Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/12675
DOI URL: http://dx.doi.org/10.21474/IJAR01/12675

RESEARCH ARTICLE

AFFECT OF ULTRASHORT ELECTRON BEAMS ON THE *ESCHERICHEA COLI* SURVIVAL

G.E. Khachatryan[1], N.I. Mkrtchyan[1], S. Sh. Tatikyan[2], V.B. Arakelyan[2,3], B.A. Grigoryan[2] and V.M. Tsakanov[2]

1. Alikhanyan National Science Laboratory (Yerevan Physics Institute) (Yerevan, Armenia).
2. Candle Synchrotron Research Institute (Yerevan, Armenia).
3. Yerevan State University (Yerevan, Armenia).

Manuscript Info

Manuscript History
Received: 05 February 2021
Final Accepted: 10 March 2021
Published: April 2021

Key words:-
Cell survival, *E.coli*, AREAL, Ultrashort beam, Femtosecond bunch

Abstract

The paper represents the study of ultrashort electron beams impact on some *Escherichia coliK-12*strains with different radiosensitivity. The charged particle beams generated by ultrashort bunch accelerators differ by short duration of particle direct exposure, relatively long intervals between bunches and by high values of instantaneous dose rates. Because of these characteristics, the nature of ultrashort beams impact on biological objects may sufficiently differ from conventional sources of radiation. As a source of ultrashort beams linear electron accellerator AREAL of Sychrotron Researche Institute CANDLE (Yerevan, Armenia) was used. The dependence of E.coli cells survival from electron bunches repetition rate and irradiation media was investigated. It is shown that the dose dependence of the survival degree of microorganisms has a qualitatively different unusual "concave" shape. Such behavior of the survival curves does not depend on the ionic composition of the irradiation medium as well as on the time of preliminary incubation of microorganisms in this medium. To explain the observed phenomenon, it was assumed that the compensatory capacity of the irradiated object increases with an increase of the irradiation dose.The proposed mathematical model described well the behavior of the survival curves. It is assumed that this change of the compensatory capacity may be determined by oxygen.

Introduction:-

In 2016 a group of researchers from A. I. Alikhanyan National Science Laboratory (Yerevan Physics Institute) and CANDLE Synchrotron Research Institute (Yerevan, Armenia) announced the initiation of a study in the field of space biology problem modelling in Earth conditions. The goals of the planned research were represented (Khachatryan et al., 2016). As a first step model studies were carried out on classic *E.coli* strains with different radiosensitivity. Currently, new technologies are intensively being developed in accelerator technology based on the application of ultrashort high frequency lasers (Hada et al., 2012,Villa et al., 2017, Uesaka et al., 2002). Such accelerators have femtosecond beams and high instantaneous dose values. One of these is AREAL electron accelerator, developed and constructed at CANDLE Institute (Tsakanov et al., 2016) with up to 5 MeV beam energy, 400 fs pulse duration and 2-50 Hz frequency. Ultra-high dose rates (UHD) of the electron beam are more

Corresponding Author:- S. Sh. Tatikyan
Address:- Candle Synchrotron Research Institute, (Yerevan, Armenia).

effective when some cancer cell lines (KPC and Panc02) are irradiated (Venkatesulu et al., 2019). The ratio of D10 values obtained using UHDR and conventional sources of electrons is 1.31 – 1.36. It may be expected that the application of ultrashort beams may lead to qualitatively new results and may be applied in radiotherapy, biotechnology and other fields.

The main aim of this work is to carry out a comparative investigation of the effect of conventional and ultrashort electron beams on microorganisms.

Materialsand Methods:-

Materials:

The research was carried out on *E.coliK-12* strains *AB 1157* - wild type, *BL 1114* – radioresistant, *AB 2463* – radiosensitive, which were kindly provided by Prof. V. Verbenko (Head of the Laboratory of Molecular Genetics of PNPI, Kurchatov Center, St. Petersburg). Genetic description of strains is given in (Verbenko, 1994, Verbenko et al., 2000).

Growth media:

For the cultivation of tested bacteria, a standard nutrient medium – Meat Peptone Agar (MPA) was used. All used chemicals were of analytical grade or higher except the technical agar-agar (Japan) that was carefully washed with a large amount of distilled water.

Table 1:- Approximate ionic composition of the low-ion drinking water.

Ion	C, g/L
HCO_3^-	0.17
SO_4^{2-}	0.02
Cl^-	0.026
K^+	0.003
Na^+	0.027
Mg^{2+}	0.014
Ca^{2+}	0.03
pH	7.0

Irradiation procedures:

The cell suspension for irradiation was obtained by inoculation of 5 ml of sterile irradiation medium with cell material from the colonies grown on Petri dishes, containing MPA, within 18-24 hours.Glass test tube with suspension was vigorously shaken on a Vortex for 2 min, after which1.8 ml suspension was immediately transferred into a 2 ml round-bottom micro-tube with lid and fixed on a special holder just in front of electron beam for irradiation. As irradiation media 50 mM Potassum-Phosphat buffer with pH 7.0 (KPh) and low-ion drink water (DW) were used. The salt composition of drink water is presented on the table 1. The concentration of the cells in the original suspension was approximately 10^9 colony-forming units (CFU). The strains were irradiated on AREAL (CANDLE Synchrotron Research Institute). During the experiments the electron beam energy was 3.5 MeV, pulse duration was 0.4 ps, the pulse frequency was 6, 12 and 20 Hz, average doze rate were 5.77, 11.54 and 19.23 Gy/min respectively. The instantaneous dose rate was not depend on pulse frequency and was equal to 4.10^{10}Gy/sec. All the procedures were held at 20 ^oC.

Dose calculation:

The charge value of electron bunches was registered during the irradiation of control and experimental samples with the Faraday Cup. As a control an empty tube was used. For the calculation of absorbeddose values the following formula was used (Petrosyan, 2017).

$$D[Gy] = \frac{\varepsilon[eV]\times\Delta Q[c]\times R[Hz]\times T[s]}{m\,[kg]} \qquad (1)$$

where D is absorbed dose, ε – electron beam energy, ΔQ – amount of the charge, absorbed by the sample, R – bunch frequency, T – time and m - masse of the sample.

Determination of Cell Survival:

Right after the irradiation, cell suspension was diluted to concentrations convenient for further counting of the grown colonies (thus only the CFU are registered), inoculated on MPA dishes and incubated in a thermostat, at

37°C, for 24-36 hours. To determine the number of survived cells the suspension was diluted for 10^5-10^7 times for control and depending on the dose from 0 to 10^5 times for irradiated samples (three dilutions in step of 10x in triplicate). After dilution 0.1 ml of suspesion was inoculated on MPA. For counting, the dishes containing from 100 to 500 grown colonies were selected. The mathematical processing of the results was done by Wolfram Mathematica v11.1.

Results:-

In Figure 1 the experimental setup of the accelerator is represented.

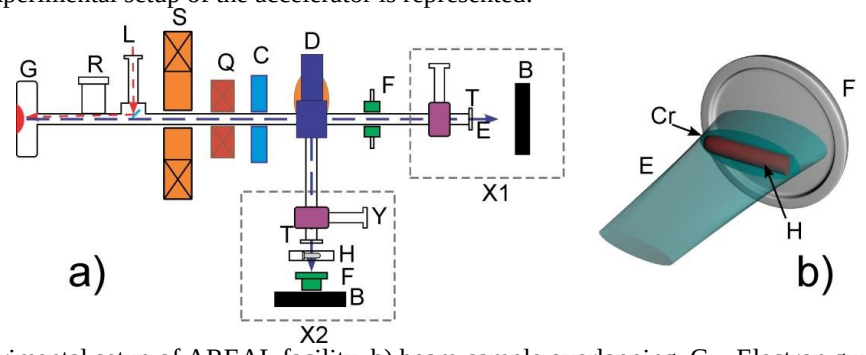

Figure 1.a) Experimental setup of AREAL facility, b) beam-sample overlapping. G – Electron gun, R – RF input, L – Laser beam, S – Solenoid magnet, C – Corrector magnet, D – Dipole magnet/spectrometer, Y – YAG screen, T – Titanium window, E – Electron beam, B – Beam dump, H – Sample holder, F – Faraday cup, X1, X2 – Experimental stations, Cr – beam cross-section.

AREAL (Advanced Research Electron Accelerator Laboratory) is an electron linear accelerator facility (Tsakanov et al., 2016) based on photocathode RF gun. The facility generates ultrashort electron bunches with a particle charge up to 800 pC. For irradiation researches, sub-picosecond long electron bunches with energies up to 5 MeV guarantee a short interaction time with the sample material. Two experimental stations of AREAL facility give a possibility to perform irradiation experiments in-air. X2 experimental station is located on the spectrometer magnet bent arm, which allows avoiding secondary emitted electrons and gamma radiation. Quite accurate dose calculation can be performed using electron bunch charge measurements from a movable Faraday Cup, placed behind the experimental sample.

In the first series of experiments the cell suspensions were irradiated by a beam with bunch frequency of 12 Hz in both irradiation media – KPh and DW. In Figure 2 the dependence of various *E.coli* strains survival on dose is shown. Hereinafter each point represents the average value of three independent experiments. As is seen from the picture, the form of the curves is different from a classical one and representing an L-shape form. With a change of irradiation medium salt composition, some change in the value of D10 was observed, but this did not affect the character of the curves obtained.

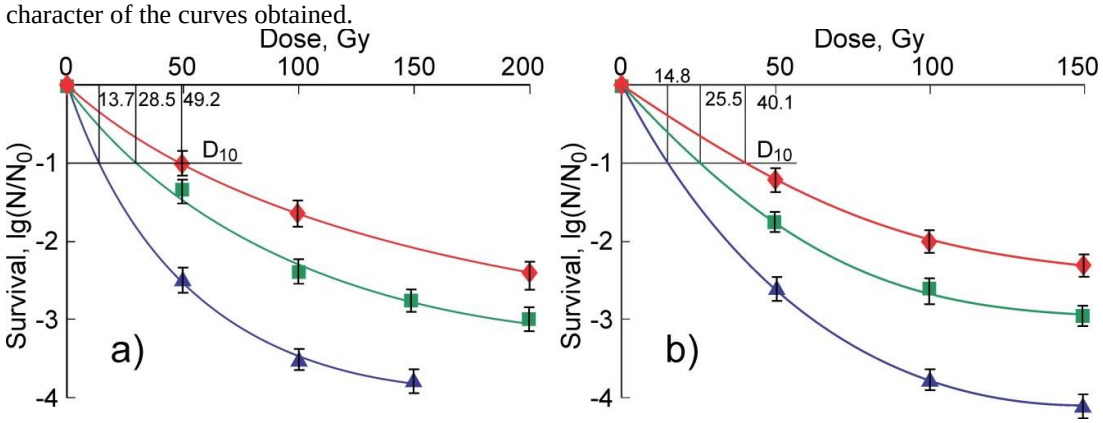

Figure 2:- The dependence of *E.coli* strains survival on the dose value when irradiated with a beam of 12 Hz in DW (a) and in KPh (b). Triangles – radiosensitive (*AB 2463*), square – wild type (*AB 1157*) and diamonds – radioresistant (*BL 1114*).

In addition, a special experiment showed that the preliminary incubation of these strains in water for up to 100 min had not significant effect on their radiosensitivity.

In our experiments the irradiation process for high doses lasted up to 40 min. To exclude various possible artifacts associated with post-radiation effects, the duration of the suspension storage before irradiation, etc. the irradiation processes were carried out in opposite directions: from low doses to high, and from high to low. The data obtained were within the statistical scatter.

The essential difference of AREAL from conventional sources of irradiation is that it generates ultrashort pulses of electron bunches of sub-picosecond duration and actually the process of irradiation is “quantized”. It can naturally be assumed that the character of this "quantization" may play a great role during irradiation. We have studied the influence of pulse frequency on the survival of wild-type strain. The results are presented in Figure 3. As it is seen, the concave form of curves is preserved for all studied frequencies, moreover, for high doses, a tendency of the proportion increase of surviving cells is observed with an increase of pulse frequency.

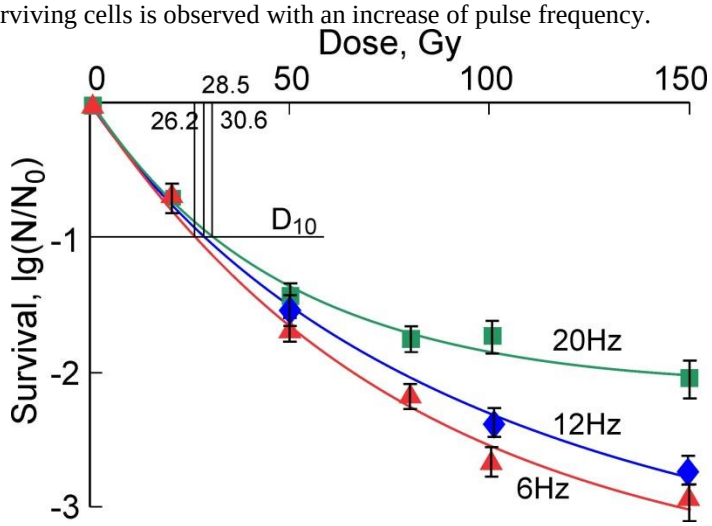

Figure 3:- The dependence of *E.coli K-12 AB-1157* wild strain survival on the dose value when irradiated by different bunch frequency;

Discussion:-

Such curves may be obtained if it is taken that the compensating capacity of cells increases along the dose (D) increase. The impact of compensatory capacity on survival can be taken into account, assuming that the reactivity *R(D)* is exponentially depends on dose (Hug O and Kellerer AM. 1969). This approach allows to describe in a unified phenomenological manner both “standard” convex survival curves, (Figure 4) as well as the “non-standard” concave (L-shape) curves (Figures 2 and 3) being a result of a growth of the compensatory capacity of the object with dose increase. The “compensatory capacity” may be any physiological response of the organism, or even physicochemical process, which results in a change of radiosensitivity during irradiation. To find out the nature of the compensatory ability, additional research is required, which is beyond the scope of this work.

In the paper (Dertinger and Jung, 1973) was proposed that the exponential decrease of reactivity along the dose from $R_0 + R_1$ to R_0 should be described in the following form:

$$R(D) = R_0 + R_1 \exp(-\gamma D) \qquad (2)$$

where γ is the parameter, indicating on how fast the compensatory capacity increases along the irradiation dose increase. Using (2) the following expression was obtained for survival, where the increase of the compensatory capacity along the irradiation dose increase was taken into consideration.

$$ln(N/N_0) = -R_0 D - \frac{R_1}{\gamma}(1 - \exp(-\gamma D)) \quad (3)$$

The curves in Figure 2a were drawn according to formula (3) by the method of least squares. The values of formula parameters are shown in Table 2. When the experimental data is processed by Wolfram Mathematica, the dose rate is more relevant to be presented in kGy. It should be noted however that in literature (Ronto et al., 1967, Dertinger

and Jung, 1973) a formula was mentioned very similar to (2), which also describes the decrease in the slope of survival curves with an increase of radiation dose, but where $R_0 = 0$.

Table 2:- The values of fitting parameters.

Object/bunch frequency/media	R_0 $(kGy)^{-1}$	R_1 $(kGy)^{-1}$	$\gamma(kGy)^{-1}$
AB 2463, 12Hz,DW	0.00008	185	20
AB 2463, 12Hz, KPh	0.00006	186	18
AB 1157, 6Hz, DW	0	100	12
AB 1157, 12Hz, DW	0	86	11
AB 1157, 20 Hz, DW	3	103	25
AB 1157, 12 Hz, KPh	0.0003	120	16
BL 1114, 12 Hz, DW	0.0001	77	12
AB 1114, 12 Hz, KPh	0	100	12

In Figure 3, the curves obtained by the irradiation of strain *AB 1157* at pulse frequencies 6, 12 and 20 Hz are shown. As is seen from the Figure, the survival dependence on dose (2) quite well describes the experimental data. It is noteworthy that there is a regular increase in D10 from the pulse frequency for the wild strain: 26, 29.5 and 30.5 for 6, 12 and 20 Hz, respectively. More significant is the fact that compensatory capacity increases with increasing pulse frequency. This increase is especially significant at relatively high doses.

It will be very interesting to compare the data obtained with the ones from our earlier publication (Avakyan et al., 2011), which were obtained by the irradiation of the same strains on a facility based on a microtron MK-7,5 (Petrosyan, 2009) (electron beam energy 7,5 MeV, dose rate 25 Gy/min, pulse duration >1μs – conventional "quasicontinious" beam). The data from the paper are shown in Figure 4.

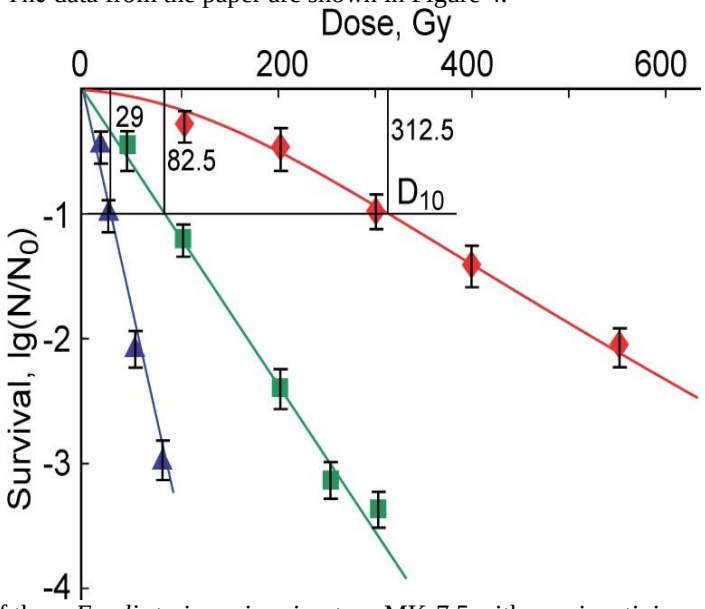

Figure 4:- Irradiation of these *E.coli* strains usig microtron MK-7,5 with quasicontinious electron beam. Legends are the same as in figure 2.

It is seen, that the shape of the survival curves for the studied strains completely fits into classic frames, while the survival curves of the same cultures, when irradiated by ultrashort electron beams, have an L-type shape (Figure 2). The results of more detailed studies, conducted on the cells of wild strains of *E.coli* at pulse frequencies of 6, 12 and 20 Hz (Figure 3), not only repeated the previously obtained data for all three strains but also allowed to definitely reveal another fact as well; the decrease of pulse frequencies from 20 to 6 Hz results in a noticeable decrease in the proportion of surviving cells, i.e. low-frequency pulses are more effective.

It is necessary to point out specially that the cross-sectional area of the beam is approximately twice larger than the longitudinal section of the microtube, where the cell suspension was irradiating (see fig.1b), which excludes the possibility of partial irradiation of the sample.

A special attention should be paid to the fact that nano- and femtosecond beams principally differ from long pulsed and continuous ones in a way that they have high values of instantaneous dose rate; $10^8 – 10^9$Gy/s. In (Manti et al 2017) a similar picture is cited when cells are irradiated with a pulsed beam (Dewey, 1959), which according to the authors is due to oxygen starvation – as a result of the rapid (because of large instantaneous dose) oxygen consumption by irradiated cells. The diffusion does not manage to entirely recover the oxygen level. The role of oxygen in irradiation process of prokaryotic and eukaryotic cells has been discussed by a number of authors (Nias et al 1970, Weiss et al 1974 Zackrisson et al 1991, Carlson et al 2006). As a rule, the absence of oxygen during cell irradiation results in increase of radioresistancy. In our experiments, the application of "anoxemia" model well explains the behaviour of survival cells when the microorganisms are irradiated by beam frequencies of 6, 12 and 20 Hz. Obviously, at a higher pulse repetition rate, the limiting effect of oxygen diffusion will play a more significant role. It is possible that the "reactivity factor" is determined by oxygen. It is worth reminding that before initiating the irradiation process, the cell suspension was vigorously shaken, i.e. the suspension is entirely saturated with oxygen. It follows, that if "oxygen starvation" does occurs, then it definitely indicates that the rate of oxygen molecules' local decrease (as a result of radiolysis) significantly exceeds the rate of its diffusion to the areas of its depletion.

Note that the ionic composition of the irradiation medium had practically no effect on cell survival, and what is more important, the nature of the dependence of the survival rate on the dose did not change. This is clearly seen in Figure 2b.

One fact affecting the survival of the cells when irradiated by ultrashort pulses should also be taken into consideration. Due to the large instantaneous dose rate, the total net time of electron-sample interaction is actually very short as compared to the overall irradiation time. In particular, during our experiments at 50 Gy, this time is approximately 1 ns. According to (Shinohara et al 2004) the short time of beam direct effect on the sample, as well as the relatively long periods between the pulses result in a better neutralization of radicals through recombination, which in its turn leads to an increase in radioresistance. Most likely, the aforementioned factor of reactivity is due to several simultaneously ongoing processes, each of which depends on irradiation features in its own way.

Besides, it cannot be ignored the fact that data cited in the articles are obtained as a result of electron or proton beam irradiation, which greatly differ from each other by their physical parameters, particularly, by pulse duration and power, which makes it difficult to compare the data.

The preliminary results lead us to the conclusion that there is a positive reactivation, which can be determined by oxygen.

Obviously, it is too early to speak on concrete mechanisms leading to the observed picture and a more detailed study is necessary.

Conclusion:-

It is shown that a qualitatively different unusual L-shape dependence of microorganisms' survival degree on the dose is noted. Such shape of survival curves don't depends on ionic composition of the irradiation media.The increase of pulse frequency of electron bunches results in a noticeable increase of cells survival level.The positive reactivation is observed, that can be due to the oxygen.

Acknowledgements:-

Authors would like to express their sincere gratitude to Mr. H. Davtyan for technical support in the irradiation process, to Ms. A. Mikayelyan for the translation and proofreading and to Prof. V. Verbenko (PNPI, Russia) for providing strains of *E.coli* and discussion.

References:-

1. Avakyan, Ts. M., Mkrtchyan, N. I., Simonyan, N. V., Khachatryan, G. E. (2011): The research of biological action of the electrons with the energy of 7.5 MeV on the cells of *E.coli* K-12 bacteria having different reparation genotype. NAS RA Reports 111(2):164-170 (in Russian).
2. Carlson, D. J., Stewart, R. D., Semenenko, V. A. (2006): Effects of oxygen on intrinsic radiation sensitivity: A test of the relationship between aerobic and hypoxic linear-quadratic (LQ) model parameters. Med. Phys. 3(9): 3105-3115.
3. Dertinger, H., Jung, H., (1973) Molecular radiation biology – the action of ionizing radiation on elementary biological objects. Moscow, Atomizdat: 248p. (in Russian).
4. Dewey, D. L. and Boag, J. W.(1959): Modification of oxygen effect when bacteria are given large doses of radiation. Nature 183:1450.
5. Hada, M., Hirscht, J., Zhang, D., Manz, S., Pichugin, K., Mazurenko, D. A., Bayesteh, S., Delsim-Hashemi, H., Floettmann, K., Huening, M. et al (2012): REGAE: New Source for Atomically Resolved Dynamics in Research in Optical Sciences, OSA Technical Digest (Optical Society of America, 2012), paper JT2A.47. VELA - ASTEC, United Kingdom https://www.astec.stfc.ac.uk/Pages/The-Versatile-Electron-Linear-Accelerator-(VELA).aspx
6. Hug, O., Kellerer, A. M. (1969): Stochastic radiobiology. Moscow, Atomizdat 184 p. (in Russian).
7. Khachatryan, G. E., Simonyan, N. V., Mkrtchyan, N. I., Arakelyan, V. B., Tatikyan, S. Sh., Tsakanov, V. M., Antonyan, P. M., Karamyan, S. K., Harutyunyan, V. V. (2016): Modeling of certain problems of space biology in Earth conditions. Biolog. Journal of Armenia 68(2): 21-29.
8. Manti, L., Perozziello, F.M., Borghesi, M., Candiano, G. et al.The radiobiology oflaser-driven particle beams: focus on sub-lethal responses of normal human cells. http://iopscience.iop.org/1748-0221/12/03/C03084.
9. Nias, A. H. W., Swallow, A. J., Keene, J. P. and Hodgson, B. W. (1970): Survival of HeLa cells from 10 nanosecond pulses of electrons. Int. J. Radiat. Biol. 17(6): 595-598.
10. Petrosyan, M. L. (2009): Interaction of accelerated electron beams with high-frequency electromagnetic fields. Abstract of Thesis for the degree of Doctor of Physical and Mathematical Sciences, https://www1.yerphi.am/PHD_Theses/doct_diss/Marzik-Avtoreferat6.pdf (in Russian).
11. Petrosyan, V. (2017): Interaction of the 5 MeV electron beam with the matter in the AREAL photoelectron gun facility experimental hall. Armenian Journal of Physics 10(4): 163-170.
12. Ronto, G., Sarkadi, K., Tarjan, I. (1967): Analysis of ultraviolet dose effect curves. Strahlentherapie 134: 151-157. (German). (https://www.ncbi.nlm.nih.gov/pubmed/5631276).
13. Shinohara, K., Nakano, H., Miyazaki, N., Tago, M. and Kodama, R. (2004): Effects of Single-pulse (< 1 ps) X-rays from Laser-produced Plasmas on Mammalian Cells. J. Radiat. Res. 45: 509-514.
14. Tsakanov, V. M., Amatuni, G. A., Amirkhanyan, Z. G., Aslyan, L.V., Avagyan, V. Sh., Danielyan, V. A., Davtyan, H. D., Dekhtiarov, V. S., Gevorgyan, K. L., Ghazaryan, N. G et al. (2016): AREAL Test Facility for Advanced Accelerator and Radiation Source Concepts. Nuclear Instruments and Methods in Physics Research A, 829(1): 284-290.
15. Uesaka, M., Watanabe, T., Hafz, N. (2002): Ultrashort electron beam generation and diagnosis by linear accelerator. JSAP International, (2): 14-21.
16. Venkatesulu, B. P., Sharma, A., Pollard-Larkin, J. M., Sadagopan, R., Symons, J., Neri1, Sh., Pankaj, K., Singh, R. T., Lin, S. H. and Krishnan, S. (2019): Ultra high dose rate (35 Gy/sec) radiation does not spare the normal tissue in cardiac and splenic models of lymphopenia and gastrointestinal syndrome. Scientific Reports, https://doi.org/10.1038/s41598-019-53562-y
17. Verbenko, V. N., (1994): Mutant alleles for radioresistance form the Escherichia coli strain Gam(r) 444: cloning and preliminary characteristics. (in Russian), Genetika, 30(6), 756-762.
18. Verbenko, V. N., Kalinin, V. L., Smolnikova, A.V. (2000): Effect of a null mutation In the PriA gene on the resistance of Escherichia coli. (in Russian) Genetika, 2000, 36(3), 315-321
19. Villa, F., Ananiaa, M. P., Artiolib, M., Baccic, A., Bellavegliaa, M., Bisestoa, F. G., Biagionia, A., Carpanesed, M., Cardellia, F., Castorinaa, G. et al. (2017): Generation and characterization of ultra-short electron beams for single spike infrared FEL radiation at SPARC_LAB. Nuclear Instruments and Methods in Physics Research A. 865(1): 43-46.
20. Weiss, H., Epp, E. R., Heslin, J. M., Ling, C.C. and Santomasso, A. (1974): Oxygen depletion in cells irradiated at ultra-high dose-rates and at conventional dose-rates. Int. J. Radiat. Biol, Relat. Stud. Phys. Chem. Med. 26(1): 17-29.
21. Zackrisson, B. U., Nyström, U. H. and Ostbergh, P. (1991): Biological Response In Vitro to Pulsed High DoseRate Electrons from a Clinical Accelerator. Actaoncologica, 30: 747-751.