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Feasibility studies of colorless LR 115 SSNTD for alpha-particle radiobiological experiments

K.F. Chan [a], A.K.W. Tse [b], W.F. Fong [b], K.N. Yu [a,*]

[a] Department of Physics and Materials Science, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon Tong, Hong Kong
[b] Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon Tong, Hong Kong

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Abstract

The feasibility of using the active layer of the colorless LR 115 SSNTD for alpha-particle radiobiological experiments was studied. The track revelation time on the bottom side (the side attached to the polyester base) was much longer than that on the top side (the side not attached to the polyester base) of the active layer so track formation on the top side was more desirable. In relation to this, culture of HeLa cells on the bottom side of the active layer was found feasible although the cultured cell number was relatively smaller. The feasibility of using this SSNTD for alpha-particle radiobiological experiments was demonstrated by culturing cells on the bottom side while performing alpha-particle irradiation and chemical etching on the top side, and by taking photographs of the cells and alpha-particle tracks together under the optical microscope.

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Keywords: Solid-state nuclear track detector; SSNTD; LR 115; Radiobiology; Alpha particle

1. Introduction

Alpha-particle radiobiological experiments involve irradiating cells with alpha-particles and require accurate positions where the alpha particles hit the cells, the latter being essential for dosimetric determination. For such purposes, it is natural that solid-state nuclear track detectors (SSNTDs) were used as substrates for cell cultures. A review on SSNTDs can be found in [1] while a review of uses of SSNTDs in cellular radiation biology can be found in [2]. Furthermore, the substrate should also be thin enough to allow passage of alpha particles with nominal energies (e.g. 5.49 MeV of alpha energy from an ^{241}Am source under vacuum).

Durante et al. [3] seeded cells on a mylar film and an LR 115 SSNTD was stuck below the mylar base. However it would be advantageous if the cells can be directly seeded on the SSNTD to facilitate correlation between positions of the cells and the alpha-particle tracks. In these cases, colorless and transparent SSNTDs are needed so that the cells can be seen clearly. Recently, Gaillard et al. [4] and Chan et al. [5] developed their own thin CR-39 detectors, and cells were grown successfully in dishes with bases made from these thin CR-39 films and used for α-particle irradiation.

On the other hand, Dörschel et al. [6] studied the colorless LR 115 SSNTD (from DOSIRAD, France), the active layer of which was based on cellulose nitrate, in view of its applicability in radiobiological experiments with alpha particles. More recently, we have studied its bulk etch characteristics [7] in details. In the present work, we will further study the feasibility of this LR 115 SSNTD for alpha-particle radiobiological experiments. In particular, custom-made

* Corresponding author. Tel.: +852 2788 7812; fax: +852 2788 7830.
E-mail addresses: peter.yu@cityu.edu.hk, appetery@cityu.edu.hk (K.N. Yu).

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petri dishes, with a hole drilled at the bottom and covered with the active layer of an LR 115 detector, will be used for culturing HeLa cells. The feasibility is assessed by taking photographs of the HeLa cells and alpha-particle tracks together under the optical microscope, which can allow the hit positions on the cells by the alpha particles to be determined accurately.

2. Track formation in colorless LR 115 detectors

All the colorless LR 115 detectors used in this project were purchased from DOSIRAD (France). The detectors consist of a 100 μm thick polyester base and the thickness of the active layer of cellulose nitrate is specified by the manufacturer as 14 μm. This specified active layer thickness is a little bit more than our own value of 12.4 ± 0.1 μm measured using surface profilometry [8] and the value of 12.9 ± 0.1 μm measured by Dörschel et al. [6]. We assume an upper limit to the thickness as 14 μm.

In the present experiments, the active layer of colorless LR 115 is peeled off from the polyester base and then glued to the bottom of a 5 cm diameter custom-made petri dish with a hole of 1 cm diameter at the center as shown in Fig. 1. Alpha particles will be irradiated from the bottom side of the petri dish. As a first step, we would like to assess the time needed for track formation, since this will be crucial in minimizing the stress on the cultured cells.

As mentioned before, the upper limit to the thickness of the active layer of the colorless LR 115 detector is 14 μm, so alpha particles should have at least an initial energy of about 3 MeV (range in cellulose nitrate is about 14.73 μm) to pass through the active layer according to the SRIM program [9]. Therefore, irradiation will be studied for alpha particles with energies of 3, 3.5, 4, 4.5 and 5 MeV under normal incidence through a collimator. The alpha source employed in the present study was a planar ^{241}Am source (main alpha energy = 5.49 MeV under vacuum). Normal air was used as the energy absorber to control the final alpha energies incident on the detector. A relationship between the alpha energy and the air distance traveled by an alpha particle was therefore needed. This relationship was obtained by measuring the energies

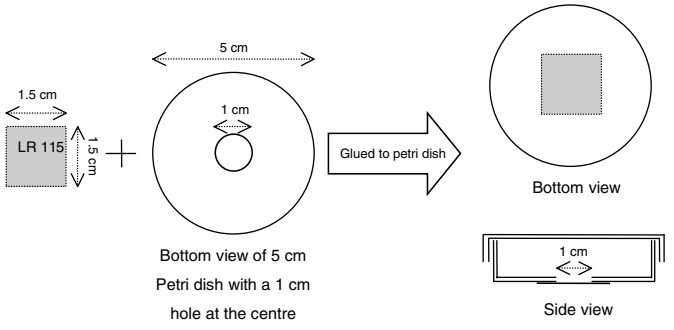

Fig. 1. Preparation of custom-made petri dish by gluing a colorless LR 115 detector onto the bottom of the petri dish with 5 cm diameter and with a 1 cm hole drilled at the center of the bottom.

for alpha particles passing different distances through normal air using α spectroscopy systems (ORTEC Model 5030) with passivated implanted planar silicon (PIPS) detectors of areas of 300 mm^2.

2.1. Irradiation on and etching from the top side of the LR 115 detectors

As the first step, alpha-particle irradiation is performed on the side not attached to the polyester base (hereafter referred as the top side of the LR 115 SSNTD). In this case, the cells have to be cultured on the side attached to the polyester base (hereafter referred as the bottom side of the LR 115 SSNTD).

In the beginning, we adopted the etching conditions employed by Dörschel et al. [6], i.e. etching in 7.25 N NaOH at 37 °C for 0.75 h. The temperature was kept constant with an accuracy of ±1 °C. After chemical etching, the detectors were taken out from the etchant, rinsed with distilled water and dried in air. However, no tracks could be observed under the optical microscope with a magnification of 200×. As a result, we have tried a longer etching period of 1 h for the same etchant. Tracks were observable but they were still relatively small and were difficult to observe for the purpose of radiobiological experiments. Furthermore, not all tracks could be seen clearly under the optical microscope, so not all alpha particle hits on the cells could be identified clearly. Therefore, we have tried an even longer etching period of 1.5 h. We observed that for the same irradiation time of 10 min, the number of tracks resulted from 1 h of etching was much smaller than that resulted from 1.5 h of etching.

Fig. 2 shows a comparison between the appearance of tracks under the optical microscope with a magnification of 200× of alpha particles with different energies on the active layer of colorless LR 115 detector after etching in 7.25 N NaOH at 37 °C for 1 h and 1.5 h. Despite some fluctuations in the trends shown by the diameters of the tracks, which are likely due to the difference in the bulk etch rates of the LR 115 detector [7,10], the tracks from etching for 1.5 h are in general larger in size and can be identified more clearly.

2.2. Irradiation on and etching from the bottom side of the LR 115 detectors

As the second step, alpha-particle irradiation is performed on the bottom side of the LR 115 SSNTD, so that the cells can be cultured on the top side. It was surprising that the tracks took longer time to develop. The tracks became observable after at least 2 h etching in 7.25 N NaOH at 37 °C, and the size and the number were then only similar to the case for irradiation on the top side and etched for 1 h etching in the same etchant. The reason is still unknown and we propose the difference arises as a result of the glue used to bind the active layer to the polyester base.

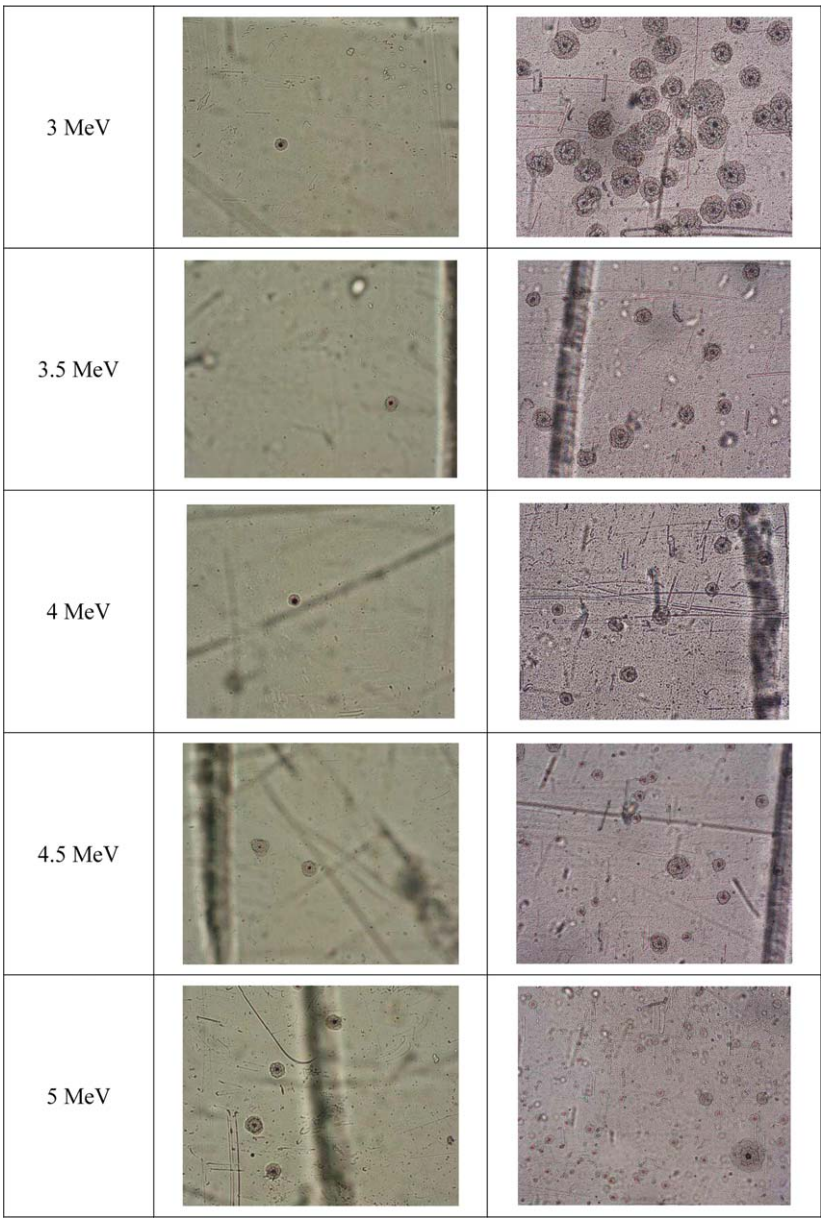

Fig. 2. Tracks observed under the optical microscope with a magnification of 200× of alpha particles with different energies on the top side of the active layer of colorless LR 115 detector after etching in 7.25 N NaOH at 37 °C for 1 h (left column) and 1.5 h (right column).

Although longer etching periods can give similar effects as those for irradiation on and etching from the top side of the LR 115 detectors (with shorter etching periods), the longer etching periods will induce more stress on the cells cultured on the detectors after alpha-particle irradiation. A possible method to alleviate this drawback is to etch the LR 115 detectors from the bottom side before actually culturing the cells and alpha-particle irradiation (hereafter referred to as pre-etching) to counteract the possible effects from the glue. It is anticipated that the track revelation time (referred to as the further etching time) after alpha-particle irradiation (after pre-etching) will be shortened. Different pre-etching duration in 7.25 N NaOH and different pre-etching temperatures were explored.

Table 1 shows the further etching time in 7.25 N NaOH at 37 °C needed for revelation of tracks for different pre-etching duration in 7.25 N NaOH and different pre-etching temperatures, and conditions of the LR 115 SSNTDs after revelation of tracks. It can be seen that the further etching time is in general less than 2 h and can be reduced to 1.5 h in many cases. However, many of the LR 115 SSNTDs became broken before the tracks were revealed. This poses potential risks in adopting these procedures in actual alpha-particle radiobiological experiments. In actual experiments, the weight of the nutritive medium for the cells will exacerbate the risk of damage of the etched SSNTDs. If these LR 115 SSNTDs were used for cell cultivation and became broken during the radiobiological experiments,

Table 1
Further etching time in 7.25 N NaOH at 37 °C needed for revelation of tracks for different pre-etching duration in 7.25 N NaOH and different pre-etching temperatures, and conditions of the LR 115 SSNTDs after revelation of tracks

Pre-etching duration in 7.25 N NaOH	Pre-etching temperature (°C)	Further etching time in 7.25 N NaOH at 37 °C for track revelation	Condition of LR 115 SSNTD after track revelation
2 h	37	1 h 50 min	Intact
3 h	37	1 h 45 min	Intact
4 h	37	1 h 30 min	Intact
3 h	40	2 h	Broken
1.5 h	45	2 h 30 min	Broken
2 h 15 min	45	2 h	Intact
2 h 25 min	45	2 h	Intact
2 h 30 min	45	1.5 h	Broken
2 h 40 min	45	1.5 h	Broken
3 h	45	1.5 h	Broken

the etchant will leak through the cracks and kill the cultured cells.

Taking into account the involved risk and the extra pre-etching procedures, cell cultivation on the top side and alpha-particle irradiation on the bottom side of the LR 115 SSNTD are deemed not feasible.

3. Cell cultivation on colorless LR 115 detectors

In Section 2.2 above, we concluded that, in order to use the colorless LR 115 SSNTD for alpha-particle radiobiological experiments, cells should be cultured on the bottom side and alpha-particle irradiation should be performed on the top side of the LR 115 SSNTD. The next task is to study the (relative) cytocompatibility of the top and the bottom sides of the LR 115 SSNTD.

These surfaces were used for culturing National Institutes of Health HeLa cervix cancer cells which were obtained from American Type Culture Collection. The cell line was maintained as exponentially growing monolayers at low passage numbers in minimal essential medium supplemented with 10% fetal borine serum, 1% (v/v) penicillin/streptomycin. The cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2. Subcultivation was performed every 3–4 d. Penicillin/streptomycin was produced by Gibco (Karlsrube, Germany). All other substances were purchased from Biochrom (Berlin, Germany). The cells were trypsinized for 4 min with 0.5%/0.2% (v/v) trypsin/EDTA (ethylenediamine-tetra-acetic acid; Biochrom), adjusted to a concentration of about 7.5×10^4 cells ml^{-1}, and plated out on the respective surfaces of the LR 115 SSNTDs.

Fig. 3(a) and (b) shows typical images under an optical microscope with a magnification of 200× of the plate out of HeLa cells cultured for 1 d with a concentration of about 7.5×10^4 cells ml^{-1} on the top and bottom sides, respectively, of the active layer of the LR 115 SSNTD. It can be observed that the cells grow well on both the top and bottom sides, although the cultured cell number is relatively smaller on the bottom side. Therefore, it is feasible to culture cells on the bottom side and perform alpha-particle irradiation on the top side of the LR 115 SSNTD.

4. Alpha-particle irradiation and location of hit positions

As the final step, the feasibility of using colorless LR 115 SSNTD for alpha-particle radiobiological experiments is assessed by taking photographs of the cells and

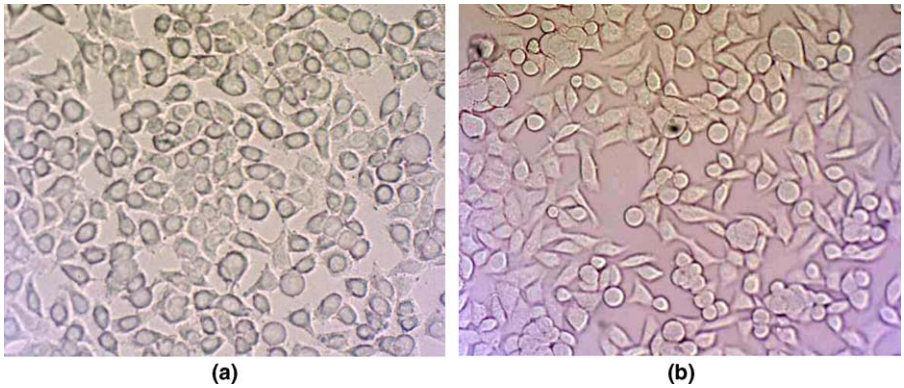

Fig. 3. Cells cultured for 1 d with a concentration of about 7.5×10^4 cells ml^{-1} observed under an optical microscope with a magnification of 200×: (a) on the top side of the active layer; (b) on the bottom side of active layer.

alpha-particle tracks together under the optical microscope, which can allow the hit positions on the cells by the alpha particles to be determined accurately.

Custom-made LR 115 petri dishes (cell dishes) are constructed as described in Section 2 and shown in Fig. 1, with the bottom sides of the LR 115 SSNTD facing upwards (i.e. glued against the bottom of the petri dishes). Cultivation of HeLa cells was carried out with procedures described in Section 3 above. The cells were adjusted to a concentration of about 7.5×10^4 cells ml^{-1}. After cell cultivation, the LR 115 cell dishes were irradiated from the bottom, i.e. on the top side of the LR 115 SSNTD, with 5 MeV alpha particles under normal incidence through a collimator for 25 min (Fig. 4). After alpha-particle irradiation, the LR 115 cell dish covered with the lid (with the cell culture inside) was kept floating on 7.25 N NaOH solution at 37 °C, leaving merely the bottom of the cell dish (with the top side of the LR 115 SSNTD) in contact with the etchant until the formation of visible tracks under optical microscope.

Fig. 5 shows an image of the HeLa cell monolayer cultured on the bottom side of a colorless LR 115 SSNTD with revealed 5 MeV alpha-particle tracks under optical microscope with a magnification of 200× (imaged from the side of the cell monolayer). These tracks are revealed by chemically etching only the top side of the active layer of the LR 115 SSNTD using the 7.25 N NaOH solution at 37 °C for 1.5 h. The image shows both the positions of the alpha-particle tracks and the cells clearly, so location of hit positions on the cells by the alpha particles can be performed accurately. The corresponding radiobiological effect on the cell could then be monitored. Therefore, we conclude that it is feasible to use colorless LR 115 SSNTD for alpha-particle radiobiological experiments.

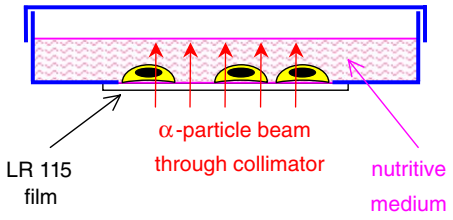

Fig. 4. The irradiation of the cell monolayer through the custom made LR 115 cell dish.

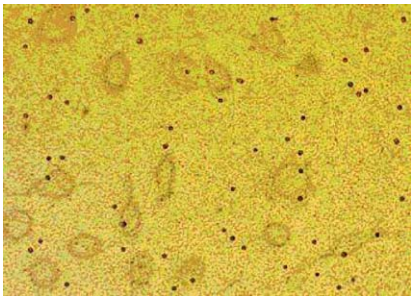

Fig. 5. The image of the HeLa cell monolayer cultured on the bottom side of a colorless LR 115 SSNTD (facing upwards in the experiment) with revealed 5 MeV alpha-particle tracks (black dots) under optical microscope with a magnification of 200× (taken from the side of the cell monolayer). These tracks are developed on the top side of the active layer of the LR 115 SSNTD (facing downwards in the experiment) after chemical etching in (while floating on) a 7.25 N NaOH solution at 37 °C for 1.5 h. The alpha-particle irradiation time was 25 min.

5. Conclusions

Alpha-particle radiobiological experiments involve irradiating cells with alpha particles and require accurate positions where the alpha particles hit the cells, the latter being essential for dosimetric determination. In the present paper, the feasibility of using the active layer of the colorless LR 115 SSNTD (from DOSIRAD, France) for alpha-particle radiobiological experiments has been studied. The conclusions are as follows:

(a) Track revelation time on the bottom side (the side attached to the polyester base) is much longer than that on the top side (the side not attached to the polyester base) of the active layer. Pre-etching can shorten the track revelation time, but poses potential risks of damage of the etched SSNTDs. Therefore, track formation on the top side is more desirable.
(b) It is feasible to culture cells on the bottom side although the cultured cell number is relatively smaller on the bottom side.
(c) The feasibility of using colorless LR 115 SSNTD for alpha-particle radiobiological experiments is demonstrated by culturing cells on the bottom side while performing alpha-particle irradiation and chemical etching on the top side, and by taking photographs of the cells and alpha-particle tracks together under the optical microscope, which can allow the hit positions on the cells by the alpha particles to be determined accurately.

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