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Simple preparation of thin CR-39 detectors for alpha-particle radiobiological experiments

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Abstract

Alpha-particle radiobiological experiments involve irradiating cells with alpha particles and require accurate positions where the alpha particles hit the cells. In the present work, we prepared thin CR-39 detectors from commercially available CR-39 SSNTDs with a thickness of 100 μm by etching them in 1 N NaOH/ethanol at 40 °C to below 20 μm. The desired final thickness was achieved within ~8 h. Such etching conditions can provide relatively small roughness of the detector as revealed by atomic force microscope, and thus provide transparent detectors for radiobiological experiments. UV radiation was employed to shorten track formation time on these thin CR-39 detectors. After exposure to UV light (UVA + B radiation) for 2–3 h with doses from 259 to 389 W/cm^2, 5 MeV alpha-particle tracks can be seen to develop on these CR-39 detectors clearly under the optical microscope within 2 h in 14 N KOH at 37 °C. As an example for practical use, custom-made petri dishes, with a hole drilled at the bottom and covered with a thin CR-39 detector, were used for culturing HeLa cells. The feasibility of using these thin CR-39 detectors is demonstrated 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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Keywords: Solid-state nuclear track detector; CR-39; Radiobiology

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, transparent and biocompatible materials, which can record alpha-particle traversals, are required as substrates for cell cultures. Furthermore, the substrate should not be dissolved in alcohol, which has to be used for sterilizing the substrate. The commercially available CR-39 solid-state nuclear track detectors (SSNTDs) fulfill these requirements. However, the substrate should also be thin enough to allow passage of alpha particles with nominal energies (e.g. those from ^{241}Am source). According to the SRIM program [1], the range of 5 MeV alpha particles in CR-39 is 28.77 μm. However, the thinnest commercially available CR-39 SSNTDs are ~100 μm thick and are thus not thin enough. A recent review on SSNTDs can be found in [2].

Recently, Gaillard et al. [3] developed their own CR-39 detectors by UV polymerization with a controlled polymer thickness of 10 μm. Cells were grown successfully in dishes with bases made from the new CR-39 films and used for α-particle irradiation. The etched CR-39 layer with a stained cell (fibroblast) monolayer was observed by a confocal microscope.

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In the present work, we propose a non-sophisticated method to prepare thin CR-39 detectors from etching commercially available CR-39 SSNTDs in NaOH/ethanol. The roughness of the CR-39 detectors is of great concern for radiobiological experiments since a rough and opaque detector will hinder the study of radiobiological effects under the optical microscope. Therefore, the roughness of the prepared thin CR-39 SSNTDs will be studied using atomic force microscope (AFM) [4]. Moreover, the track formation time should also be short since it is desirable to be able to locate the hit positions of the cells within a very short time after irradiation. In the present work, UV irradiation is proposed to shorten track formation time. As an example for practical use, custom-made petri dishes, with a hole drilled at the bottom and covered with a thin CR-39 detector, were used for culturing HeLa cells. The feasibility of using these thin CR-39 detectors is demonstrated 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. Methodology

2.1. Preparation of thin CR-39 detectors

In the present work, we prepared thin CR-39 detectors (<20 μm) from commercially available CR-39 SSNTDs with a thickness of 100 μm (from Page Mouldings (Pershore) Limited, Worcestershire) by chemical etching in NaOH/ethanol. For successful preparation of the thin detectors, the bulk etch characteristics should be studied in details. For convenience and better accuracies, the bulk etch characteristics were determined using CR-39 SSNTDs with a thickness of 1000 μm (also from Page Mouldings).

The bulk etch rate of the CR-39 SSNTDs was measured by the masking method [5,6]. CR-39 SSNTDs of dimensions $1 \times 1 \times 0.1$ cm (thickness) were prepared. The bulk etch was measured as the height difference between the etched portion and the portion masked by epoxy through surface profilometry measurements (using Form Talysurf PGI) [6]. Considering the bulk etch rates as well as the uniformity of the etched detector, the most appropriate etching conditions were determined. These etching conditions were then employed to prepare thin CR-39 detectors (<20 μm) from commercially available CR-39 SSNTDs.

For etching CR-39 detectors in NaOH/ethanol, a layer of precipitate consisting of sodium carbonate (which is one of the etched products) always accumulates on the surface of the CR-39 detector [7]. In order to ensure the most even and the fastest etching, the CR-39 SSNTDs were regularly rinsed with distilled water for every 2 h during etching in NaOH/ethanol. After etching, the thickness of the detector was measured using a micrometer (Mitutoyo, Japan) with an accuracy of ±1 μm. These 20 μm CR-39 detectors (after UV irradiation, see Section 2.2. below) were then glued by epoxy (Araldite®Rapid, England) to the bottom of petri dishes (Orange Scientific) with a diameter of 5 cm, with a hole of 1 cm diameter drilled at the bottom, to form the cell dishes as shown in Fig. 1 (see also [3,8]).

As mentioned in the introduction, the roughness of the thin CR-39 detectors is of great concern for radiobiological experiments. The roughness of the detectors after etching was studied by AFM for the different molarities used. For convenience, the roughness was determined using CR-39 SSNTDs with a thickness of 1000 μm. The AFM used in the present investigation was the Autoprobe CP model from Park Scientific Instruments (1171 Borregas Avenue, Sunnyvale, CA 94089). The probe of the AFM employed was an ultralever, with an opening angle of 10° and a length of 4 m.

Contact mode operation was used where a high-resolution image was expected. A constant force of 13.2 nN was applied on the tip and the scan rate was 1 Hz. The surfaces of the detectors were imaged directly in air and room temperature. The CR-39 detectors etched in different molarities of NaOH/ethanol were scanned for an area of 20×20 μm^2 with a 256×256 pixel resolution to determine the root-mean-square (RMS) roughness of the detector

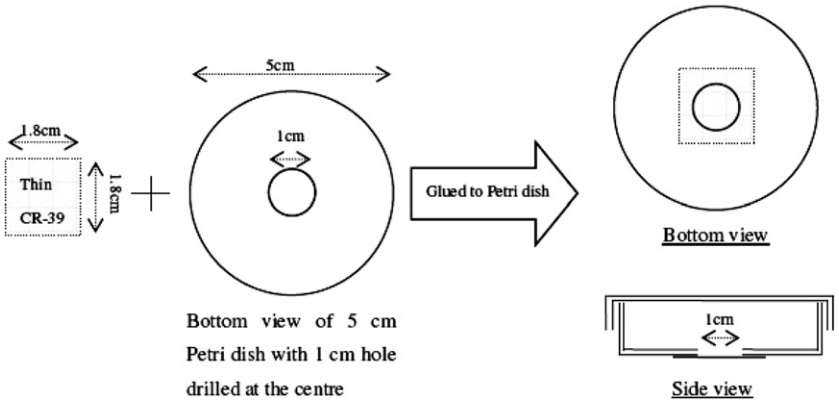

Fig. 1. Preparation of thin CR-39 cell dish by gluing the thin CR-39 detector onto the bottom of the petri dish with 5 cm diameter and with a 1 cm hole drilled at the center of the bottom.

surfaces. AFM has very high three-dimensional spatial resolutions and is ideal for quantitatively measuring the surface roughness.

2.2. UV irradiation to shorten track formation time

The track formation time should be short since it is desirable to be able to locate the hit positions of the cells within a very short time after irradiation in order to minimize the possible adverse effects on the cultured cells after they are taken out of the incubator and placed under the microscope. This is particularly difficult to achieve since the etching temperature used in the present work to reveal track after irradiation of the cells was 37 °C. This temperature was used to match that required for cell culture, and the track formation time is expected to be long for this low etching temperature.

In the present work, ultra-violet (UV) irradiation of the detectors is proposed to shorten track formation time. According to Khayrat et al. [9], the bulk etch and track etch rates of Pershore CR-39 detectors increased with UV exposure and the effect depended on exposure duration. Therefore, the detectors that glued to the petri dishes were exposed to UV light (UVA + B radiation) from Model 16S Solar Simulator (Solar Light Co., 721 Lane, Philadelphia, PA 19126) for 2–3 h, with UV doses from 260 to 390 W/cm^2. KOH was then used as the etchant because it is more reactive than NaOH and can reveal tracks within a shorter time frame.

2.3. Cell cultivation, alpha irradiation and location of hit positions

The thin CR-39 cell dishes were first sterilized by submerging into 75% (v/v) ethyl alcohol for 2 h. These cell dishes were then 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 a 10% fetal borine serum and 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 days. 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 1×10^5 cells ml^{-1}, and plated out on thin CR-39.

After cell cultivation, the CR-39 cell dishes were irradiated from the bottom with 5 MeV alpha particles under normal incidence through a collimator for 1 h as depicted in Fig. 2. The alpha source employed in the present study was a planar ^{241}Am source (main alpha energy = 5.49 MeV under vacuum). The final alpha energies incident on the detector were controlled by the source to detector distances

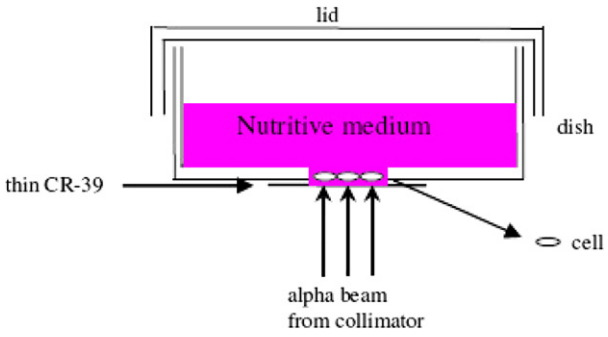

Fig. 2. The irradiation of the cell monolayer through the custom-made cell dish.

in normal air. The relationship between the alpha energy and the air distance traveled by an alpha particle with initial energy of 5.49 MeV from ^{241}Am was obtained by measuring the energies for alpha particles passing different distances through normal air using alpha spectroscopy systems (ORTEC Model 5030) with passivated implanted planar silicon (PIPS) detectors of areas of 300 mm^2.

After alpha-particle irradiation, the CR-39 cell dish covered with the lid (with the cell culture inside) was kept floating on the 14 N KOH solution at 37 °C, leaving merely the bottom of the thin CR-39 detector in contact with the etchant until the formation of visible tracks under optical microscope. With the tracks revealed beneath the cell monolayer, the hit positions on the cell could be pinpointed under optical microscope. The corresponding radiobiological effect on the cell could then be monitored.

3. Results and discussion

3.1. Preparation of thin CR-39 detectors

The bulk etch rates of the CR-39 SSNTDs with a thickness of 1000 μm etched in NaOH/ethanol were found to range from 22 to 75 μm/h for different molarities at 55 °C as shown in Table 1. Therefore, the desired final thickness of 20 μm could be achieved within as short as ~4 h. However, such fast etching rates might result in excessive precipitation of etched products on the detector [7] leading to uneven etching. Finally, the etchant has been decided

Table 1
The bulk etch rates of CR-39 SSNTDs derived from the masking method using Form Talysurf PGI in different etchants

Etchant	Bulk etch rate from masking method (μ/h)
0.5 N NaOH/ethanol at 55 °C	22.364 ± 0.738
1.0 N NaOH/ethanol at 55 °C	47.522 ± 0.248
1.5 N NaOH/ethanol at 55 °C	61.328 ± 3.723
2.0 N NaOH/ethanol at 55 °C	65.606 ± 1.736
2.15 N NaOH/ethanol at 55 °C	75.615 ± 4.384
2.52 N N NaOH/ethanol at 55 °C	75.290 ± 2.637
1.0 N NaOH/ethanol at 55 °C	9.490 ± 0.040
1.5 N NaOH/ethanol at 55 °C	11.700 ± 0.100

Table 2
Root-mean-square (RMS) roughness of the CR-39 SSNTDs etched with different etchants at 55 °C for 2 h

Etchant	RMS roughness from AFM
0.5 N NaOH/ethanol	0.00537
1.0 N NaOH/ethanol	0.00558
1.5 N NaOH/ethanol	0.00760
None	0.00261
6.25 N NaOH/water	0.00442

as 1 N NaOH/ethanol at 40 °C, for which the bulk etch rate of CR-39 is ~10 μm/h. Under such etching conditions, the desired final thickness could be achieved for ~8 h. The 100 μm CR-39 SSNTDs were first cut into the size of 1.8×1.8 cm^2 and were then etched in 1 N NaOH/ethanol at 40 °C down to ~20 μm.

The roughnesses of the CR-39 detectors are summarized in Table 2. It can be observed that the CR-39 detectors etched in NaOH/ethanol are rougher than those etched in 6.25 N NaOH/water solution, and the roughness increases with the molarities of NaOH/ethanol. This also supports our choice of the relatively mild etching conditions as 1 N NaOH/ethanol at 40 °C for the 100 μm CR-39 detectors to obtain the thin detectors instead of etching in higher molarities that can achieve the required thickness in a shorter time. The milder etching conditions can provide relatively transparent and less rough detectors for radiobiological experiments, which require observations under the optical microscope.

3.2. UV irradiation to shorten track formation time

After exposure to UV light (UVA + B radiation) for 2–3 h with doses from 260 to 390 W/cm^2, 5 MeV alpha-particle tracks can be seen to develop on our thin CR-39 detectors clearly under the optical microscope within 2 h in 14 N KOH at 37 °C.

3.3. Cell cultivation, alpha irradiation and location of hit positions

Fig. 3 shows the image of a HeLa cell monolayer on a prepared piece of thin CR-39 detector under the optical microscope in the transmission mode with a magnification of 200×. The image shows a group of alpha-particle tracks formed by etching the underside of the thin CR-39 detector beneath the cell monolayer. This thin CR-39 detector was exposed to UV light for 3 h before culturing the cells, and the cell dish was kept floating on the 14 N KOH solution for 4 h at 37 °C. 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. In fact, as mentioned in Section 3.2 above, the tracks start to be observable in less than 2 h of etching under the optical microscope. This is comparable to the track revelation time obtained by Gaillard et al. [3]. A longer etching time (4 h) has been used here to produce larger tracks that are more conspicuous on the image shown.

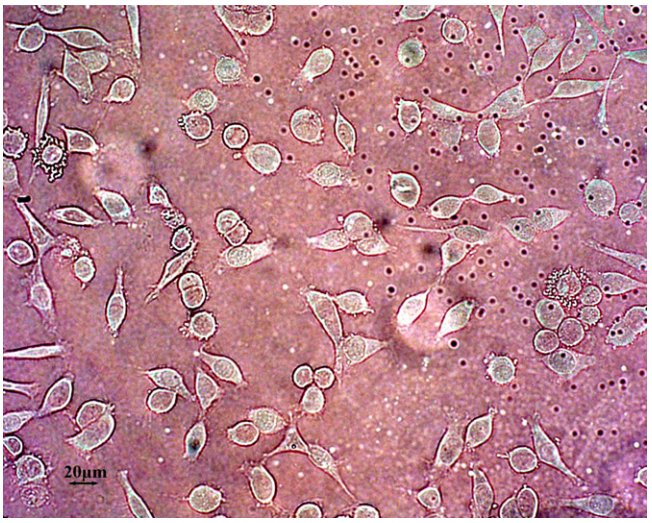

Fig. 3. The image of the HeLa cell monolayer on a thin CR-39 SSNTD with revealed alpha-particle tracks under optical microscope with a magnification of 200× (taken from the side of the cell monolayer). The 5 MeV alpha-particle tracks are seen on the right hand side as black dots. These tracks are developed on the underside of the CR-39 SSNTD (which is glued to the bottom of the cell dish) after chemical etching in (while floating on) a 14 N KOH solution at 37 °C for 4 h.

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