of outstanding performances and/or with very sophisticated methods hardly applicable in practical machines. A special effort is devoted to pre-form conditions in which the focused laser pulse can propagate at the peak intensity beyond the length (Rayleigh length) allowed by the difraction of the focusing optics. Partially successful techniques include capillary discharges and pre-formed plasma channels.

The scientific and technological path towards the realization of competitive laser driven accelerators still demands a considerable op-grade of many performances, including energy spread, collimation and pointing stability of the electron bunches, high repetition rates with reproducibility of the bunch parameters. Though we will briefly review the main results obtained in this path so far, the main object of this report will be to evaluate the possibility of having now tabletop sources of relativistic electrons driven by ultrashort, intense laser pulses. In particular, there is a specific interest to sources of electrons suitable for an e_cient activation of radionuclides. To this purpose, extreme performances in terms of energy, energy spread, collimation and pointing stability are not required. On the other hand, electrons of energy of tens of MeVs, fairly collimated, with high average current (i.e. high charge per bunch at high repetition rate) have to be produced e_ciently and reproducibly. Recent experiments proved that such requirements are close to be achieved with tabletop lasers (pulse peak power 10 TW) and gas jets. Detailed studies on the laser pulse propagation in a gas (plasma), on the acceleration mechanism and on the radio-activation method gave a basic contribution in obtaining such promising results. We will report and discuss those studies and their main results in order to address further investigations and technological improvements towards the realization of a source for practical uses.

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In all this variety of investigations a crucial role is played by novel diagnostic tools that are devoted to characterize a) the laser pulse intensity distribution (both in space and time) and spectrum before and after the interaction, b) the plasma during the interaction, c) the energetic electron bunches produced by the interaction.

Recently, a high efficiency regime of acceleration in laser plasmas has been discovered at CEA-Saclay SLIC laser facility by an European team of scientists [1]. This regime allows table top equipment to deliver doses of interest for radiotherapy with electron bunches of suitable kinetic energy. An experimental facility delivering relativistic electrons from a few MeV up to a few tens of MeV is available at Intense Irradiation Laboratory in Pisa. The National Research Council (Italy) launched an R&D program aimed to the realization of an innovative class of accelerators for medical uses, based on laser techniques of acceleration. In this framework, a preliminary radiobiological validation is needed. At the present time, the biological effects of electron bunches from the laser-driven electron accelerator are largely unknown. In radiobiology and radiotherapy, it is known that the early spatial distribution of energy deposition following ionizing radiation interactions with DNA molecule is crucial for the prediction of damages at cellular or tissue levels and during the clinical responses to this irradiation [2-4]. The purpose of the present study is to evaluate the radio-biological effects obtained with electron bunches from a laser-driven electron accelerator compared with bunches coming from a IORT-dedicated medical Radio-frequency based linac's on human cells by the cytokinesis block micronucleus assay (CBMN). To this purpose a multidisciplinary team including radiotherapists, biologists, medical physicists, laser and plasma physicists is working at CNR Campus and University of Pisa. The team provides a complete spectrum of expertise from radiotherapy of tumors to preparation samples, testing of biological effects, dosimetry, set up and control of the high power laser equipment, set up and control of the laser-plasma mini-linac. Dose on samples is delivered alternatively by the "laser-linac" operating at ILIL lab of Istituto Nazionale di Ottica and an RF-linac operating for IORT at Pisa S. Chiara Hospital. Experimental data are analyzed on the basis of suitable radiobiological models as well as with numerical simulation based on Monte Carlo codes. Possible collective effects are also considered in the case of ultrashort bunches of ionizing radiation.

Towards clinical issues

Results obtained by the group of Pisa leaded by A Giulietti in collaboration with the group of CEA-Saclay, showed that a high efficiency regime for laser driven electron acceleration particularly suited for the generation of electron beams of potential clinical interest can be obtained by using table-top, so-called TW-class laser systems. Such results boosted further studies (both experimental and theoretical) on the possible use of such beams in radiotherapy and recently led to the submission of an European Patent proposal by the CNR for the development of a clinical device [5].

A laser-linac (L-linac) has been setup at the Intense Laser Irradiation Laboratory of the INO-CNR (http://www.ino.it) and a preliminary characterization of the produced electron pulses has been carried out in the past few months. In particular, the following features have been studied and consolidated:

- bunch total charge: about 100 pC/per laser shot (the L-linac based source could in principle be operated at a 10Hz repetition rate)

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- bunch transverse homogeneity: the bunch features a gaussian angular spread with typical aperture of a few degrees. Possible setups leading to the required homogeneity on the sample have been being studied by means of Monte Carlo simulations (see below)
- bunch spectrum: electron bunches with kinetic energy up to around 25 MeV can be reliably obtained; the exact spectral shape can be already controlled to some extent
- shot-to-shot stability and reproducibility: an acceleration regime in which close to a 100% reproducibility in terms of accelerated energy and charge has been steadily reached. Furthermore, the possible spatial non-homogeneities of the delivered dose arising from the shot-to-shot small (mrad) pointing deviations have been observed to be fully ruled out over an area of interest by integrating the dose over about ten L-linac shots

Based on these outputs, for the L-linac based research to be carried out within this work, the figures reported in the Table 1 can be foreseen. The table also shows the corresponding values as obtained in the experimental campaign carried out by the INO-CNR group at CEA-Saclay using a slightly more powerful laser system (the same conditions are expected to be obtained at INO-CNR in the next months by an upgrade of the existing laser system). Also, the corresponding figures for a conventional IORT accelerator are reported for the sake of comparison .

In the past few weeks, the L-linac based e-source has been used to carry out "test" experiments on different samples under vacuum. In particular, electron radiography imaging has been performed of few mm thick biological samples. The results agree with expectations. Recent efforts have been also devoted to the design and study, by means of Monte Carlo simulations, of a vacuum-air interface for the produced e-bunches as well as to the design of a plastic collimator and homogeneizer [6]. As an example of the obtained results, Fig. 1 shows the changes experienced by the electron pulse passing through a 500-micron thick Cu-made vacuum-window interface.

At the same time, based upon such a kind of simulations, a mechanical system allowing the irradiation of biological samples in air has been designed and is currently in operation. The system enables controlled and reproducible e-bunch irradiation in air of samples a few cm^2 surface. Finally, dose measurements can be easily performed within the present irradiation setup in collaboration with the Department of Oncology of University of Pisa.

	LIAC (Sordina SpA)	**INO-CNR L-linac (CEA-Saclay L-linac†)**
e^- energy	$< 12\,\mathrm{MeV}$ $(4, 6, 9, 12\,\mathrm{MeV})$	$< 25\,\mathrm{MeV}$ $(< 45\,\mathrm{MeV}^\dagger)$
peak current	$1.5\,\mathrm{mA}$	$0.1\,\mathrm{kA}$ $(1.6\,\mathrm{kA}^\dagger)$
bunch duration	$1.2\,\mu\mathrm{s}$	$< 1\,\mathrm{ps}$
bunch charge	$1.8\,\mathrm{nC}$	$0.1\,\mathrm{nC}$ $(1.6\,\mathrm{nC}^\dagger)$
rep. rate	$5 - 20\,\mathrm{Hz}$	$10\,\mathrm{Hz}$
average current	$18\,\mathrm{nA}$ (@10 Hz)	$1\,\mathrm{nA}$ $(16\,\mathrm{nA}^\dagger)$

† figures corresponding to the results obtained at CEA-Saclay (Giulietti A. *et al.*, *Phys. Rev. Lett.* **101**, 105002 (2008))

Table 1. Comparison between a currently Hospital employed Linac (left) and L-linac's set at INO-CNR, Pisa and CEA-Saclay

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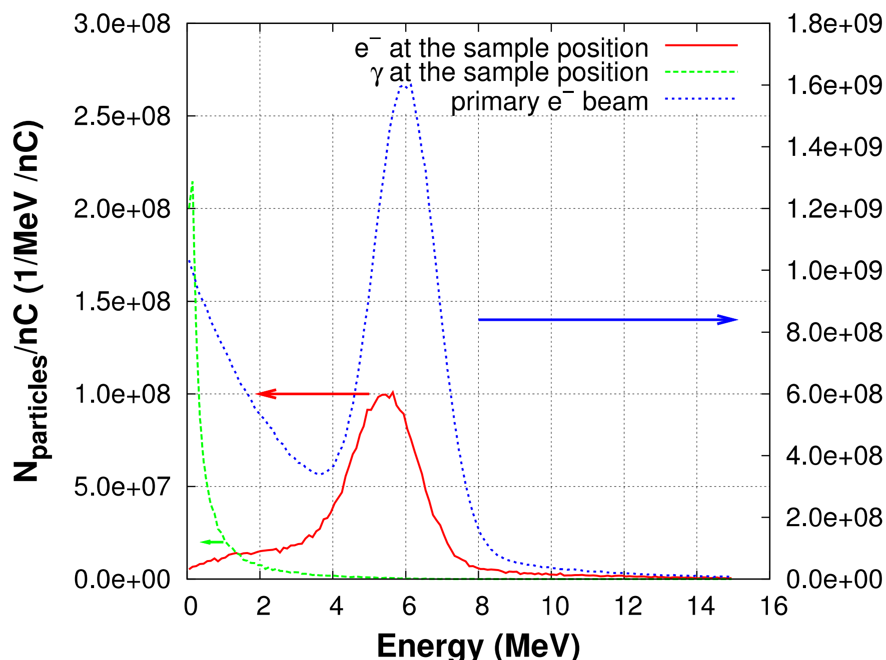

Figure 1. Simulated spectra of electrons (red) and gamma rays (green) generated by electrons (original spectrum in blue) passed through 500-µm thick Cu window.

This work brings together for the first time people with an expertise in the fields of physics, medicine and biology, in order to study and assess the biological effects of electrons produced by Laser-linacs and aims at the setup of an innovative source of relativistic electrons, based upon laser-plasma acceleration, to be used in biology and medecine. In particular, the laser-plasma source already available at the Intense Laser Irradiation Laboratory of the Istituto Nazionale di Ottica of the CNR has been equiped in order to make it possible to perform electron irradiation experiments of biological samples in air. This will allow a full radiobiological characterization of the electron source.

Radiobiological studies

Full dosimetric characterization

As a first step, a full characterization of the electron source (laser-linac) already available at the CNR Unit will be carried out, both from a physical and radiological point of view. The accelerator is based upon a >3 TW ultrashort (40 fs duration) laser system. The system can deliver up to 150 mJ laser pulses at the laser fundamental frequency (800 nm) at a 10 Hz repetition rate. The laser pulse is focused onto a supersonic gas-jet target inside a vacuum chamber, at an

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intensity up to 10^{19} W/cm^2. Electron bunches with an energy up to 25 MeV can be reliably obtained, with total charge per pulse around 100 pC.

The vacuum chamber has been equipped with a simple vacuum-air interface made up by a thin window, similar to the one acting both as an homogenizer and as an interface in conventional RF linac for medical use. The effect of the window on the electron bunches has been modelled by means of Monte Carlo simulations

based upon the CERN library GEANT4. Based upon these simulations, a collimator tube for the electron bunches will be eventually built, similar to the perspex tubes currently used in the RF linac for IORT. The total charge per shot will be monitored by means of an ICT device just acquired by the ILIL laboratory. Furthermore, a measurement of the electron bunch duration is expected to be carried out in the framework of the work by means of an electro-optics method. This is a particular challenging task of the work, as electro-optics methods, which have been used so far only for the measurement of the duration of electron bunches from RF linacs, requires a “clean” bunch to give reliable results. From a dosimetric point of view, the characterization of the source has beeing carried out in collaboration with Department of Oncology of University of Pisa. After such a dosimetric characterization, the study of the radio-biological effects of the electron bunches from the laser source has been carried out by comparing the effects of electrons from a RF linac used for IORT.

Cell lines, culture conditions and irradiation

The following cell lines are used in the experiments: OVCAR-3 (human ovarian cancer cell line) and F98-glioma (rodent glioma model. Cell lines are maintained in Eagle's MEM (Gibco) or RPMI 1640 supplemented with heat-inactivated FBS (10%), penicillin (100 units/ml), and streptomycin (100 µg/ml) in a humidified atmosphere consisting of 5% CO2 at 37°C. Peripheral blood of healthy volunteers are collected using heparin as an anticoagulant; cultures from each sample are set up by mixing 0.3 mL of whole blood with 4.7 mL of Ham's F12 medium (ICN, Irvine, USA), supplemented with 10% fetal calf serum (ICN), 1.5% phytohaemagglutinin (Wellcome, UK) and antibiotics (penicillin 100 IU/mL and streptomycin 100 mg/mL, Sigma, St Louis, MO, USA).

All cultures are incubated at 37°C in a humidified atmosphere of 5% CO2 and 95% O2. Cell cultures are exposed alternatively to electron of kA currents from the novel laser-driven accelerator and electrons of mA currents from conventional RF-accelerators for IORT, at varying doses and times that are evaluated of interest for the radiobiological analysis in order to determine dose-response. Exponentially growing OVCAR-3 cells and F98-glioma are irradiated in 100-mm culture flask. Dosimetry and irradiation of the human lymphocytes [7] are carried out at 30 cm from the beam exit point of the accelerator. For irradiating the samples, polypropylene vials of 1 cm diameter are used. After exposure to electron pulses, cells are harvested after 48h. Cells are rinsed, trypsinized, pelleted, and resuspended in cold 1x PBS.

Cytotoxicity Assay

Cytotoxicity and cell survival [8] are determined for OVCAR-3 (human ovarian cancer cell line) and F98-glioma (rodent glioma model) cell lines by the3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide (MTT) assay

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(Mossmann 1983). In brief, cells are planted at 5000 cells/well in 96-well microtiter plates. During the final 2 h of incubation, MTT (0.5 mg/ml, Sigma) are added to each well. At the termination of the experiment, the culture media are removed, and the precipitated dye are solubilized in 100% DMSO. The absorbance of each well are determined using the microplate reader at 595 nm. The percentage of cell survival are defined as the relative absorbance of control versus treated cells.

MN assay abd CA test

In radiobiology and radiotherapy, it is known that the early spatial distribution of energy deposition following ionizing radiation interactions with DNA molecule is crucial for the prediction of damages at cellular or tissue levels and during the clinical responses to this irradiation. The evaluation of the radiobiological effects obtained with electron bunches from a laser-driven electron accelerator and from bunches coming from a
IORT-dedicated medical Radio-frequency based linac on human cells are performed by the cytokinesis block micronucleus assay (MN assay) and chromosome aberrations test.
Cytochalasin B (6 µg/ml) are added 44 hs after culture initiation and following irradiation (24 hrs) in human lymphocytes. In cell lines, 2 hours after irradiation, Cytochalasin B diluted in fresh medium are added to cultures to obtain final concentrations of 0.5-3 µg/ml. Cells are harvested and fixed according to the
standard method in use in our laboratory (Andreassi et. 2006). For each sample, 1000 binucleated are be scored by use of an optical microscope (final magnification ×400) for MN analysis, following the criteria for micronucleus acceptance We quantify the micronucleated binucleated cell frequency as the number of micronucleated cells per 1000 cells. For the CA analysis, the cultures are fixed after 48 h of incubation, following a terminal 2 h treatment with colcemid (final concentration 0.1 mg/mL), in order to arrest the lymphocytes in metaphase.

Immunofluorescence staining and scoring of -H2AX

For immunofluorescence staining, approximately 500 µl of cells are centrifuged. The cells are fixed on polysine slides using 3 % Paraformaldehyde in phosphate buffered saline (PBS) and afterwards put in PBS. Permeabilization of the cells are carried out by placing the slides on ice and dripping TritonX-100 solution on each slide. Immunofluorescence staining of the cells are performed using anti-phospho-histone H2AX (1:300) as primary antibody and RAM-TRITC (1:1000) as secondary antibody. The slides are dried and DAPI (2 %) will be added to the cells before placing a cover glass on the slides. The slides are observed using a fluorescent microscope attached to a CCD camera connected to a computer. A free software (FociCounter available at http://focicounter.sourceforge.net) are used to randomly analyze foci of 250 cells per sample. The number of foci induced by X-rays exposure are calculated by subtracting the number of foci per cell in blood samples untreated and treated.

Lipid membrane fluidity alterations

Cells will be loaded with TMA-DPH as described by Petty et al. (PETTY 1987). Steady-state anisotropy, decay time and time-dependent anisotropy are measured with Edinburgh OB 920 time-resolved spectrofluorometer.

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Plasma membrane permeability

The assay (LINDHAGEN 2008) is based on hydrolysis of the probe, fluorescein diacetate (FDA), by esterases and retaining of fluorescein in cells with intact plasma membranes. FDA are added to the cell suspension to a final concentration of 20 µg/ml, and cells are incubated in a 37°C humidified incubator for 10 min.
Cells will be centrifuged, the supernatant are removed, and the cells will be resuspended in 0.5 ml PBS. The cell suspensions are placed in test tubes and kept ice-cold during fluorescence analyses.

2D-PAGE and mass spectrometry

To obtain cell extracts for proteome analyses, cells are washed three times with physiological saline (PBS) for 10 min with gentle shaking, and lysed in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 1% DTT). The samples are shaken for 1 h at room temperature and centrifuged for 10 min at 16,000 g. The supernatants
are precipitated in 10 volumes of cold acetone, and proteins are dissolved in Isoelectric focusing (IEF) buffer and quantified by BioRad protein assay. Immobilized pH gradient (IPG) strips are rehydrated passively overnight in strip holders with 300 µl IPG buffer containing 250 µg of protein. IEF run and subsequent 2D-PAGE are carried out as described by Zhang et al. (ZHANG 2003). Proteins are visualized by either silver staining or PhastGel Blue R (GE Healthcare Bio-Sciences). Protein patterns in gels are recorded as digitalized images using a high-resolution scanner and the intensity of each spot are compared and analyzed using PDQuest software (BioRad, Hercules, CA). Proteins with different expression levels of more than 1.5-fold between control and treated cell line progress to in-gel trypsin digestion and analyzed and identified using a MALDI-TOF mass spectrometer.

Conclusion

A variety of techniques have been eployed to assess the effects on biological samples of ultrashort, ultradense, ionizing electron pulses produced by a laser-driven accelerator and compare them with irradiation by conventional accelerators. Preliminary results are under analysis also supported by ad-hoc numerical simulations. Final results will be of great relevance in validating Laser-linac's for clinical purposes.

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