Factors affecting ultraviolet-A photon emission from -irradiated human keratinocyte cells

2015 Phys. Med. Biol. 60 6371

(http://iopscience.iop.org/0031-9155/60/16/6371)

IOP Publishing | Institute of Physics and Engineering in Medicine Physics in Medicine & Biology

Phys. Med. Biol. **60** (2015) 6371–6389 doi:10.1088/0031-9155/60/16/6371

Factors affecting ultraviolet-A photon emission from β-irradiated human keratinocyte cells

M Le[1], C E Mothersill[1], C B Seymour[1], S B Ahmad[2], A Armstrong[1], A J Rainbow[3] and F E McNeill[1]

[1] Medical Physics and Applied Radiation Sciences Department, McMaster University, 1280 Main Street West, Hamilton Ontario, L8S 4L8, Canada
[2] Pakistan Institute of Engineering and Applied Science, Nilore, Islamabad, Pakistan
[3] Department of Biology, McMaster University, 1280 Main Street West, Hamilton Ontario, L8S 4L8, Canada

E-mail: lem8@mcmaster.ca

Received 18 January 2015, revised 26 June 2015
Accepted for publication 1 July 2015
Published 3 August 2015

Abstract

The luminescence intensity of 340 ± 5 nm photons emitted from HaCaT (human keratinocyte) cells was investigated using a single-photon-counting system during cellular exposure to ^{90}Y β-particles. Multiple factors were assessed to determine their influence upon the quantity and pattern of photon emission from β-irradiated cells. Exposure of 1×10^4 cells/5 mL to 703 μCi resulted in maximum UVA photoemission at $44.8 \times 10^3 \pm 2.5 \times 10^3$ counts per second (cps) from live HaCaT cells (background: 1–5 cps); a 16-fold increase above cell-free controls. Significant biophoton emission was achieved only upon stimulation and was also dependent upon presence of cells. UVA luminescence was measured for ^{90}Y activities 14 to 703 μCi where a positive relationship between photoemission and ^{90}Y activity was observed. Irradiation of live HaCaT cells plated at various densities produced a distinct pattern of emission whereby luminescence increased up to a maximum at 1×10^4 cells/5 mL and thereafter decreased. However, this result was not observed in the dead cell population. Both live and dead HaCaT cells were irradiated and were found to demonstrate different rates of photon emission at low β activities ($\leqslant$400 μCi). Dead cells exhibited greater photon emission rates than live cells which may be attributable to metabolic processes taking place to modulate the photoemissive effect. The results indicate that photon emission from HaCaT cells is perturbed by external stimulation, is dependent upon the activity of radiation delivered, the density of irradiated cells, and cell viability.

It is postulated that biophoton emission may be modulated by a biological or metabolic process.

Keywords: radiation-induced luminescence, ultraviolet radiation, beta radiation, yttrium-90, intercellular signaling, bystander effect, phosphorescence

(Some figures may appear in colour only in the online journal)

1. Introduction

Biological entities are known to emit photons and the photon emission can be linked to signaling processes between entities. For example, since the early 1920s, multiple reports of low-intensity photon emission from plant (Popp *et al* 1984, Gurwitsch 1988, Bajpai *et al* 1991), animal (Devaraj *et al* 1991, Evelson *et al* 1997, Van Wijk *et al* 2014) and human-derived material (Niggli 1993, 1996, Niggli *et al* 2008, Van Wijk *et al* 2013) have been made and referred to as ultraweak luminescence or biophoton emission.

Biophoton emission at very low fluxes has been observed in the absence of a stimulus, a phenomenon termed spontaneous photon emission (Bajpai *et al* 2013). Weak electromagnetic fields such as these can act as informational signals. Further, these signals are intended for inter- and intra-cellular communication within a population of cells, a tissue system or within an organism (Borodin 1930, Rahn 1936, Gurwitsch 1988, Slawinski *et al* 1992). Of interest to us, was the fact that photon emission can also be elicited in response to a stimulus. The stimulated emission is generally in quantities that are orders of magnitude greater than those of a spontaneous nature.

Previous work conducted in our laboratory (Ahmad *et al* 2013, Le *et al* 2015) has confirmed that stress in the form of β-radiation elicits significant photon emission from human keratinocyte cells, HPV-G and HaCaT, in the ultraviolet (UV) range. Photon emission from biological materials has been shown to be induced not only following exposure (in our hands) to ionizing radiation but also (in other hands) by chemical agents (Popp *et al* 1984, Devaraj *et al* 1991), ultraviolet radiation (Niggli 1993, Niggli *et al* 2008) and intense white light (Niggli 1996).

Biophoton emission is seen as a response to stress or stimuli (Slawinski *et al* 1992) and therefore has, in fact, been utilized as a diagnostic indicator of disease and stress states in animal (Van Wijk *et al* 2013) and human (Van Wijk *et al* 2014, 2008) models. Similar to reported data regarding spontaneously emitted photons, the previous work from our lab has shown that the radiation-induced UV photons have cell communication capabilities (Le *et al* 2015). That particular experiment is detailed in full but we summarize the information here.

Our previous experiment had a layer of tritium-incubated cells, a barrier, and then another layer of cells. The upper were only exposed to the subsequent radiation-induced UV, not the initial β-radiation from the tritium. We state that UV induced cell communication occurred, because a bystander effect was observed in the upper layer of non-β exposed cells. When a UV filter was inserted between the layers, the bystander effect i.e. the induced cell communication, was not observed.

To date therefore, our data show that an external stressor of β-radiation is indeed able to initiate biophoton emission. We previously observed UV photon emission from a number of organic materials including plastics and dried tissues (Ahmad *et al* 2014), so one mechanism of UV emission is probably simply a consequence of electron rearrangement after ionization

events in atoms and/or molecular structures. In addition, our previously reported data show that the subsequent emitted photon signal is capable of communicating with bystander cells to produce observable responses.

We decided to investigate factors that can modulate the UV signal. This manuscript therefore reports on a series of experiments that were designed to investigate potential factors that can affect the magnitude of the β-induced UV emission. We explore the pattern of photon emission and the relationship to factors such as cell viability, activity, of radiation delivered, density of irradiated cells and concurrent versus post-irradiation photon quantification.

2. Materials and methods

2.1. Cell line

The HaCaT cell line was obtained from the lab of Dr Orla Howe (Dublin, Ireland). HaCaT cells are immortalized, non-transformed human skin keratinocyte cells which express p53 mutations on both of its alleles (Boukamp *et al* 1990, Lehman *et al* 1993, Datto *et al* 1995). Cells were confirmed to be free of mycoplasma using PlasmoTest mycoplasma detection kit (catalog no: rep-pt1, Invivogen, San Diego, CA, USA).

2.2. Cell culture

All reagents were obtained from Gibco unless otherwise stated. HaCaT cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco Life Technologies Inc., Grand Island, NY, USA) containing a final concentration of 10% fetal bovine serum, 0.5 μg mL^{-1}hydrocortisone (Sigma-Aldrich, St-Louis, MO, USA), 2 mM L-glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin sulphate. Cell culture work was performed in a class II laminar flow cabinet to maintain sterility of the cells. Subculture of cells was performed at 80–90% confluency using a 1:1 solution of dissociation reagent, 0.25% trypsin and 1 mM ethylenediaminetetraacetic acid (EDTA), for detachment of cell monolayer from the flask substrate. Cell stock was subsequently seeded into 250 mL stock flasks and grown in 15 mL medium. Cells were incubated at 37 °C, 95% relative humidity, 5% CO_2 and medium renewed every 2–3 d. Stock flasks received full volume medium renewals 24 h prior to set-up of experimental cultures.

2.2.1. Preparing living cell cultures for experiments. HaCaT cells were plated onto 60×15 mm polystyrene petri dishes (BD Falcon, Mississauga, ON) and supplemented with RPMI culture medium (without phenol red) to a total volume of 5 mL. A 5 mL volume placed into the dish created a 3 mm thick layer of liquid. Living cells were plated at 7 different densities ranging from 5×10^2 to 5×10^5 cells per 25 cm^2 petri plate containing a total volume of 5 mL. From now on, *cell density* throughout this article refers to the number of cells per 5 mL volume of cell culture medium in a petri-dish. As the volume is fixed for all experiments, we quote total number of cells/5 mL when we use the term *cell density*. Cells were incubated at 37 °C, 95% humidity, 5% CO_2 for 6 h before experimentation took place to ensure cell attachment to the dish substrate. Cells destined for irradiation in a living state were irradiated in the petri dish containing 5 mL of RPMI 1640.

2.2.2. Preparing dead cell cultures for experiments. For experiments conducted upon dead cells, cells were seeded in the same manner as previously described. Cells were plated at

densities ranging from 5×10^5 to 2×10^6 cells per 5 mL. Following 6 h incubation, cells were killed by treating the HaCaT monolayer with 5 mL of 100% ethanol for 30 min. Following ethanol treatment, the ethanol was aspirated carefully from the cell culture dish and left to stand for an additional 10 min to facilitate complete evaporation of residual ethanol from the petri dish. 5 mL of RPMI 1640 without phenol red was then added to the petri dish containing dead cells in order to ensure consistency with the live-cell conditions.

2.3. Source Preparation

The radioactive source, Yttrium-90 (^{90}Y) decays by β-emission (maximum beta energy = 2.28 MeV; average beta energy = 0.9337 MeV; half life = 64.1 h). It was chosen because it is an almost pure β-emitter with no γ-signal which could be used as an external source of radiation. We manufactured the ^{90}Y in house: Yttrium salt was irradiated in the core of the McMaster Nuclear Reactor and prepared into a liquid solution by dissolving the Yttrium-salt in 0.5 M HCl. The resultant ^{90}Y possessed a high specific activity to reduce self-shielding effects. The stock source was always prepared to a final volume of 400 μL, however, the physical dimensions and mass of the source were not always consistent for a given activity. For each preparation, the target activity was 1000 μCi per 400 μL, however this could not always be achieved and resulted in variability in the ^{90}Y activity between 839.3 μCi to 1100 μCi per 400 μL. Activity of the prepared source was measured using an AtomLab 400 Dose Calibrator possessing an expected uncertainty of $\pm$3% (Biodex Medical Systems, Shirley, NY).

In regard to the volumes actually used during irradiation, different volumes were required to achieve each of the desired radiation activities. Therefore, for activities ranging from 14 to 703 μCi, the required volume from the 400 mL stock, varied from 5.09 to 335 μL. The variability in the physical dimensions and mass of the source may have contributed to uncertainties in the activity delivered to the cells (table 1) and therefore the observed counts.

2.4. Irradiation and Photon Quantification

Photons emitted from keratinocyte cells were quantified using a single photon counting apparatus (Ahmad *et al* 2013) comprised of a Hamamatsu R7400P photomultiplier tube (PMT) (Hamamatsu Photonics, Bridgewater, NJ, USA) fitted with an optical filter (Edmund Optics Inc., Barrington, NJ, USA) specific to 340 nm $\pm$ 5 nm. It is noted that the transmission of incident light through this optical filter is reduced to 25% using a collimated beam as compared to a non-collimated light source. As determined previously (Le *et al* 2015), one reason we focused on the measured photon wavelength centered at 340 nm $\pm$ 5 nm for further experiments was because it demonstrated an optimal photon output compared to 300 nm and 280 nm wavelengths. An additional reason for choosing to focus on the 340 nm UVA wavelength is because it is biologically interesting since UVA, but not UVB, has been proven to induce bystander effects in human cells (Whiteside and McMillan 2009). In this manuscript, we use the term UV from now on to mean the emission we measured at 340 nm. This is not to suggest that there are no other frequencies of light emitted, merely that this is the wavelength we could measure with our apparatus, and also the wavelength we chose to measure because the stronger signal would result in greater statistical strength.

Photon counting was conducted in a light-tight aluminum container with the photomultiplier tube located within (figure 1). The radioactive source was placed into a 60×15 mm petri dish at the bottom of the light-tight box and a 3.2 mm thick aluminum collimator (2 mm diameter pinhole) was placed above the source. This collimator was used for the photon quantification

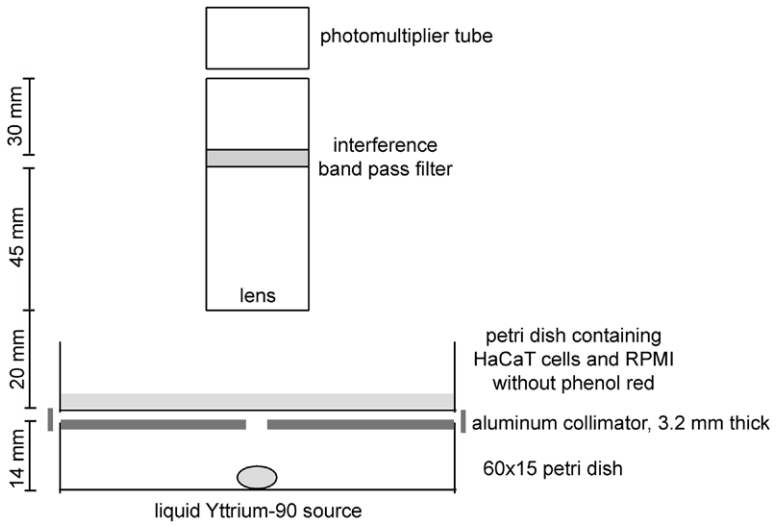

Figure 1. Experimental configuration for ^{90}Y irradiation of cells and quantification of photon emission from irradiated cells. Image not to scale.

experiments in order to limit scattering of photons within the lens system. Our previous work had shown that collimation was a necessary part of the experiment (Ahmad *et al* 2013). The 0.95 mm thick petri dish containing cells and a 3 mm layer of cell culture medium was positioned above the collimator. Although the cell culture medium was free of the pH indicator phenol red, it was still slightly colored (light orange) due to the natural orange pigmentation of the FBS supplemented into the medium. The pigmentation of the culture medium would have contributed to absorption and scattering of the UV photons. In addition, natural chromophores in cells, cell membranes and boundaries can all contribute to absorption and scattering of UV photons. Therefore the UV flux detected by the PMT does not accurately represent the number of photons actually emitted from the irradiated cells, and it will require validated Monte Carlo methods to accurately back calculate the UV emission at source.

The photon counting apparatus was developed in-house; apparatus specifications are described in detail in an existing publication (Ahmad *et al* 2013). The PMT was turned on 30 min prior to each experiment and the high voltage supplied to the PMT was set to −800 V. Following a 30 min warm-up period, three background photon counts lasting 3 min each were taken to determine the background noise level (no radiation, dishes or cells in the chamber).

2.4.1. Different activities. Cell irradiation was carried out at six different activities for live cells (14, 70, 140, 197, 351, 703 μCi) and eight activities for dead cells (same as live cells, also 165 and 540 μCi) and photon emission was quantified concurrently. Unirradiated controls with cells and without cells were also included. Photon quantification was conducted at each of the activities for a total of 9 min; each of three petri dishes were exposed for 3 min while photon quantification took place. In order to accomplish this, the high voltage was switched off between each 3 min exposure, the petri dish containing cells was removed, and another petri dish containing the same number of cells was placed into the chamber to be exposed by the same source. Once in place, the high voltage was switched back on and photon quantification resumed. After three dishes were exposed to a given activity, the activity was increased to the next specified level, taking into account decay over the last 9 min. Irradiation and photon quantification usually began within 15 min of the completion of source preparation. This time

Table 1. Estimated average beta particle flux seen by cells and estimated error in beta particle flux seen by cells due to pipetting uncertainty, source decay, activity measurement and differences in source geometry.

^{90}Y target activity (μCi)	Activity uncertainty (μCi)
14	1
70	3
140	5
165	6
197	7
351	13
540	20
703	27

Note: The estimate of error includes contributions from source decay, measurement of the source activity using a dose calibrator, pipetting uncertainties and an estimate of variation in source dimensions. This may, however, still be an underestimate of the overall error.

required to transport and set-up the source was taken into consideration when calculating the decay and thus the volume of ^{90}Y required for the next activity. It is noted that the source activity was only replenished every 9 min between each triplicate. It was not adjusted between irradiation of each petri dish (every 3 min), therefore variability in the activity stemmed from decay of the source, especially at the higher activities which were done later in the experiment (table 1). Experiments were always completed within 3 h of source formation, therefore, the source was always maintained above 96.81% of the initial activity.

2.4.2. Different cell densities. For dead cells, 3 different densities from 5×10^5 to 2×10^6 cells per 5 mL volume and for live cells, 7 different densities ranging from 5×10^2 to 5×10^5 cells per 5 mL volume were exposed to each of the activities described in the previous section. 0 cell controls were also included where petri dishes containing only 5 mL of cell culture medium and no cells were exposed to each of the activities ranging from 0 to 703 μCi.

2.4.3. Post-irradiation quantification. Photon quantification was also carried out for 92 min immediately *after* irradiation. In this experiment, photon quantification took place for 30 min during the exposure of 1×10^4 living cells/5 mL to 703 μCi. Irradiation set-up for the 30 min duration was identical to that in figure 1. Post-irradiation quantification for 92 min was then accomplished by turning off the HV supply, removing the petri dish containing the ^{90}Y source, and replacing the source petri dish with an empty petri dish thereby maintaining the geometry of detection from UV-emitted cells. It is noted that photon quantification could not be measured for the minute immediately following removal of the cells from the radiation source. This experiment was conducted with a freshly perpared source. Taking into consideration transport, preparation, and 30 min irradiation time, the activity delivered to cells in this experiment varied between 703 and 699.2 μCi (approximately 99.46% of the initial activity).

2.5. Statistical Analysis

Photon quantification experiments were conducted three times with a triplicate tested for each trial, $n = 9$. For each trial, a different source was prepared and used. The data are presented as the mean where error bars are representative of the mean $\pm$ the standard error. All data sets were found to be normally distributed. To determine the nature of the relationship between photon emission and the ^{90}Y activity applied, experimental data was assessed first using

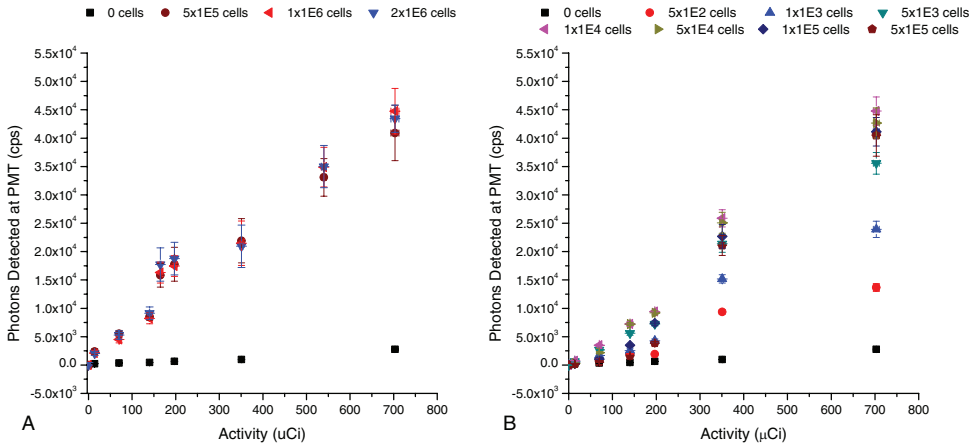

Figure 2. UVA (340 ± 5 nm) photon quantification from (A) ethanol-treated and (B) living HaCaT cells during exposure to different activities of beta emitter, Yttrium-90. Legend: reported numbers refer to total number of cells per 5 mL of medium. Error bars: mean $\pm$ standard error (SEM) for $n = 9$.

Pearson's correlation test and then linear regression fixed to a common y-intercept (y-intercept for unirradiated control). 2-way analysis of variance (ANOVA) tests were conducted to assess photon emission dependent upon activity and density. A p-value less than 0.05 was considered significant. Statistical analyses were conducted using SPSS Statistics 17.0 and Origin Pro 8.

3. Results

3.1. Background counts at 340 nm detection

Background photon counts, obtained in the absence of any materials in the path of the PMT, were quantified within the light-tight apparatus at the beginning of each experimental setup. The background counts were found to be between 1 and 5 counts per second (cps) (data not shown). As the background count values quantified were low, these quantities were considered negligible compared to count rates obtained following β-irradiation and were not subtracted from subsequent photon emission measurements.

3.2. 340 nm photon emission from irradiated ethanol-treated (dead) HaCaT cells

Ahmad and colleagues demonstrated significant photon emission resulting from the exposure of dead HPV-G human keratinocytes to ionizing radiation (Ahmad *et al* 2013). Dead cells were initially studied because the experiments could then be conducted in a non-biohazard approved laboratory. We previously did not have access to laboratories that were approved for work with both open liquid sources of radioactivity and biohazardous materials. To confirm this phenomenon in our chosen human keratinocyte cell line (HaCaT), photon counting was conducted during the irradiation of dead HaCaT cells with low LET-β-particles.

3.2.1. Relationship between activity and photon emission. The results of this experiment demonstrated an increase in UVA photon emission with increasing activity applied to the dead cells (figure 2(A)), this result is evidence of a positive relationship between ^{90}Y activity

and photon emission intensity (table A1). Given the relatively small number of activity points, we fitted linear relationships to the data. Other functions may be better representations of the underlying physical process, but there are too few degrees of freedom to determine this with certainty. The linear fits were conducted such that the regression fits for all of the cell densities intersected a common point along the *y*-axis (*b*) which corresponds to the intercept of the unirradiated control data ($b = 2.77$). Linear fits to the data were significant $p < 0.05$ (table A2) for all but one cell density. Among those that were significant, the lowest R^2 value of 0.800 provides the information that approximately 80 percent of the variation in UV photon emission can be explained by the activity in a linear model. The borderline significance (p=0.051) demonstrated by the linear regression fit to the 5×10^5 cell/5 mL data is probably because a linear fit is not the ideal model for this data and because the intercept has been forced.

Photon quantification of unirradiated controls, was limited to 1–2 cps and did not differ significantly from background counts ($p = 0.856$). Upon quantification of cells exposed to 14 μ Ci ($p = 0.023$) and all activities greater ($p < 0.0001$), emission was found to be significantly different from unirradiated and background controls.

As stated earlier, linear fits may not be the best model of the data pattern. The data may turn over and follow some other function. A greater number of data points would be required to evaluate the pattern fully. We can say that at these activity levels and densities, the numbers of detected UV photons increase with activity. There is, perhaps, a suggestion of a small systematic offset or an artifact that arises between 150 and 200 μCi. Our measurements were performed in the same activity order for each cell density series. An explanation is that there is perhaps some evidence of a time dependent transition. Another interpretation could be differences in source distribution. However, whether this is a true pattern arising from the interactions between UV and more and less dense layers of cells, or an artifact or systematic offset, is difficult to determine in a post hoc analysis. This small effect requires further investigation.

3.2.2. Relationship between cell density and photon emission. The control curve labelled 0 cells in figure 2(A) illustrates the UV photon emission from petri dishes containing no cells and only culture medium that received radiation. Upon exposure of culture medium in the absence of cells to the lowest activity level used (14 μCi), the detected emission was significantly different from background emission levels (up to 5 cps) at the 95% confidence level ($p = 0.037$). The observed emissions in the absence of cells can be attributed to interaction of the beta particles with the polystyrene petri dish and cell culture medium. However, the amount of emission observed upon irradiation of 0 cells (dish and medium only) was significantly less than that observed in the presence of cells ($p < 0.0001$) indicating that UV emission is significantly increased further when cells are placed in the path of the radiation. Therefore we conclude that the molecular structures of cells compared to the composition of medium make UV emission more likely.

It was also found that the emission count rates of all three dead cell densities (5×10^5 to 2×10^6 cells/5 mL) were not significantly different from each other. Upon analyzing micrographs of these three cell densities using ImageJ (figures 3((A)–(C)), it was determined that 5×10^5, 1×10^6, and 2×10^6 cells/5 mL covered 15.7%, 35.1% and 67.7% of the petri dish surface area, respectively. Although the plated densities are quite variable in confluency on the plate, emission magnitudes measured from each of these densities were comparable. This result is suggestive either of a threshold in density above which photon count rate does not change greatly, or that the large relative measurement uncertainties of approximately 19% mean that there is emission variation of less than 0.5% per 5×10^5 cells/5 mL.

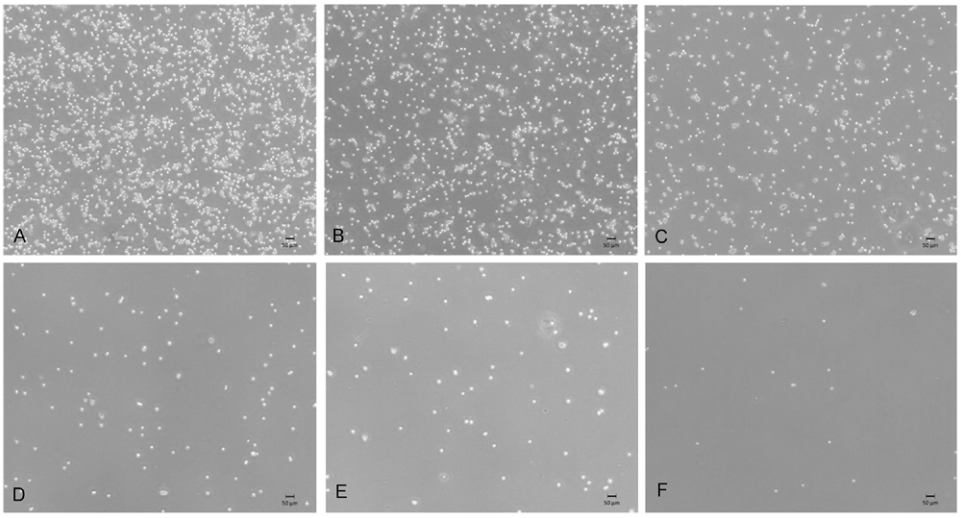

Figure 3. Micrographs of different cell densities plated on 60×15 mm petri plates. Cells per 5 mL volume: (A) 2×10^6, (B) 1×10^6, (C) 5×10^5, (D) 1×10^4, (E) 5×10^3, (F) 1×10^3.

3.3. 340 nm photon emission during irradiation of living HaCaT cells

Following gain of access to laboratory facilities approved for both open radioisotope work and biohazard work, we quantified the photon emission at 340 nm emanating from live HaCaT cells during irradiation at six different activities of ^{90}Y and seven cell densities to determine whether there was a difference between living and dead cells.

3.3.1. Relationship between activity and photon emission. Unirradiated cell controls demonstrated a minimum count rate of 1 cps and a maximum count rate of 4 cps for cell densities of 5×10^2 to 5×10^5 cells/5 mL, respectively. We conclude that because there is no significant increase ($p = 0.983$) in photon emission above background in the presence of unirradiated cells alone, living HaCaT cell cultures, like dead cells, do not luminesce strongly in the absence of stimulation. When live HaCaT cells were exposed to 14 μCi of the ^{90}Y low-LET β-radiation, photon emission was significantly greater than the 0 dose control ($p = 0.025$). For all activities greater than 14 μCi, $p < 0.025$ when a given activity was compared to the 0 dose control. As with dead cells, positive relationships between photon emission and activity were observed (table A3).

The curves do appear to be non-linear (figure 2(B)), with some evidence of a turn-over at the high activity end. However, with 6 data points, there are not enough degrees of freedom to determine the exact mathematical relationship. Linear fits were significant (p <0.05, table A4) for all but one data set. Among those that were significant, the lowest R^2 value of 0.803 suggests that just over 80% of the increase in light intensity can be explained by an increase in activity in a linear model. Similar to the dead cell analyses, the linear regression analysis for one of the data sets was nearly significant (5×10^4 cells/5 mL, $p = 0.050$), likely because the underlying pattern of the relationship between measured UV emission and activity in living cells is not completely linear. There may be an underlying pattern, but the possibility of artifacts in the data will need to be investigated. Future measurements will need to assess

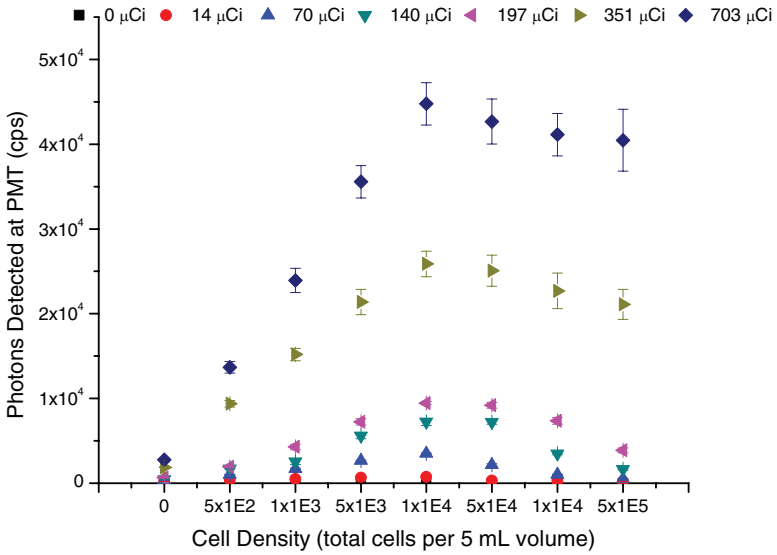

Figure 4. UVA (340 ± 5 nm) photon quantification from HaCaT cells plated at various densities during exposure of live HaCaT cells to Yttrium-90. Surface area of petri dishes upon which cells were plated: 25 cm^2, total volume: 5 mL. Error bars: mean ± standard error ($n = 9$).

source distributions, and issues such as phosphoresence in the lens arising from beta and bremmstrahlung interactions with the lens.

3.3.2. Relationship between cell density and photon emission. Figure 4 illustrates the effect of cell density upon the resultant photon emission quantity. Control irradiations consisting of irradiated petri dishes containing medium in the absence of cells were conducted at each activity level (6 activity points from 14 μCi to 703 μCi). The resultant light emission intensity was 219 cps to 2767 cps for activities of 14 and 703 μCi, respectively. In the absence of cells, irradiation of the polystyrene petri dish and the RPMI cell culture medium with 14 μCi produced a count rate of 219 cps. System background counts were quantified to be 1–5 cps in the absence of radiation, petri dishes, cells and medium. Photon emission from the 14 μCi cell-free control was significantly greater than background (1–5 cps) at the 95% confidence level, however this difference was just significant at $p = 0.046$. Despite significant emission from the cell-free controls, the cell-free counts were comparatively lower than those measured in the presence of cells. This data suggests that although there is some degree of interaction of the incident β-particles with the polystyrene and cell culture medium, the molecular structures of these materials result in lower UV emission than from cells.

As figure 4 shows, at low cell densities starting at 5×10^2 cells/5 mL (figure 3(F)), the photon emission demonstrates a positive relationship with cell density until reaching a density of approximately 1×10^4 cells/5 mL (figure 3(D)) where the UV emission starts to decrease slightly. Upon reaching 1×10^4 cells/5 mL, the photon emission quantity reaches a maximum; this observation was a consistent trend at all activity levels.

The relationship between measured photon emission and cell density is approximately linear up to densities of 1×10^4 cells/5 mL. While the relationship between measured UV emission and cell density may not be completely linear, and there may be better models of the behavior, this is an observation based on a set of 5 data points, so further analysis would

Table 2. Linear regression of measured UV emission versus cell density (for 0 to 10 000 cells) for each of the activities studied.

Activity (μCi)	Slope of light output(cps) versus cell density	p (for slope of light output versus cell density)	R^2 value for regression ($n = 5$)	Slope/ activity
70	0.28 ± 0.06	0.02	0.88	0.004 ± 0.001
140	0.63 ± 0.11	0.01	0.92	0.004 ± 0.001
197	0.81 ± 0.17	0.02	0.89	0.004 ± 0.001
351	2.02 ± 0.65	0.05	0.76	0.006 ± 0.001
703	3.59 ± 0.95	0.03	0.82	0.005 ± 0.001

possibly over interpret the results of this data. For each activity level, linear regressions were significant at the 95% confidence level, and the activity explained over 75% of the variation in measured light output in a linear model. Table 2 presents the results from linear regressions of measured UV emission versus cell density for each of the activities studied.

There is a positive relationship between measured UV emission and cell density up to 1×10^4 cells/5 mL. This would indicate that the cells are the source of the emission of a significant proportion of the measured UV. It can be seen in table 2 that the results of the slope of light output versus cell density increase linearly with increasing activity. If the slopes are normalized to activity, they are found to be the same to within uncertainties. Up to cell densities of 1×10^4 cells/5 mL, the measured level of emitted UV is a function of the source activity incident upon the cells and the number of cells in the dish.

Above cell densities of 1×10^4 cells/5 mL, small decreases in measured UV emission are observed. The decrease in measured UV photons is related to cell density: it is not a function of source activity. This is shown in figure 5 below. The decrease in measured photon counts per second is plotted against cell density in the figure for the region above cell densities of 1×10^4 cells/5 mL, where decrements are observed. The decrease is defined as the number of counts per second at a particular cell density minus the number of counts per second at the maximum signal recorded at a cell density of 1×10^4 cells/5 mL. It can be seen that the magnitude of the decrease is similar for all measured source activities, so this reduction is a function of cell density not activity. We suggest that the reduction is possibly a form of self-shielding. As cell densities increase, the amount of material which emits the UV signal increases. However, the amount of scattering and absorption also increases, and above cell densities of 5×10^4 cells/5 mL this reduces the signal that is measurable at an external detector.

3.3.3. Photon emission post-irradiation. An experiment was performed where UV photon emission was measured during irradiation of 1×10^4 living cells/5 mL to 703 μCi for 30 min and then was subsequently measured in the same cells as promptly as possible following removal of cells from β-exposure. Average photon emission during irradiation was 3.96×10^4 cps $\pm 1.52 \times 10^3$ cps (figure 6). The quantification of post-irradiation photon emission began one minute after the end of irradiation; measurement immediately following irradiation was not possible due to the need to turn off the PMT high voltage, remove the radioactive source from the light-tight unit and then replace the petri dish back into the counting apparatus. The average post-irradiation emission over a 92 min duration (figure 6 inset) was measured to be 1.74 ± 0.42 cps. In the absence of irradiation, it is evident that photon emission from live HaCaT cells is less than during irradiation. However, there is a slight suggestion that it is not quite to background levels. There is some evidence of a slow decay in light output post-irradiation in figure 6. This can be seen most clearly in the first 20 min. The light output in the

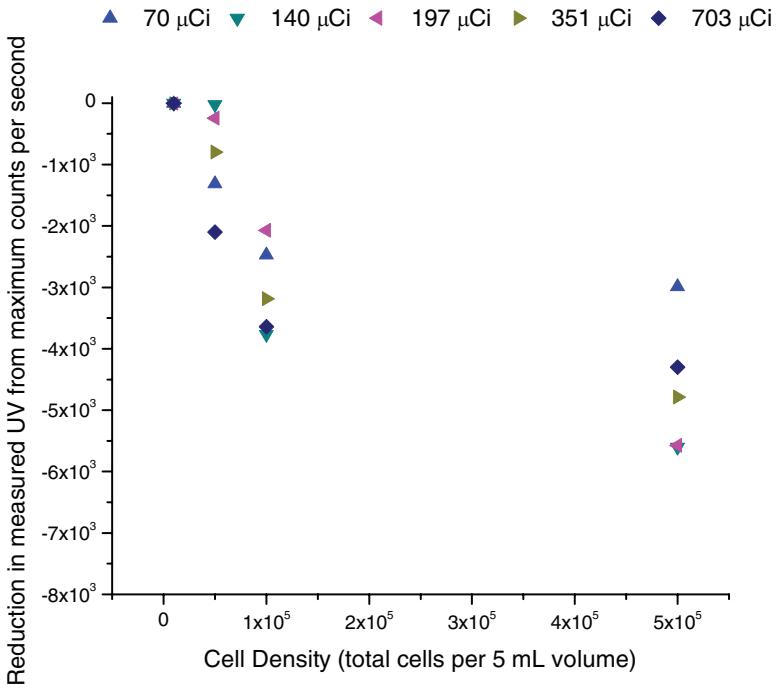

Figure 5. A plot of the reduction in measured UV counts per second from the maximum observed emission at cell density of 1×10^4 cells/5 mL plotted against cell density. All higher activities (which have high measured UV emission rates) show the order of magnitude of reduction indicating that the reduction is a function of cell density.

last time decile is significantly lower than in the first ($p < 0.004$), and fitting a linear decay results in a significant regression. If some of the UV photon emission is from scintillation in organic molecules, then it might be expected, through intersystem crossing, that in addition to scintillation, there could be phosphorescence. We do not suggest that scintillation and phosphorescence are the only mechanisms of photon emission under direct irradiation, but they would be expected to occur when certain types of organic molecules (which are present in cells) are irradiated.

It is also possible that the photon counts observed immediately following removal of the radioactive source is attributed to a systematic error. Gain variation (drift) over short operating times could explain the decay observed: Hamamatsu Photonics testing demonstrated an approximate 2% decay in relative output over 100 min of PMT operation (Hamamatsu Photonics 2007). To achieve more stable operation, Hamamatsu recommends warm-up for several ten minutes where voltage is set close to the operating voltage (Hamamatsu Photonics 2007). However, in the current experiment this was not possible due to the nature of the experiment. In order to account for this potential source of counts, a control experiment was performed where counts were detected for 90 min immediately following the application of high voltage at −800 V (figure A1). These measurements were taken without any cells or radiation in the light-tight chamber. The counts detected from the control's first time decile (mean = 1.219 ± 0.003 cps) were significantly different ($p = 0.001$) from the counts detected during the following 9 time deciles (mean = 1.205 ± 0.009 cps). The counts detected in the last 81 min of measurement were not significantly different from each other ($p > 0.994$). This result supports the idea that there is a noise contribution due to high voltage switch-on to the

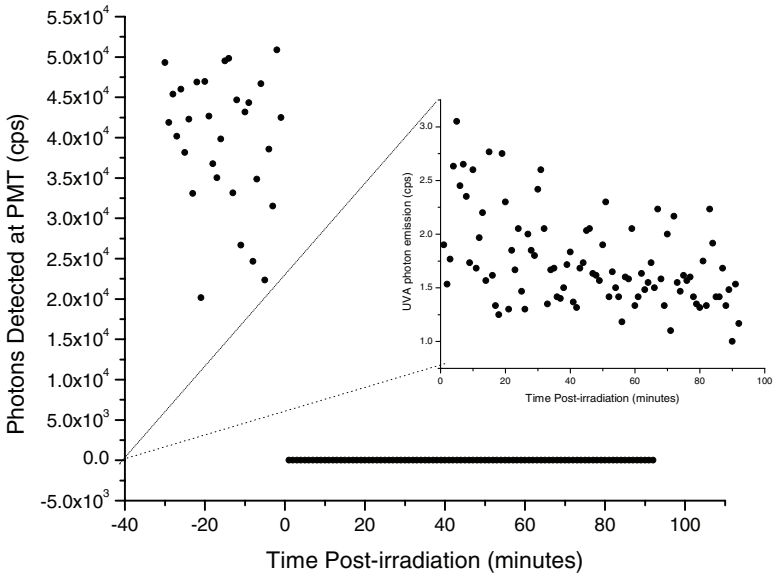

Figure 6. Photon emission during irradiation (1×10^4 cells/5 mL exposed to 703 μ Ci ^{90}Y for 30 min) and post-irradiation (92 min measurement). Inset: photon emission post-irradiation only.

observed light signal decay in the post-irradiation measurements. However, when the intensity of emission from HaCaT cells post-irradiation was compared to the intensity in the control experiment, it was found that the emission from the cells during the first time decile (mean = 2.23 ± 0.52 cps) remained significantly greater than the highest background control counts (1.219 ± 0.003 cps) ($p < 0.0001$). This would suggest that the photon emission observed in the post-irradiation experiment was not only attributed to noise in the system, but processes such as phosphorescence from cells may have also contributed a portion of the signal.

4. Discussion

4.1. Relationship of 340 nm photon emission quantity with ^{90}Y activity

Significant UVA photon emission at 340 ± 5 nm was detected emanating from both dead and living HaCaT cell cultures subjected to ionizing radiation. In both HaCaT cell populations, we noted a positive dependence of luminescence upon the activity of ^{90}Y applied. The underlying relationship between luminescence and activity may not be completely linear: more data will be required to establish the best model for the relationship with statistical certainty. The increase in emission related to an increase in activity seen in the results are in line with the ideas proposed by existing publications which suggest that greater photon emission from biological entities is prompted by a disruption of biological order (Bajpai *et al* 1991) or by any agents capable of insult or injury (Popp *et al* 1984). It also makes sense from a physical perspective; more ionizations resulting from a higher level of irradiation would be expected to result in a subsequently greater number of molecular and atomic level transitions which could produce photons in a range of wavelengths including the UVA range that we have measured here.

It is not surprising that irradiated materials can produce light, including that in the UV range, when irradiated. This is, of course, the physical basis by which both organic and crystal scintillation detectors can be used (Knoll 2011). These detectors emit light when irradiated, although by different mechanisms, within the material depending on whether they are organic or crystalline detectors. In addition, there is a whole field of materials analysis research, called ionoluminescence, which uses the phenomenon of emission of light as a consequence of charged particle irradiation, to interrogate and characterize materials. Our previous work showed that many materials used in radiation biology applications emit light when irradiated (Ahmad *et al* 2013) and that, in fact, dead cells also emit light. An important outcome of the data presented here, however, is that light output relates to cell density. This implies that the majority of the light detected in the presence of cells is coming from the cells.

These may be purely physical processes arising from transitions in the materials. However, an interesting question is whether these purely physical processes that lead to subsequent light emission as a consequence of irradiation, link to biological mechanisms and further, whether there is light emission in biological processes that may be changed, interrupted or enhanced as a consequence of irradiation. It has been hypothesized that reactive oxygen species (ROS) may be involved in the promotion of the photo-emissive process (Quickenden and Tilbury 1983, Devaraj *et al* 1991, Evelson *et al* 1997, Niggli *et al* 2001, Van Wijk *et al* 2008). Presence of reactive oxygen species (ROS) is one possible explanation for luminescence as it can explain the occurrence of the phenomenon in both dead and live cells via radical chain reaction processes or, in living cells, via oxidative stress mechanisms.

We have observed that at higher levels of radioactivity, dead and live cells (plated at 5×10^5 cells/5 mL) emit the same levels of UV per unit cell density and unit activity. However, it is particularly interesting to note that irradiation of 5×10^5 cells/5 mL produced significantly greater magnitudes of photoemission in dead cells than in living cells at beta activities up to and including 197 μCi ($p < 0.01$) (figure 7); the difference in photoemission was not significant at greater activities (351 μCi, $p = 0.146$; 703 μCi, $p = 0.987$). We speculate that the observation of weaker photon detection from living cells may be due to the ability of living cells to mediate intracellular reduction-oxidation (redox) reactions. This contrasts with dead cells where there is an absence of potential difference across cell membranes. Living cells possess active redox potential and therefore would be able to initiate antioxidant defenses to moderate the degree of cellular photoemission. At higher beta activities however, accumulation of ROS could cause antioxidant activity to decrease (Egea *et al* 2007) and therefore result in the inability to suppress ROS-driven photon emission. Despite uncertainty regarding the mechanism for reduction in photon emission, these results indicate that cellular viability may play some role in the measured level of emitted UV.

In previous findings by our research group (Le *et al* 2015), we had observed a lack of significant difference between HaCaT cell *survival* when exposed to signals from dead and living tritium-irradiated HaCaT cells. In the current study, we found that *signal* strength differed in dead versus living cells. These differences observed suggest that the response of cells to signals are likely mediated by mechanisms separate from those which mediate the actual signal production.

4.2. Relationship of 340 nm Photon emission quantity with cell density plated

No significant difference in photon emission rate was observed for the range of cell densities where irradiated ethanol-treated (i.e. dead) cells were studied. However, in the live cell

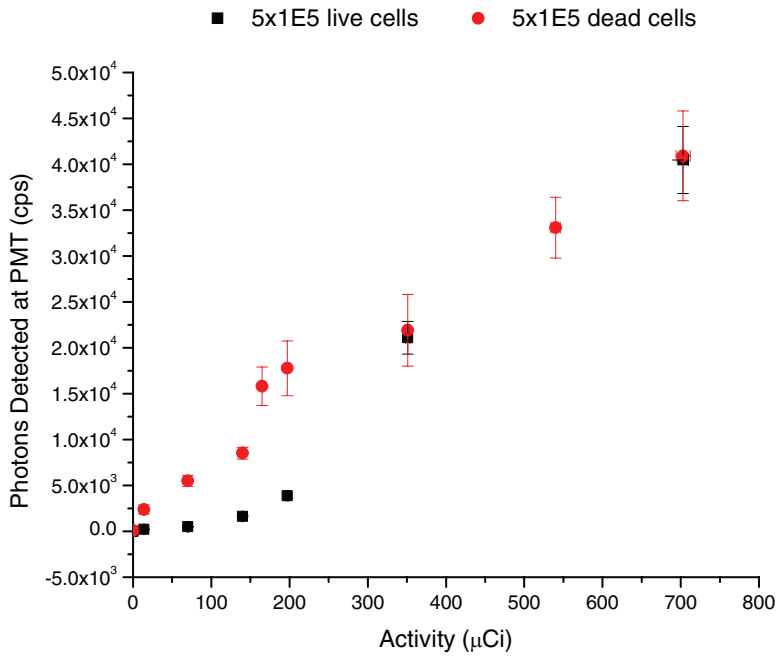

Figure 7. Comparison of the UVA photoemission from dead and live cells, each plated with 5×10^5 cells/5 mL and exposed to various ^{90}Y activities (n=9). Same data as that from figure 2.

population, where a different range of densities was studied, a trend was observed where the photon emission rate increased with increasing cell density up to a maximum at 1×10^4 cells/5 mL, after which a decrease in detected photons was demonstrated. This distinct pattern of photon detection in live cells was demonstrated at all six of the activity levels applied and the reproducible nature of these emissions strongly supports a relationship between the density of cells and the photon emission quantity. We suggest that the decrease in photon quantification at cell densities greater than 1×10^4 cells/5 mL could be attributed to a greater degree of interaction, such as scattering and absorption occurring between the photons emitted from the cells and structures within the cells. Scattering or absorption of the photons would effectively prevent the photons from reaching the detector to be read by the photocathode. The observed maximum at approximately 1×10^4 cells/5 mL may have biological implications if the emitted UV has a subsequent effect. It could be expected that observable damage or other biological effects would be evidently demonstrated when the density of cells is near 1×10^4 cells/5 mL. It is therefore expected that the effects of the cell-emitted UV would be relevant for cell signalling in all tissues which possess densities greater than 1×10^4 cells/5 mL (i.e. all tissues of the human body).

Importance of cell presence during irradiation. Exposure of the cell culture medium and petri dish in the absence of cellular material produced a relatively low count rate at the PMT (2767 cps at 703 μCi exposure activity). Upon irradiation of 5×10^2 cells/5 mL at 703 μCi, the photon emission quantity was 5 times greater than that emitted upon irradiation of 0 cells to the same activity. Upon irradiation of 1×10^4 cells/5 mL at 703 μCi, the luminescence rate increased to a maximum of just under 4.5×10^4 cps, a 16-fold increase over the photon emission levels

achieved in the absence of cells. It is clear that the presence of cells during irradiation induces a significantly greater photon emission signal than in the case of an absence of cells. This inference is further supported by the literature (Devaraj *et al* 1991, Ahmad *et al* 2013). Following removal of suspension medium from the cell culture, an increase in the photon emission intensity was identified (Devaraj *et al* 1991). Deveraj suggests that absorption of light emitted from the cellular material is attributed to the presence of suspension medium and therefore, upon removal of the absorbing medium, a greater photon signal could be detected.

4.3. Photon emission post-irradiation

In the assessment of photon emission from cells during and immediately after irradiation, we found that photon emission decreased sharply down to levels only marginally above background when measured only 1 min post-irradiation. Such results are supportive of direct cell irradiation being a causative factor for photon emission and indicates that the vast majority of photoemission ceases without coincident stimulation. The one caveat is that there may be a very small level of phosphorescence observed post-irradiation. In addition, some of the signal detected during the first tens of minutes of post-irradiation measurement is likely attributed to noise in the system caused by insufficient warm-up time following high voltage application. Even still, the contribution to the detected photon emission by cellular phosphorescence has not been dismissed since the post-irradiation emission intensity with cells was significantly greater than the high voltage controls quantified in the absence of cells.

5. Conclusions

The present study demonstrates significant secondary ultraviolet photon emission from both dead and living human keratinocyte HaCaT cells upon irradiation by low-LET β-particles. We have presented data showing various factors including cell density and intensity of irradiation that affect the level of secondary photon emission. In addition, the level of photon emission from dead cells was found to be significantly greater than that emanating from living cells over certain cell density ranges, leading us to suggest that photon emission may be reduced by antioxidant activity occurring within living cells. In living cells, a distinct relationship was observed between photon emission and the density of cells present within the irradiated field. It was also evident that the cells themselves were required to be present in order to achieve significant levels of photon emission. Overall, these results indicate that cells, when irradiated with β-particles emit secondary photons, some of which are emitted in the UV range. In addition, there may be a biological component which contributes to the level of measured photon emission. Further investigation is required to determine the responsible biologic or metabolic factor.

Acknowledgments

We are grateful to Dr O Howe (Dublin Institute of Technology) and Dr H Furlong (McMaster University, Dublin Institute of Technology) for generously donating the HaCaT cell line to our lab. Thank you to Dr S-H Byun (McMaster University) for his help regarding source geometry-dependent changes in solid angle, to the fellow members of the Seymour/Mothersill lab for their instruction on the topic of cell culture and for their continuing

support in the lab and to the faculty, staff and students of the Medical Physics and Applied Radiation Sciences department at McMaster University for their support and many helpful discussions. This work was partially supported by the Natural Sciences and Engineering Research Council of Canada and by the Chronic Fatigue and Immune Deficiency Syndrome (CFIDS) Foundation USA.

Appendix A

Table A1. Pearson's correlation analysis output for assessment of activity-photon emission relationship in dead cells.

Cells/5 mL	R	p-value
0 (control)	0.948	<0.0001
5×10^5	0.985	<0.0001
1×10^6	0.988	<0.0001
5×10^6	0.980	<0.0001

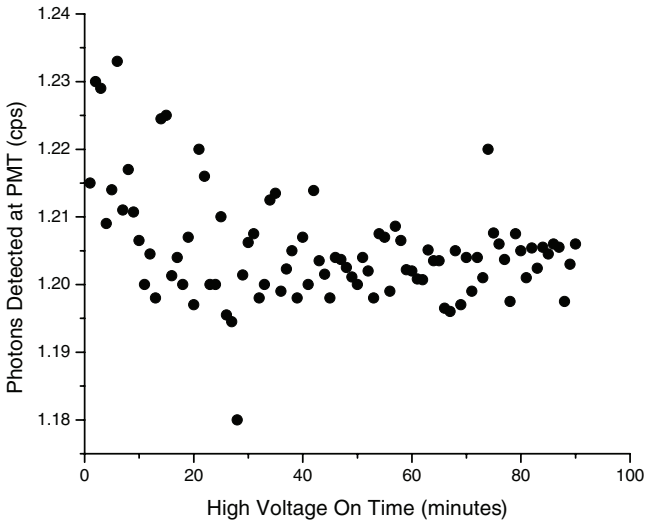

Figure A1. Background photon quantification conducted immediately following activation of the −800 V high voltage supply.

Table A2. Output for activity-dependent photon emission data fit to fixed-intercept linear regression (dead cells).

Cells/5 mL	R^2	p-value
0 (control)	0.914	0.003
5×10^5	0.895	0.051
1×10^6	0.893	0.044
5×10^6	0.800	0.031

Table A3. Pearson's correlation analysis output for assessment of activity-photon emission relationship in living cells.

Cells/5 mL	R	p-value
0 (control)	0.948	<0.0001
5×10^2	0.954	<0.0001
1×10^3	0.959	<0.0001
5×10^3	0.968	<0.0001
1×10^4	0.972	<0.0001
5×10^4	0.966	<0.0001
1×10^5	0.960	<0.0001
5×10^5	0.935	<0.0001

Table A4. Output for activity-dependent photon emission data fit to fixed-intercept linear regression (living cells).

Cells/5 mL	R^2	p-value
0 (control)	0.915	0.003
5×10^2	0.844	0.010
1×10^3	0.849	0.045
5×10^3	0.916	0.042
1×10^4	0.866	0.041
5×10^4	0.857	0.050
1×10^5	0.881	0.032
5×10^5	0.803	0.021

References

Ahmad S B, McNeill F E, Byun S H, Prestwich W V, Mothersill C, Seymour C, Armstrong A and Fernandez C 2013 Ultra-violet light emission from HPV-G cells irradiated with low let radiation from (90)Y; consequences for radiation induced bystander effects *Dose-response* **11** 498–516

Ahmad S B, McNeill F E, Prestwich W V, Byun S H, Seymour C and Mothersill C E 2014 Quantification of ultraviolet photon emission from interaction of charged particles in materials of interest in radiation biology research *Nucl. Instrum. Methods Phys. Res.* **319** 48–54

Bajpai R P, Bajpai P K and Roy D 1991 Ultraweak photon emission in germinating seeds: a signal of biological order *J. Biolumin. Chemilumin.* **6** 227–30

Bajpai R P, Van Wijk E P A, Van Wijk R and van der Greef J 2013 Attributes characterizing spontaneous ultra-weak photon signals of human subjects *J. Photochem. Photobiol. B: Biol.* **129** 6–16

Borodin D N 1930 Energy emanation during cell division processes (M-rays) *Plant Physiol.* **5** 119–29

Boukamp P, Stanbridge E J, Foo D Y, Cerutti P A and Fusenig N E 1990 c-Ha-ras oncogene expression in immortalized human keratinocytes (HaCaT) alters growth potential *in vivo* but lacks correlation with malignancy *Cancer Res.* **50** 2840–7 (PMID: 2183932)

Datto M B, Li Y, Panus J F, Howe D J, Xiong Y and Wang X F 1995 Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism *Proc. Natl Acad. Sci. USA* **92** 5545–9

Devaraj B, Scott R Q, Roschger P and Inaba H 1991 Ultraweak light emission from rat liver nuclei *Photochem. Photobiol.* **54** 289–93

Egea M I, Sánchez-Bel P, Martínez-Madrid M C, Flores F B and Romojaro F 2007 The effect of beta ionization on the antioxidant potential of Búlida apricot and its relationship with quality *Postharvest Biol. Technol.* **46** 63–70

Evelson P, Ordóñez C P, Llesuy S and Boveris A 1997 Oxidative stress and *in vivo* chemiluminescence in mouse skin exposed to UVA radiation *J. Photochem. Photobiol. B: Biol.* **38** 215–9

Gurwitsch A A 1988 A historical review of the problem of mitogenetic radiation *Experientia* **44** 545–50

Hamamatsu Photonics 2007 *Stability Photomultiplier Tubes: Basics and Applications* 3a edn (Hertfortshire: Hamamatsu Photonics) chapter 4 p 63

Knoll G F 2011 *Radiation Detection and Measurement* 4th edn (New York: Wiley)

Lehman T A, Modali R, Boukamp P, Stanek J, Bennett W P, Welsh J A, Metcalf R A, Stampfer M R, Fusenig N and Rogan E M 1993 P53 Mutations in human immortalized epithelial cell lines *Carcinogenesis* **14** 833–9

Le M, McNeill F E, Seymour C, Rainbow A J and Mothersill C E 2015 An observed effect of ultraviolet radiation emitted from beta-irradiated HaCaT cells upon non-beta-irradiated bystander cells *Radiat. Res.* **183** 279–90

Niggli H J, Scaletta C, Yu Y, Popp F A and Applegate L A 2001 Ultraweak photon emission in assessing bone growth factor efficiency using fibroblastic differentiation *J. Photochem. Photobiol. B: Biol.* **64** 62–8

Niggli H J, Tudisco S, Lanzanò L, Applegate L A, Scordino A and Musumeci F 2008 Laser-ultraviolet-A induced ultra weak photon emission in human skin cells: a biophotonic comparison between keratinocytes and fibroblasts *Indian J. Exp. Biol.* **46** 358–63 (PMID: 18697620)

Niggli H J 1996 The cell nucleus of cultured melanoma cells as a source of ultraweak photon emission *Naturwissenschaften* **83** 41–4

Niggli H 1993 Artificial sunlight irradiation human skin fibroblasts induces ultraweak photon emission *J. Photochem. Photobiol. B: Biol.* **18** 281–5

Popp F A, Nagl W, Li K H, Scholz W, Weingärtner O and Wolf R 1984 Biophoton emission. New evidence for coherence and DNA as source *Cell Biophys.* **6** 33–52

Quickenden T I and Tilbury R 1983 Growth dependent luminescence from cultures of normal and respiratory deficient saccharomyces cerevisiae *Photochem. Photobiol.* **37** 337–44

Rahn O 1936 *Invisible Radiations Of Organisms* (Berlin: Gebreuder Borntrarger)

Slawinski J, Ezzahir A, Godlewski M, Kwiecinska T, Rajfur Z, Sitko D and Wierzuchowska D 1992 Stress-induced photon emission from perturbed organisms *Experientia* **48** 1041–58

Van Wijk E, Kobayashi M, van Wijk R and van der Greef J 2013 Imaging of ultra-weak photon emission in a rheumatoid arthritis mouse model *PloS One* **8** e84579

Van Wijk R, Van Wijk E P A, van Wietmarschen H A and van der Greef J 2014 Towards whole-body ultra-weak photon counting and imaging with a focus on human beings: a review *J. Photochem. Photobiol. B: Biol*. **139** 39–46

Van Wijk R, Van Wijk E P, Wiegant F A and Ives J 2008 Free radicals and low-level photon emission in human pathogenesis: state of the art *Indian J. Exp. Biol.* **46** 273–309

Whiteside J R and McMillan T J 2009 A bystander effect is induced in human cells treated with UVA radiation but not UVB radiation *Radiat. Res.* **171** 204–11