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Experimental set up for the irradiation of biological samples and nuclear track detectors with UV C

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ABSTRACT

Aim: In this work we present a methodology to produce an "imprint" of cells cultivated on a polycarbonate detector by exposure of the detector to UV C radiation.
Background: The distribution and concentration of ^{10}B atoms in tissue samples coming from BNCT (Boron Neutron Capture Therapy) protocols can be determined through the quantification and analysis of the tracks forming its autoradiography image on a nuclear track detector. The location of boron atoms in the cell structure could be known more accurately by the simultaneous observation of the nuclear tracks and the sample image on the detector.
Materials and Methods: A UV C irradiator was constructed. The irradiance was measured along the lamp direction and at different distances. Melanoma cells were cultured on polycarbonate foils, incubated with borophenylalanine, irradiated with thermal neutrons and exposed to UV C radiation. The samples were chemically attacked with a KOH solution.
Results: A uniform irradiation field was established to expose the detector foils to UV C light. Cells could be seeded on the polycarbonate surface. Both imprints from cells and nuclear tracks were obtained after chemical etching.

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Conclusions: It is possible to yield cellular imprints in polycarbonate. The nuclear tracks were mostly present inside the cells, indicating a preferential boron uptake.

1. Background

Boron Neutron Capture Therapy (BNCT) is a cancer treatment modality based on the high thermal neutron capture cross section of ^{10}B and the high linear energy transfer (LET) radiation of the particles emitted in the reaction. Nontoxic tumor-seeking boron compounds are administrated to patients and the region to be treated is exposed to a thermal/epithermal neutron flux. Due to the short path of the emitted particles, a lethal damage is produced on tumor cells, preserving surrounding normal tissue.[1]

The precise knowledge about the location of boron compounds in tumor and surrounding tissue is essential in order to optimize the treatment protocol. The variety of tissue structures will accumulate different amounts of boron and this will affect dose distribution. Dosimetric effect of tissue heterogeneity has also been reported for other radiation therapies.[2,3]

The neutron autoradiography can provide this essential information.[4] This methodology is based on the superposition of a biological sample and a solid state nuclear track detector (SSNTD). When the assembly sample-detector is irradiated with thermal neutrons, the detector registers the damage (nuclear tracks) produced by the alpha and lithium particles originated in the neutron capture reaction with the boron atoms present in the sample. As alpha and lithium particles are ejected in opposite directions, only one of them is registered in the detector per each ^{10}B reaction making it possible to localize the emission place in the sample. In this way the distribution and concentration of ^{10}B atoms in biological samples can be determined through the quantification and analysis of the tracks forming its autoradiography image on the SSNTD.[5] As a chemical attack is necessary to enlarge the damage and convert it to observable tracks, the biological sample is lost in the etching process. For this reason, a methodology based on the use of a reference system have been developed,[6] to correlate the histological and the autoradiographic images for a better determination of local boron concentration values.

However, the location of boron atoms in the cell structure could be known even more precisely by the simultaneous observation of the nuclear tracks and the sample image on the detector. Sophisticated techniques of high resolution autoradiography[7] can be found in the literature, but an attractive alternative is the detector sensitization with UV C, in order to create reliefs in the SSNTD. This work had been proposed for culture cells in polyallyldiglycol carbonate and combined with special observation methods.[8–10]

2. Aim

The advantages of polycarbonate for neutron autoradiographic studies of samples coming from BNCT protocols were previously demonstrated,[11,12] so we present here the experimental setup to produce an "imprint" of cells cultivated on a polycarbonate detector by exposure of the detector to UV C radiation.

3. Material and methods

Cells of a human metastatic line of melanoma (MELJ) were seeded on polycarbonate foils (Lexan^{TM}) of 250 μm thickness. The detectors were cut with circular shapes in order to fit in the base of Petri dishes (diameter: 60 mm). They were incubated with borophenylalanine (BPA, 10 μg/L) for 4 h, and then washed and fixed. Glutaraldehyde and Methanol were tested as fixation solutions. Previous studies reported ^{10}B concentrations in blood during BNCT treatments calculated as the mean of measured values just before and after BNCT. The values were among 3 and 9 ppm of boron in blood.[13] The concentration of 10 ppm of boron in the medium of cells cultures adopted in our study represents a value close to those above mentioned values determined in blood samples during clinical treatments.

As shown in Fig. 1, the samples were attached to Lexan films of 9 cm × 13 cm and fixed to an acrylic container, in order to expose them to the thermal column of the biomedical facility of the RA-3 Reactor (Ezeiza, Argentina).[14,15] Two neutron fluences were evaluated: 10^{12} n cm^{-2} and 10^{13} n cm^{-2}.

The neutron flux was assumed to be uniform. Neutron flux was previously measured using a SPND (Self Powered Neutron Detector) and the uncertainty in the delivered neutron fluence is 8%. A UV C irradiator was built with a 254 nm wave length lamp (G15T8, General Electric, USA) of 15 W. A wooden container with a sample holder was fabricated and painted with opaque black coating, in order to absorb light. The dose was evaluated as a function of the distance from the lamp. The uniformity of the irradiance along the lamp and in the transversal direction was also analyzed in order to establish the field where the samples would receive a uniform dose. A UV C radiometer (Extech 40736C) was used for this purpose. A scheme of the UV C irradiation facility is presented in Fig. 2.

The samples were divided in sections of about 2 cm × 2 cm, exposed to the UV C lamp for 8 h, stained with hematoxylin and explored with increasing magnification (1.25×, 10×, 20×, 40× and 100×) with a light microscope (Olympus DP70). Reference points were established on different regions in order to localize them after the chemical etching and correlate the cellular images with its corresponding autoradiographies. For this purpose, the foils were drilled with a drill bit (diameter: 0.5 mm). The lateral resolution using the reference points is ≤10 μm.

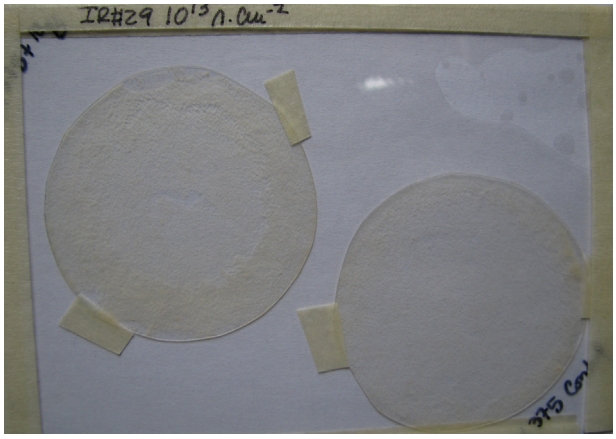

Fig. 1 – Irradiation assembly.

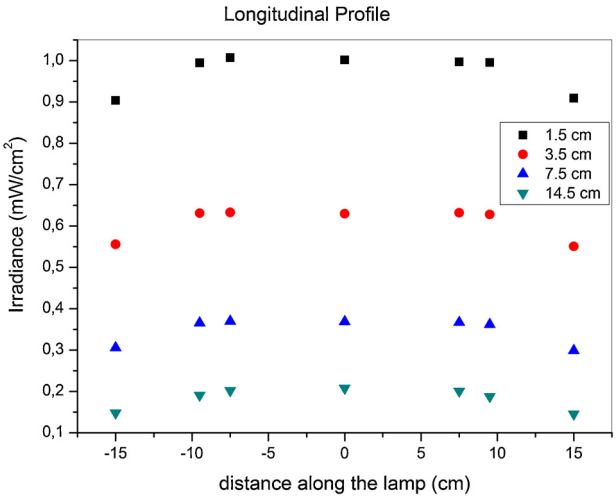

Fig. 3 – Longitudinal profile, at several distances from the UV-C lamp.

The foils were processed with PEW solution (30 g KOH, 80 g ethyl alcohol, 90 g distilled water) at 70 °C for 3 min. Pictures of the previously explored regions were taken.

4. Results and discussion

A stabilizing time of 30 min was established for the UV C lamp after being turned on. The results of the irradiance measurements are shown in Figs. 3 and 4. It can be observed from Fig. 3 that the irradiance remains approximately constant in the central region of the lamp for all the evaluated distances. Variations are around 10% for the distances closer to the lamp (1.5 cm and 3.5 cm). This fact allowed determining a longitudinal extension of 15.0 ± 0.2 cm of uniform field.

In Fig. 4 important variations in the irradiance were measured in the transversal direction (perpendicular to the lamp). At 4 cm from the central point, the irradiance is 50% lower. These observations led to the need to align the samples in the direction of the lamp (uniform field: 1.0 ± 0.2 cm). Given that the samples are small, a uniform field can be assumed inside them. A distance from the lamp of 1.5 ± 0.2 cm was chosen and the foils were irradiated for 8 h.

The glutaraldehyde fixation process seemed to be the most convenient to avoid cell shrinkage. In Fig. 5, an example of the exploration process is presented. On the left, the stained culture images matched with the cell imprints of the same zones (obtained after the etching) that appear on the

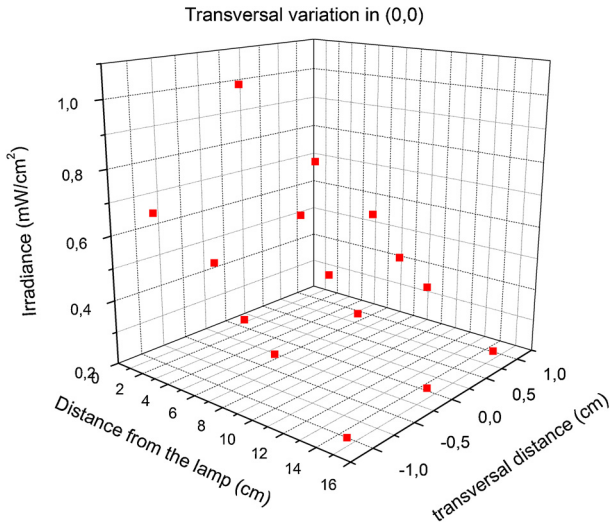

Fig. 4 – Transversal variation of the irradiance, at several distances from the UV-C lamp.

right. At low magnification, the reference marks can be localized. At greater magnification, it can be observed how the imprints reproduce the cells morphology. The reference points being circular holes, their centers were taken into account to

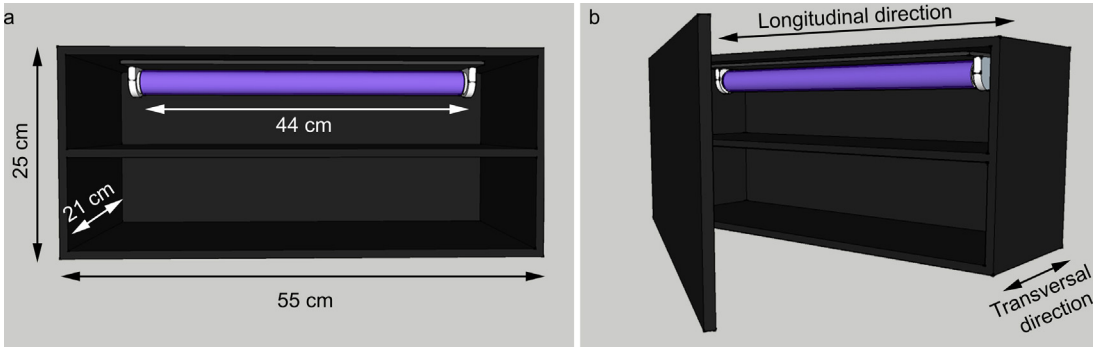

Fig. 2 – UV C irradiation facility scheme. Lamp diameter: 2.7 cm.

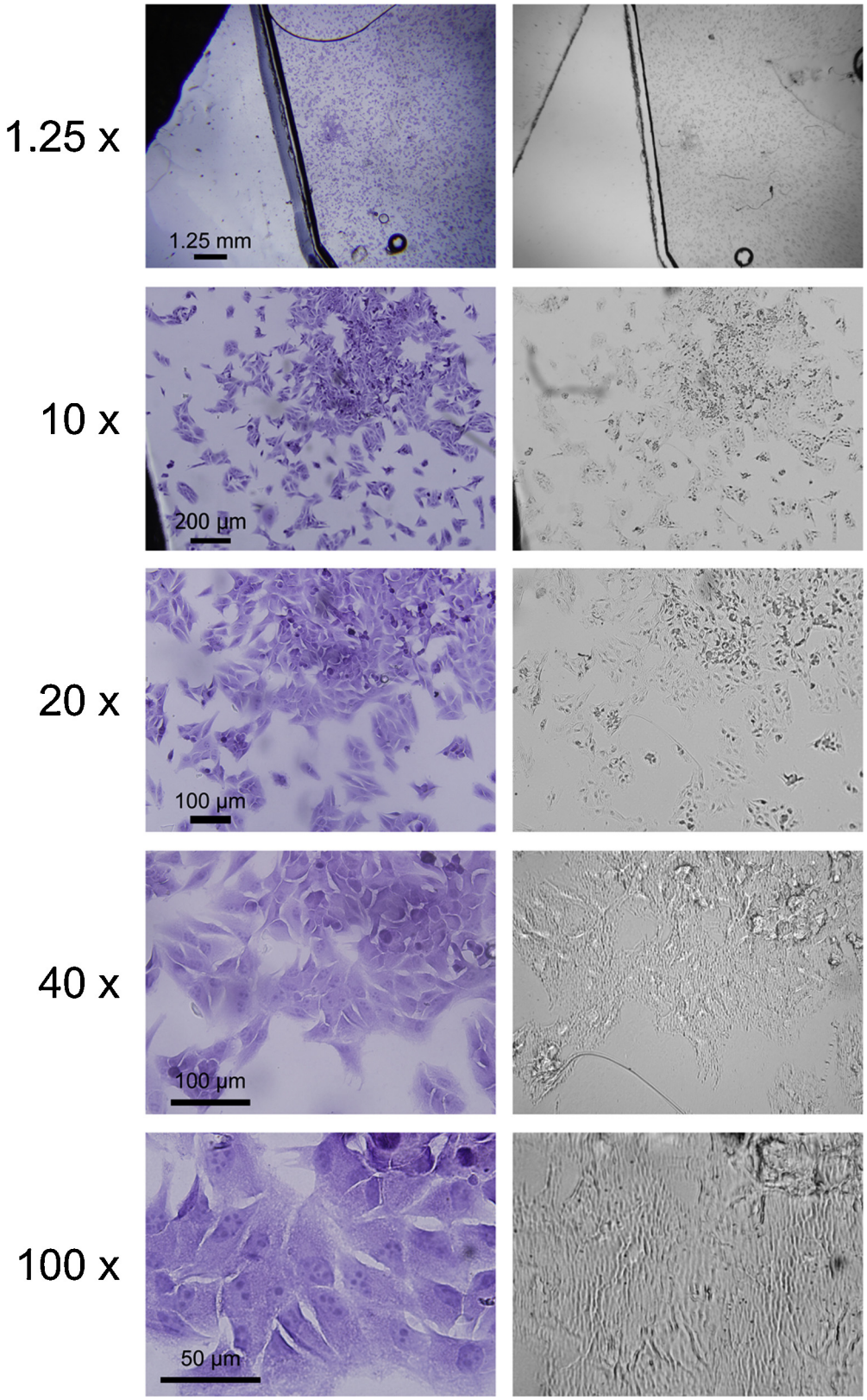

Fig. 5 – Exploration process. MEL J cells cultured on Lexan and stained with hematoxylin (left) and cell imprints after 8 h of UV C exposure (right), at increasing magnification (1.25×, 10×, 20×, 40× and 100×). Etching time: 3 min.

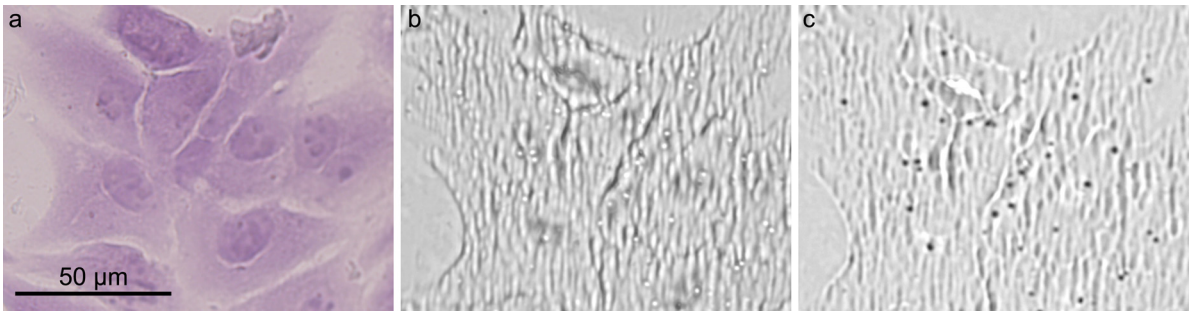

Fig. 6 – Cells and imprints. (a) MEL J culture incubated with BPA (10 μg ^{10}B/mL) and stained with hematoxylin. (b) Cellular imprints. (c) Same field as (b), focusing on nuclear tracks. 8 h of UV C, 3 min etching with PEW at 70 °C. Original magnification: 100×.

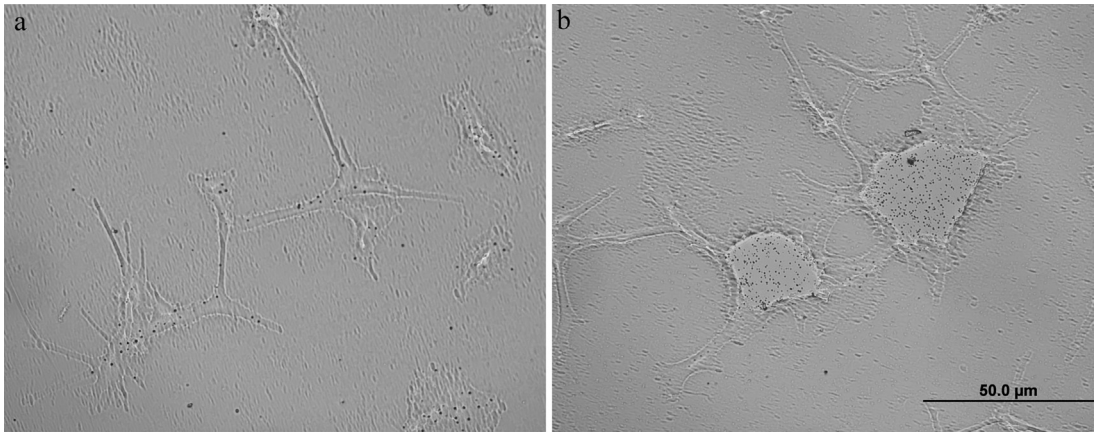

Fig. 7 – Cells imprints in (a) a monolayer and (b) superimposed cells.

establish the origin of coordinates. Moreover, the imprints allow a better correlation of the autoradiography with its corresponding image of the culture.

Sensitization of the detector by UV C irradiation produces a photodegradation of the polymer.[16] As a consequence an imprint of the biological material is formed, which reproduces the cellular and nuclear contours. As an example, Fig. 6(a) and (b) show regions of a cellular culture and its relief image, respectively. Tracks appear as bright points. By varying the focus, they can be clearly identified as black dots (Fig. 6c).

Regions where cells form a monolayer were paler (Fig. 7a) than those zones where there is cellular superposition (Fig. 7b). A neutron fluence of 10^{13} n cm^{-2} was chosen in order to obtain more events per cell. The etch pits seemed to be mostly present inside the cells, indicating a preferential boron uptake.

5. Conclusions

Cultures were able to grow on polycarbonate, but cell overlapping should be avoided. An irradiation system was set up and characterized. The first irradiation and etching conditions were tested, yielding cell imprints observable on polycarbonate surface. Future work will focus on determining the optimal conditions to obtain high contrast images of imprints and nuclear tracks.

Conflict of interest

None declared.

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