Jurnal Sains Nuklear Malaysia, 2018, 30(2): 47 – 56
ISSN: 2232-0946

INFLUENCE OF OUT-OF-FIELD PHOTON BEAM RADIOTHERAPY TO THE CANCER CELL SURVIVAL

Raizulnasuha Ab Rashid, Nurhikmah Azam, Norhayati Dollah and Wan Nordiana W Abd Rahman

School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kota Bharu, Kelantan, Malaysia
Correspondence author: wandiana@usm.my

ABSTRACT

The purpose of the study was to determine the effect of out-of-field photon beams radiotherapy to the cancer cell survival. In this study, HeLa and T24 cancer cells were irradiated with 6 MV and 10 MV photon beams in two different conditions, one with intercellular communication with the in-field cell and one without the communication. Cells survival was determined by clonogenic assay. In the presence of intercellular communication, the cell death was increased which indicate the presence of radiation induced bystander effects (RIBE). The effects were also dependent on the cell types and photon energy where the HeLa cells exhibit less survival compares to T24 cells and the effects were prominent at higher photon energy. This study demonstrates that the out-of-field cells in conjunction with RIBE plays important roles in the cells response towards megavoltage photon beam radiation therapy.

Keywords: Cell survival, intercellular communication, photon beam radiotherapy, out-of-field

INTRODUCTION

Radiotherapy aims to deliver a highly conformal radiation dose to the tumour while minimising unnecessary dose to the surrounding normal tissue. The presence of scattered photon limits the ability of radiotherapy to achieve this aims. Accumulated dose due to the scattered photon to the out-of-field region may have significant effects to the normal tissue tolerance and hence increase complication. Out-of-field radiation is known to be composed of leakage radiation from the head of the treatment machine and scatter produced from the collimator system or inside the phantom or patient (Scarboro et al., 2011). Outside of the treatment field, the photon spectrum is significantly different from the spectrum in the planned target area with uncertain dose that could deliver undesirable effects to normal tissues.

Dosimetric measurements of the out-of-field dose demonstrate 20% variation in beam quality compared to penumbra and central axis dose (Liu and Verhaegen, 2002). Increase in radiation beam quality outside the treatment field also has been observed (Kirkby et al., 2007; Liu and Verhaegen, 2002). The results were in agreement with the *in-vitro* experimental study that shows significant enhancement of DNA damage for out-of-field region in normal human fibroblasts irradiated with a 6 MV photon beam (Syme et al., 2009). Radiobiological response of non-irradiated cells in the out-of-field region as a consequence of scattered photon doses is also dependent on cellular communication. This cellular communication between the irradiated and non-irradiated cell create radiation induced bystander effects (RIBE) in which the non-irradiated cells exhibits similar response as irradiated cells.

Studies on RIBE suggested that the cell communication play roles in determining the effect to non-irradiated cells (Butterworth et al., 2011; Suchowerska et al., 2005). Different cell response were observed when the cellular communication were inhibit or intact. Experimental evidence confirmed the involvement of RIBE as determinant factor for non irradiated cell responses (Mackonis et al., 2007; Moiseenko et al., 2007). Reduction in the cell survival driven by cellular communication and signalling with irradiated cells in the out-of-field region showing relative contribution of RIBE on cell's radiobiological impact.

In this study, the effects of out-of-field photon beams to the cell survival were investigated. The study were conducted using HeLa and T24 cancer cells irradiated with 6 MV and 10 MV photon beams in two different conditions, one with intercellular communication with the in-field cell and one without the communication.

MATERIALS AND METHODS

Cell Culture

HeLa cell line and T24 bladder cancer cells was purchased from the American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). HeLa cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) and T24 bladder cancer cells were grown in Roswell Memorial Park Institute (RPMI) 1640 Medium (RPMI-1640) supplemented with 10% fetal bovine serum (Gibco, Life Technologies, City, U.S.A.), 1% penicillin and streptomycin (Gibco, Life Technologies, City, U.S.A.). The cells were grown to confluence in a 75 cm^2 flask (Greiner Bio-One, Austria) and split in a ratio of 1:3. The cells incubated at 37^0C in a humidified environment of 5% CO_2 in air.

Irradiation Preparation

The irradiations were conducted using medical linear accelerator (Siemen Primus) at Nuclear Medicine, Radiotherapy and Oncology Department, Hospital USM. The cells were irradiated at 100 cm source to surface distance (SSD) with field size of 10 cm x 10 cm at depth of maximum dose (d_{max}). MU was set to deliver 300 cGy to only half of the plate as shown in Figure 1. The middle wells received half of the field and was noted as the region of the in-field cells communicated with the out-of-field cells. Cells were irradiated using two different energies; 6 MV and 10 MV photon beam. Schematic diagram on the irradiation set up is presented in Figure 2.

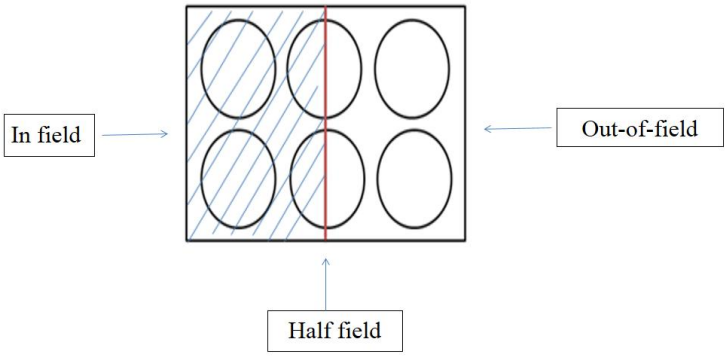

Figure 1: Schematic diagram on plate division into in field and out-of-field region

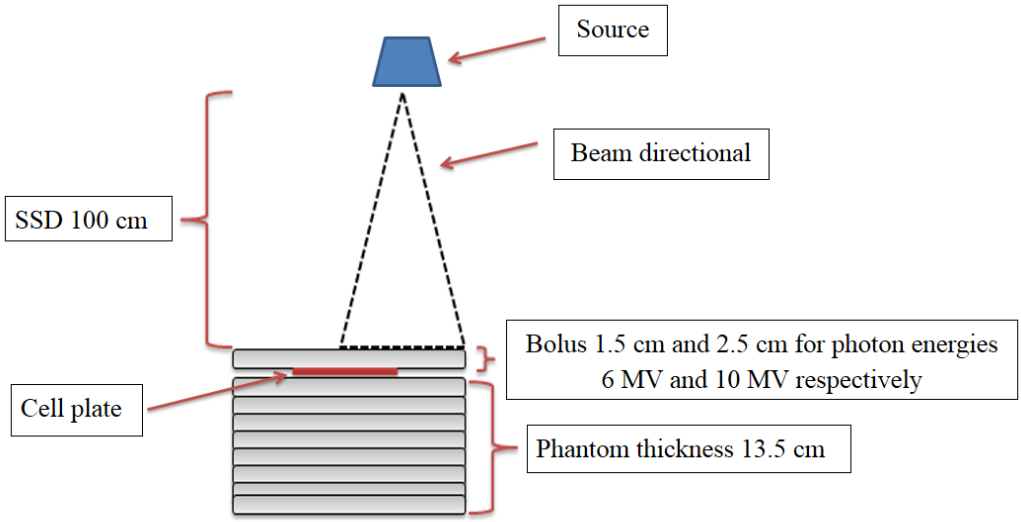

Figure 2: Schematic diagram on irradiation set up

Clonogenic Assay

Cell survival was determined by clonogenic assay. Culture plates were filled with serum-free medium and sealed immediately prior to irradiation. Following irradiation, cell plates were incubated for 10 days and later being washed in PBS solution and stained with 0.5% crystal violet in 50% methanol. The cells that formed colonies were counted. For each experiment, unexposed controls were prepared and treated as sham exposures. The colonies at penumbra region where the steep dose gradient were included in the data analysis.

Statistical Analysis

Survival fractions (SF) were calculated as the ratio of the number of colonies in exposed flask to the number of seeded cells corrected for the plating efficiency of sham irradiated control cells. Data uncertainties were calculated as the standard error of the mean (SEM). One way analysis of variance (ANOVA) was used to determine the significance of the difference between control and experimental group, followed by post-hoc analysis mean comparison using Bonferroni's test. All analyses were carried out using Origin Pro Version 8.

RESULTS

Cell survival data in the field and out-of-field were obtained for HeLa and T24 bladder cancer cell. Intercellular communication intact of cells is assumed to occur in the condition where the in-field and out-of-field cells were within the same well, sharing the same culture medium (partially irradiated). The inhibition of intercellular communication occurs when the irradiated and non-irradiated cells were placed in the different wells of the same plate. The cell colonies within the penumbra region (steep dose gradient) were included in the data analysis due to the small diameter of the well.

Figure 3, 4, 5 and 6 show the cell survival for HeLa and T24 cells at 6 MV and 10 MV of photon beams irradiated in three condition; in-field, partially and out-of-field. From the figures, +CI (with communication intact) indicates that the cells were partially irradiated to allow the intercellular communication between in-field and out-of-field cells. Oppositely,–CI (without communication intact) specified the in-field and out-of-field cells totally separated to inhibit the intercellular communication between them.

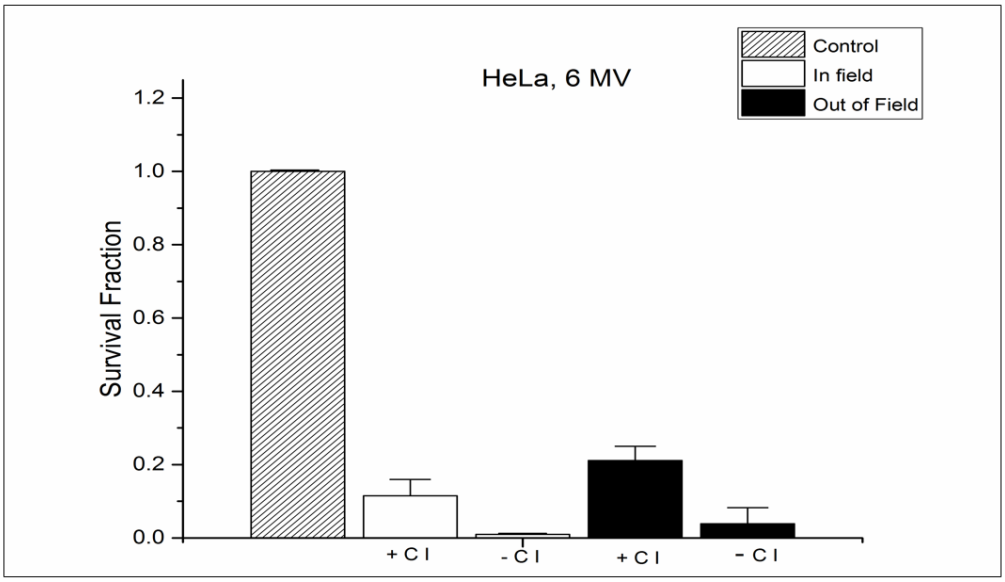

Figure 3: Survival fraction of HeLa cells irradiated with 6 MV photon beam

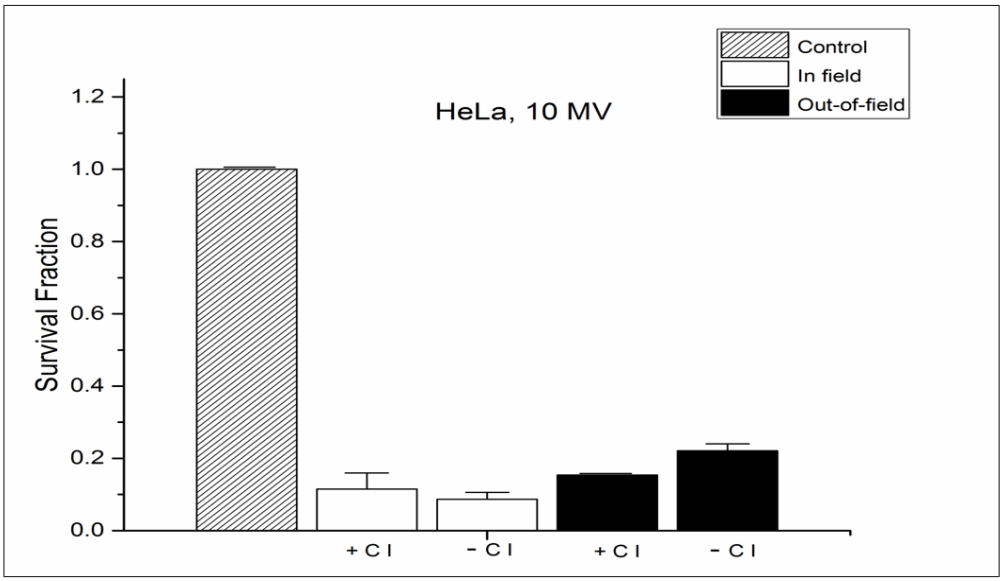

Figure 4: Survival fraction of HeLa cells irradiated with 10 MV photon beam

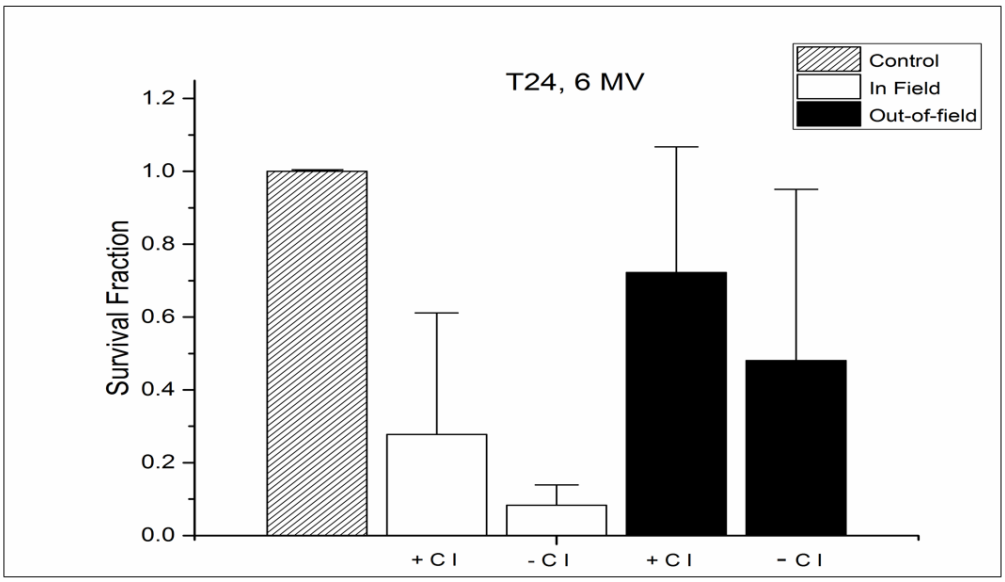

Figure 5: Survival fraction of T24 cells irradiated with 6 MV photon beam

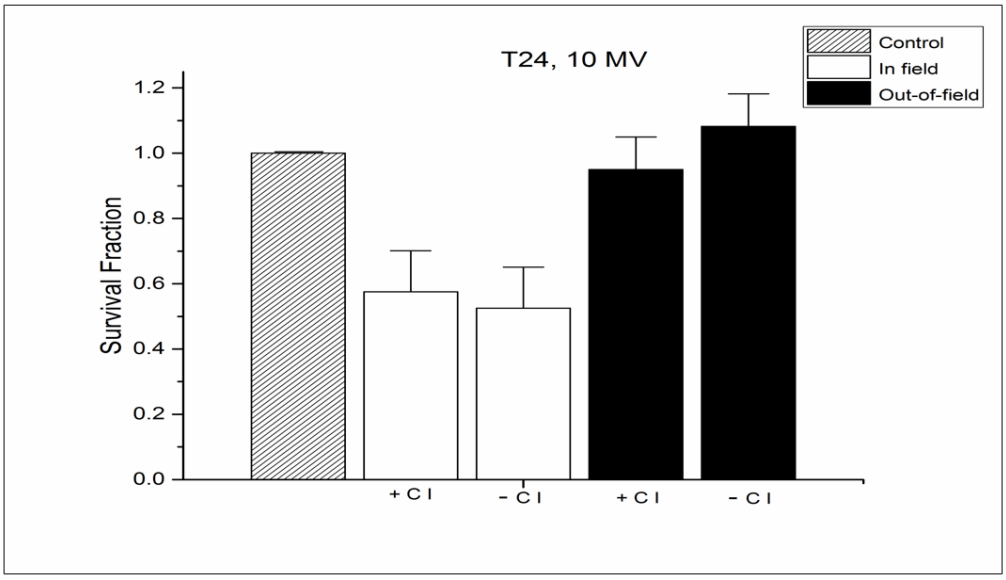

Figure 6: Survival fraction of T24 cells irradiated with 10 MV photon beam

The results for 6 MV as shown in Figure 3 indicated that the out-of-field survival for HeLa cells was higher when intercellular communication intact (0.211 ± 0.03) compared to when intercellular communication inhibited (0.04 ± 0.04). It was different from what had been reported by Butterworth et al. (2011), who demonstrated higher out-of-field survival value when intercellular communication with the in-field cells was inhibited. In-field region exhibit higher cell death when the intercellular communication inhibited but smaller value than out-of-field survival when the communication was intact with the out-of-field cells (0.12 ± 0.04). Meanwhile, the results for 10 MV photon beams show the out-of-field survival were lower when intercellular communication intact (0.15 ± 0) in comparison to when communication inhibited (0.22 ± 0.012) by direct physical inhibition.

T24 cells irradiated with 6 MV of photon beams were shown in Figure 5 presents that the survival fraction for T24 cells has analogous pattern with HeLa cells when similar energy level was used. For out-of-field survival, regions of communication intact demonstrated higher survival fractions (0.72 ± 0.35) than the region where the intercellular communication with in-field cells was inhibited (0.48 ± 0.47). Conversely in Figure 6, the cell shown lower out-of-field survival when intercellular communication intact (0.95 ± 0.10) compared to when intercellular communication inhibited (1.08 ± 0.10). Similar behaviour has been observed in HeLa cells irradiated at 10 MV previously. For the in-field evaluation, high survival fraction at the region where the intercellular communication was intact compared to when the intercellular communication was inhibited for the same energy level used.

In evaluation of in-field cells survival, when intercellular communication was intact there was no significant differences between the 6 MV and 10 MV of photon energies for the dose delivery of 300 cGy of dose to the HeLa cells. However, when intercellular communication was inhibited, cells irradiated with 10 MV photon energy shows higher survival fraction than 6 MV. T24 cells have no statistically significant difference in the in-field cells survival for the regions with or without intercellular communication with the out-of-field cells for 10 MV of photon energy as compared to

6 MV where it was slightly higher. HeLa cells had shown higher cells death than T24 bladder cancer cells except for the in-field region when the communication inhibited.

DISCUSSION

This study demonstrates significant deviations of out-of-field survival responses between cells with and without intercellular communication for the 6 MV and 10 MV photon beams. The out-of-field cell survivals remarkably lessen when the intercellular communication with the in-field cells was intact. The data reported in this study appear to support the assumption by Butterworth et al. (2011), who relates intercellular communication with the existence of RIBE mechanism after the delivery of conformal, IMRT and VMAT plans. It is relevant since the out-of-field cell survival significantly increase when the intercellular communication was inhibited.

The results could correspond to RIBE or the radiobiological response observed in the cellular system that has not been directly traversed by ionising radiation but is in close proximity to irradiated cells. These irradiated cells send the signal which mediated either through direct physical cell contact via gap junction of intercellular communication or through signalling molecules which have been secreted into the surrounding media (Yang et al., 2007). The irradiated cells from the experiment set up shares the same culture medium with the non-irradiated cells. According to the data presented by Mothersill and Seymour in 1997, a significant reduction in clonogenic survival has been demonstrated in un-irradiated cells exposed to irradiated cell conditioned medium (ICCM) from epithelial cells. In contrast, no toxic effect was observed after treatment with medium irradiated in the absence of cells or with medium from un-irradiated cells. It has been established that cells exposed to low-LET γ radiation (contributed by the scattered) releases substances into the medium that can be transferred to un-irradiated cells. The cytotoxic factor(s) substances from the irradiated cells into the media includes lipid peroxide products, inosine nucleotides and cytokines such as tumour necrosis factor-α, as well as reactive oxygen species (ROS) such as superoxide radicals.

In addition, out-of-field cell survival was reduced than what would be predicted merely based on scattered radiation. From the results, inhibition of intercellular communication increases the level of cell survival, proving that intercellular communication between in field and out-of-field cell populations is an essential factor of survival response similar to the observations that had been reported by Butterworth et al. (2011). However, it is likely that a local dose component may also affect out-of-field response (Butterworth et al., 2011). The dose delivered to out-of-field occurs mainly as a result of scattered photons and charged particles that have a larger low energy component. Because of LET is inversely related to electron energy, the out-of-field dose could potentially have different biological effectiveness compared with that of the primary field. The results also suggest that the genetic damage in cells exposed to scattered radiation is caused by factors released by irradiated cells into medium rather than by DNA damage induced directly by X-rays (Marin et al., 2014).

The results also indicate that the cells which received half of the irradiation exhibit higher survival as compared to the in-field region which deviated from earlier hypothesis when using 6 MV photon beams. This is might be caused by the possibility of cells repopulation after being irradiated. In 1966, Malaise and Tubiana first demonstrated that regrowth of a transplantable mouse fibrosarcoma was faster after a single dose of radiation than the growth of non-irradiated control tumours; which others have also reported similar findings. Supporting the finding as well were a study by Brade and Tannock in 2006, which states that there is evidence in several tumour types for an increase of rate

of repopulation during the latter part of a radiation treatment course, which is an effect long recognised as a mechanism of clinically significant resistance to treatment. Besides, another possible factor is, the penumbra or the region at where the steep dose gradient for half irradiated well in a plate was included in data analysis due to its smaller size; although there is variation in beam quality up to 20% between penumbra and the central axis, which due to change in the energy spectrum of the photon fields (Liu and Verhaegan, 2002).

In relation to the intercellular communication, the significant deviations of out-of-field survival as being discussed only occur at 10 MV of photon beam for both HeLa and T24 cells. Contrary to expectations, out-of-field survival response was higher for cells with intercellular communication intact than inhibited at 6 MV photon beam. This is deviated from what had been predicted. Application photon energy of 6 MV was proven to be insufficient to cause the RIBE effect. The intercellular communications existed for both energies but increasing the dose for 6 MV photon beam might be necessary to produce the same effect as 10 MV. The linear accelerator output of 10 MV photon beam deliver 500 MU/min dose rate and 6 MV deliver as much 300 MU/min. Thus, a shorter time is taken when using 10 MV photon beam to deliver the dose as compared to 6 MV photon energy. Moreover, reducing the dose rate makes the survival curve shallower and causes the shoulder to eventually disappear.

Based on the data obtained, it is proved that different types of cell produced different level of survival. HeLa and T24 cells exhibit different survival even though the parameters used were same. This is exactly similar as being reported by Butterworth et al. (2011). They conducted the experiments using two cell lines, the human prostate cancer cell line, DU-145, and the human fibroblast cell line, AGO-1522. Both of the cells exhibit different level of survival when irradiated with uniform field response (AGO-1522; $\alpha = 0.53 \pm 0.05$, $ß = 0.01 \pm 0.02$ while for DU-145, $\alpha = 0.20 \pm 0.03$, $ß = 0.01 \pm 0.01$).

It has been reported that all of the cells were not equally sensitive to radiation. Mammalian cell shows significant differences in radiosensitivity depending on the type of cell. The survival curves of malignant cells do not show any systematic differences from those of normal cells and the parameters which characterise those covers a wide a range as those of normal cells. Clinical experience accumulated over more than half a century show that there is great variation in the radiosensitivity of human tumours, both within any one type of histology but even more so between one histological type and radiosensitivity has been suggested. Cells which divide rapidly or were relatively non-specialized tend to show the effect at lower doses radiation than those which were less rapidly dividing and more specialised (Tubiana et al., 1990).

All measures of our study are consistent with previous studies but show different magnitudes (Butterworth et al., 2011; Trainor et al., 2012). Similarly, this effect was dependent on intercellular communication and could be abrogated by physical inhibition between cells irradiated directly and those cells exposed to scattered dose out-of-field. Although the level of out-of-field responses is greater than those previously reported, the experiments conducted in this study differ from the non-irradiated cells by IMRT.

CONCLUSIONS

The results of this study indicate that there is significant effect of out-of-field cell responses following irradiation with megavoltage photon beam radiotherapy. The current data highlight the importance of intercellular communication and RIBE in determining the out-of-field survival

responses. Consideration on the out-of-field cell response and RIBE factors are important in clinical radiotherapy as to ensure accurate and effective cancer treatment.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support from the Malaysian Ministry of Higher Education (FRGS grant No: 203/PPSK/6171160) and USM Short Term Grant (304/PPSK/61313139).

REFERENCES

Brade, A.M. and Tannock, I.F. (2006). Scheduling of radiation and chemotherapy for limited-stage small-cell lung cancer: Repopulation as a cause of treatment Failure?, *J. Clin. Oncol.* 24(7):1020-1022.

Butterworth, K.T., McGarry, C.K., Trainor, C., O'Sullivan, J.M., Hounsell, A.R. and Prise, K.M. (2011). Out-of-field cell survival following exposure to intensity-modulated radiation fields, *Int. J. Radiat. Oncol. Biol. Phys.* 79(5): 1516-1522.

Kirkby, C., Field, C., Mackenzie, M., Syme, A. and Fallone, B.G. (2007). A Monte Carlo study of the variation of electron fluence in water from a 6 MV photon beam outside of the field, *Phys. Med. Biol.* 52(12): 3563-3578.

Liu, H.H. and Verhaegen, F. (2002). An investigation of energy spectrum and lineal energy variations in mega-voltage photon beams used for radiotherapy, *Radiat. Prot. Dos.* 99: 425-427.

Mackonis, E.C., Suchowerska, N., Zhang, M., Ebert, M., McKenzie, D.R. and Jackson, M. (2007). Cellular response to modulated radiation fields, *Phys. Med. Biol.* 52: 5469-82.

Malaise, E. and Tubiana, M. (1966). Growth of the cells of an experimental irradiated fibrosarcoma in the C3H mouse, *C.R. Acad. Sci. Hebd. Seances Acad. Sci. D.* 263:292-295.

Marin, A., Martin, M., Linan, O., Alvarenga, F., Lopez, Mario., Fernandez, L., Buchser, D. and Cerezo, L. (2014). Bystander effects and radiotherapy, *Rep. Pract. Oncol. Radiotherapy* 20: 12-21.

Moiseenko, V., Duzenli, C. and Durand, R.E. (2007). In-vitro study of cell survival following dynamic MLC intensity modulated radiation therapy dose delivery, *Med. Phys.* 34: 1514-1520.

Mothersill, C. and Seymour, C. (1997). Medium from irradiated human epithelial cells but not human fibroblasts reduces the clonogenic survival of unirradiated cells, *Int. J. Radiat. Biol.* 71(4): 421-427.

Scarboro, S.B., Followill, D.S., Howell, R.M. and Kry, S.F. (2011). Variations in photon energy spectra of a 6 MV beam and their impact on TLD response, *Medic. Phys.* 38(5): 2619-2628.

Suchowerska, N., Ebert, M.A., Zhang, M. and Jackson, M. (2005). In vitro response of tumour cells to non-uniform irradiation, *Phys. Medic. Biol.* 50(13): 3041-3051.

Syme, A., Kirkby, C., Mirzayans, R., Mackenzie, M., Field, C. and Fallone, B.G. (2009). Relative biological damage and electron fluence in and out of a 6 MV photon field, *Phys. Medic. Biol.* 54(21): 6623-6633.

Trainor, C., Butterworth, K.T., McGarry, C.K., McMahon, S.J., O'Sullivan, J.M., Hounsell, A.R. and Prise, K.M. (2012). DNA damage responses following exposure to modulated radiation fields, *PLoS ONE 7*(8): e43326.

Tubiana, M., Dutreix, J. and Wambersie, A. (1990). Introduction to radiobiology, Taylor & Francis, London.

Yang, H., Anzenberg, V. and Held, K.D. (2007). The time dependence of bystander responses induced by iron-ion radiation in normal human skin fibroblasts, *Radiat. Res.* 168(3): 292-298.