How air influences radiation dose deposition in multiwell culture plates: a Monte Carlo simulation of radiation geometry

Sebastia SABATER1,*, Roberto BERENGUER2, Paloma HONRUBIA-GOMEZ3, Miguel RIVERA2, Ana NUÑEZ2, Esther JIMENEZ-JIMENEZ4, Ana MARTOS1 and Carmen RAMIREZ-CASTILLEJO3,5

1Department of Radiation Oncology, Complejo Hospitalario Universitario de Albacete (CHUA), C/ Hnos Falcó 37, 02006 Albacete, Spain
2Department of Medical Physics, Complejo Hospitalario Universitario de Albacete (CHUA), C/ Hnos Falcó 37, 02006 Albacete, Spain
3Centro Regional de Investigaciones Biomedicas (CRIB), Universidad de Castilla-la Mancha (UM), C/ Almansa 14, 02006 Albacete, Spain
4Department of Radiation Oncology, Hospital Son Espases, Carretera de Valldemossa 79, 07120 Palma de Mallorca, Spain
5Instituto de Salud Carlos III. Av Monforte de Lemos 5, 28029 Madrid, Spain
*Corresponding author. Department of Radiation Oncology, Complejo Hospitalario Universitario de Albacete (CHUA), C/ Hnos Falcó 37, 02006 Albacete, Spain. Tel: +34-967-597-523; Fax: +34-967-597-530; Email: ssabaterm@gmail.com

(Received 14 November 2013; revised 12 February 2014; accepted 2 March 2014)

Radiation of experimental culture cells on plates with various wells can cause a risk of underdosage as a result of the existence of multiple air–water interfaces. The objective of our study was to quantify this error in culture plates with multiple wells. Radiation conditions were simulated with the GAMOS code, based on the GEANT4 code, and this was compared with a simulation performed with PENELOPE and measured data. We observed a slight underdosage of ~4% on the most superficial half of the culture medium. We believe that this underdosage does not have a significant effect on the dose received by culture cells deposited in a monolayer and adhered to the base of the wells.

Keywords: Monte Carlo; plate irradiation; culture cells

INTRODUCTION

Irradiation is a primary tool in biological research. A great number of experimental biological studies involve and have involved culture plate irradiation. In spite of this fact, very few studies have analyzed their radiation geometry and validated the method employed. Normally when calculating the dose received to a culture plate, the total of the irradiated plate is considered to be an homogenous volume of water; in fact, cells plated in multiwell culture plates are surrounded by large amounts of air because of the existence of air cavities in such labware products. Air cavities exist in, around and within the well-plates and above the culture medium, which partially fills the well volume (Figs 1 and 2).

It is known that the presence of air cavities may produce significant underdosage as a result of the air–interfaces, depending on the energy of the photon beam, the form and size of the cavity, the size of the radiation field, and the distance from the evaluated point to the interface [1]. These underdosages are related to a lack of electronic equilibrium. This is a physical matter, but one with important issues for biologists and physicians who rely on dose deposition accuracy when planning and analyzing experimental data. To date, no information exists on how air–water interfaces influence dose deposition on culture plates.

Our aim was to quantify the grade of dose variation associated with the lack of backscatter material (build-down zone) in the zones occupied by air in the interior of the well plates.

MATERIALS AND METHODS

The Monte Carlo (MC) code used was GAMOS v.1.9.0, which is based on the GEANT4 code [2]. Prior to our study of the radiation geometry of cell cultures, a simple homogeneous phantom 30 × 30 × 30 cm³ of water was prepared. This
was for comparing the depth dose and profiles obtained using GAMOS with the experimental measurements performed using a silicon diode for a field size of 10 x 10 cm$^2$ and 20 x 20 cm$^2$ of a Siemens PRIMUS X6MV, using the phase-space provided by the IAEA for such fields [3]. (The phase-spaces chosen are computer files containing a detailed description of the particles emitted from the treatment source via an MC simulation in a defined plane.) Frequently, researchers use the files supplied by IAEA because of its creation complexity, that is also time-consuming, and in addition IAEA files have been validated versus measurements. [3]. Results were compared with another MC simulation rebuilt with the PENELOPE 2006 version. In order to be able to use the IAEA phase-spaces, a recent application of the PenEasy code (named 'penEasy_IAEAaddon') was employed. The irradiation geometry was generated with the ‘gview3d’ software from the PENELOPE package.

Next, a radiation geometry like that used in cellular radiation was simulated, but minus the culture plate, i.e. 5 cm of water, 2 cm of air, and another 5-cm thickness of water. We compared the results of the simulation with the experimental measurements obtained in the zone of interest for us (the first water–air interface). In order to evaluate the underdosage, we compared the problem geometry ‘water–air–water’ (5 + 2 + 5 cm) to ‘all water’ geometry (a thickness of 12 cm). Experimental measurements were performed using X6MV photons from a Siemens PRIMUS linear accelerator at a dose-rate of 200 UM/min. Radiation was delivered via a single field at 180°, which went through 5 cm of water-equivalent material (Plastic Water® from CIRS, Computerized Imaging Reference System Inc., Norfolk, VA, USA). A Ross plane-parallel ionization chamber (PTW-Freiburg, Freiburg, Germany) (Fig. 3b) was used to measure the dose in the interface between water and air. These measurements were done in order to validate the simulations results. The plane-parallel chamber is the recommended chamber for measuring percentage of depth doses (PDDs) in the build-up or build-down areas [4]. The drawback is that the chamber used has a distance of 2 mm between the electrodes. It is very important to mention that the chamber was irradiated downside. We compared the chamber response in this situation with the irradiation in the standard situation and the difference was <1%.

Finally, the goal radiation geometry was simulated with only two cubic wells (measuring 2 x 2 cm with 5 mm of

---

Fig. 1. A 6-well plate photo and 96-well plate drawn showing the multiple air spaces in and around each well.

Fig. 2. Simulated irradiation of an ideal well plate showing the water volume related to the air spaces. The drawing on the right shows the radiation geometry generated with the ‘gview3d’ software.
thickness and 2 cm of separation) symmetrically positioned around the axis of the beam (Fig. 2). The cell-plates, which were 2 cm thick, had the wells partially filled (some 5 mm thick) and, finally, above the plates was another 5 cm of water-equivalent material. Therefore, between the surfaces of the culture medium and the water-equivalent material, there was a significant region of air (measuring 15 mm). In addition, there was air surrounding the different wells (Figs 1, 2 and 3). With this geometry, two distinct simulations were performed—with distinct voxel sizes (and tally sizes)—to observe if there was underdosage parallel to the direction of the beam (z axis) or perpendicular to the beam (x or y axis). The tally size was set to 1 mm in the direction of interest, and the dimensions of the voxel in the other directions were greater in order to improve statistical results. The step length used in the GAMOS simulations was the default production cut value, and in the Penelope case the values used were EABS(ph) 10 KeV, EABS(e-) 100 KeV and EABS(e+) 100 KeV, according to previously published data [5]. The simulations performed took into account the electron transport.

RESULTS

First, in the homogenous phantom, good agreement between simulated and measured PDDs and profiles was found, both for the 10 × 10 cm² field and the 20 × 20 cm² field (data not shown), and also between the GAMOS and PENELOPe simulation (Fig. 4a). However, a slight difference was observed in the shoulder area of the profile (better appreciated in the profile at the maximum dose depth (Fig. 5a). These divergences probably reflect slight differences in the energy spectrum between the data of phase-spaces proportioned by IAEA and the measured data in our linear accelerator Siemens PRIMUS (Fig. 5a).

According to the simulation in the water–air–water geometry without cell-plates, the underdosage in the last voxel of water adjacent to the water–air interface was 0.955 ± 0.02. However, the measured value was 0.983. Taking into account the fact that the effective point of measurement of the Roos chamber is located at 1 mm from the surface, we corrected the results to the last and penultimate voxel before reaching the water–air interface. A value of 0.984 ± 0.02 was obtained, which tallies very well with the measured data. These prior comparisons between measurements and simulations, therefore, serve to validate the code. Figure 4b shows the comparison between the PDD of the water–air–water phantom with the PDD of the 12-cm homogenous water phantom that was used to estimate the underdosage in this situation. In addition, for this geometry, the comparison between the simulations performed with GAMOS and PENELOPe is included (Fig. 4c). The comparison reveals good agreement except in the air, which is attributable to air density differences between the MC codes, but this result is not of concern for our study.

Finally, after evaluating the results of the plate radiation simulations, we found that the underdosage parallel to the beam direction in the last voxel was 0.965 ± 0.01 (mean of both wells) (Fig. 4d). Perpendicular to the beam direction, we found a negligible lateral underdosage, 0.994 ± 0.01 (average of the two extremes of the two wells) (Fig. 5b). MC calculations of the same plate geometry were repeated with the PENELOPe code and similar results were obtained (data not shown).

DISCUSSION

No concerns about underdosage as a result of build-down during in vitro culture plate irradiation have been raised by these results. There is little data about the real dosage received inside well culture plates, despite their widespread utilization in radiotherapy research, other than ascertaining that the intended dose is the dose actually delivered. Kulmala et al. [6] found that, for 96-well plates, there was a 3% discrepancy between the dosimetric results calculated...
with a CT-based radiotherapy planning system and those experimentally obtained after dropping thermoluminescent dosimeters (TLDs) into the wells. Tomic et al. [7] investigated dose deposition using a radiochromic film and found that the maximum dose gradient was of the order of 1.5% for a 6-MV photon beam. However, analysis of these results needs to take into account the fact that the uncertainties in the measured dose, with the model of film used, were of the order of 2%. Blockhuys et al. [8] also used a radiochromic film, but his aim was to investigate the dosimetry related to a ‘smooth linear dose gradient’ using an intensity-modulated beam. Altman et al. [9] characterized a phantom for in vitro cell experiments comparing TLD and film measurements with those acquired from their treatment planning system. He found differences ranging from 1.3–2.9%, without statistical significance. None of the previous works, however, have dealt with the air–water interface underdosage, thus no direct comparisons can be made with regards to our paper, which is the first to focus on this issue in relation to in vitro experiments. TLD doses are an averaged measurement that do not consider spatial dose distribution; radiochromic films have limitations inherent to their nature, such...

![Fig. 4](https://academic.oup.com/jrr/article-abstract/55/5/1009/2751009)
as non-uniformity (which has been reported as ±3% in a multi-institutional investigation), the need for calibration, and the influence related to the scanning system, among others [10, 11].

In our study, we did not evaluate the effect of the energy of the photon beams on the underdosage at the proximate interface. However, the results from Klein et al. [12] (using a parallel-plane chamber) indicate a greater underdosage for low-energy photons compared with high-energy photons, owing to a major contribution from back-scattering to the total dose. According to these results, it is advisable to use a photon energy of X18MV (instead of X6MV, which we normally use). The same author also indicated that the thickness of the air gap posterior to the proximate interface scarcely influences the underdosage.

On the other hand, according to Li and coworkers [13], the underdosage at the proximate interface is less dependent on the field size than on the far interface. This can be verified from our graphs (Fig. 4b, c and d), despite this not being the main objective of our study. Although our results relate to a field of 10 × 10 cm², in the light of Li et al.’s work, the underdosage obtained at the proximate interface for any other field size is not expected to differ significantly, contrary to what was previously believed.

The differences found between the MC simulations and the measurements are minimal, as revealed by the PDDs and profile plots (Figs 4a and 5a). Despite the fact that the published IAEA phase-spaces have been validated with real measurements, there are some slight differences between IAEA energy spectrum considered to produce the IAEA files used, and the real energy spectrum of our LINAC, but these differences do not affect the results of our study in any significant way.

Comparison of the simulations performed with GAMOS and Penelope indicates good agreement (except in the air, attributable to air density differences between the MC codes, which is of no particular relevance to this study).

**CONCLUSION**

In conclusion, we would like to emphasize our finding that in the irradiation of cell culture plates, there is a slight underdosage (~4%) which corresponds to the last voxel (1 millimeter depth), at the most superficial part of the culture medium, whereas in lower layers where the cells are attached, the underdosage is negligible. The value shown (~4%) represents the maximum underdosage value found in the whole configuration set-up. We conclude that the underdosage will hardly affect the monolayer of cells at the base of the culture wells. Furthermore, the lateral underdosage is also insignificant.

**FUNDING**

This work was partially supported by Research Grant AN-2010/16 from Fundacion Sociosanitaria de Castilla-La Mancha (FISCAM). Funding to pay the Open Access publication charges for this article were provided by Junta local de
Albacete de la Asociación Española Contra el Cáncer (AECC).

REFERENCES

1. Apipunyasopon L, Srisatit S, Phaisangittisakul N. An investigation of the depth dose in the build-up region, and surface dose for a 6-MV therapeutic photon beam: Monte Carlo simulation and measurements. J Radiat Res 2013;54:374–82.
2. Arce P. Geant4-based Architecture for Medicine-Oriented Simulations (GAMOS) version 1.9.1. User’s Guide 2009.
3. IAEA. Phase-space database for external beam radiotherapy: Vienna, Austria. https://www-nds.iaea.org/phsp/photon1/ (15 October 2012, last accessed).
4. Podgorsak E. External photon beam: physical aspects. In: Podgorsak E (ed). Radiation Oncology Physics: A Handbook for Teachers and Students. Vienna: IAEA, 2005, 161–217.
5. Carrasco P, Jornet N, Duch MA et al. Comparison of dose calculation algorithms in slab phantoms with cortical bone equivalent heterogeneities. Med Phys 2007;34:3323–33.
6. Kulmala J, Rantanen V, Pekko-la-Heino K et al. Dosimetry of irradiation models. The 96-well clonogenic assay for testing radiosensitivity of cell lines. Acta Oncol 1995;34:105–9.
7. Tomic N, Gosselin M, Wan JF et al. Verification of cell irradiation dose deposition using a radiochromic film. Phys Med Biol 2007;52:3121–31.
8. Blockhuys S, Vanhoecke B, Paelinck L et al. Development of in vitro models for investigating spatially fractionated irradiation: physics and biological results. Phys Med Biol 2009;54:1565–78.
9. Altman MB, Vesper BJ, Smith BD et al. Characterization of a novel phantom for three-dimensional in vitro cell experiments. Phys Med Biol 2009;54:N75–82.
10. Niroomand-Rad A, Blackwell CR, Coursey BM et al. Radiographic film dosimetry: recommendations of AAPM Radiation Therapy Committee Task Group 55. American Association of Physicists in Medicine. Med Phys 1998;25:2093–115.
11. Tanooka M, Doi H, Miura H et al. Three-dimensional radiochromic film dosimetry for volumetric modulated arc therapy using a spiral water phantom. J Radiat Res 2013;54:1153–9.
12. Klein EE, Chin LM, Rice RK et al. The influence of air cavities on interface doses for photon beams. Int J Radiat Oncol Biol Phys 1993;27:419–27.
13. Li XD, Yu C, Holmes T. A systematic evaluation of air cavity dose perturbation in megavoltage x-ray beams. Med Phys 2000;27:1011–7.