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Studies of biocompatibility of chemically etched CR-39 SSNTDs in view of their applications in alpha-particle radiobiological experiments

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Abstract

Alpha-particle radiobiological experiments involve irradiating cells with alpha particles and require thin biocompatible materials which can record alpha-particle traversals as substrates for cell cultures. The biocompatibilities of chemically etched CR-39 solid-state nuclear track detectors (SSNTDs) using aqueous NaOH or NaOH/ehtanol are studied through the abundance and morphology of the cultured HeLa cells. The wetting properties of these etched CR-39 SSNTDs are also studied. The moderately hydrophobic CR-39 SSNTDs as well as the hydrophobic NaOH/ethanol-etched CR-39 SSNTDs are more biocompatible than the hydrophilic aqueous-NaOH-etched SSNTDs. Too small water contact angles, too large surface energy (γ_s) or the polar component $\left(\gamma_s^p\right)$ do not favor the cell culture. On the other hand, the dispersive component $\left(\gamma_s^d\right)$ of the surface energy and the ratio γ_s^p/γ_s^d do not seem to significantly affect the biocompatibility.

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Keywords: Solid-state nuclear track detector; CR-39; Etching; Radiobiology; Alpha particle; HeLa cells

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. The commercially available CR-39 solid-state nuclear track detectors (SSNTDs) fulfill these requirements. Early radiobiological experiments using SSNTDs were performed by Durante et al. [1,2]. 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 [3], the range of 5 MeV alpha particles in CR-39 SSNTDs 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 [4].

Recently, Gaillard et al. [5] 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 alpha-particle irradiation. The etched CR-39 layer with a stained cell (fibroblast) monolayer was observed by a confocal microscope. However, for laboratories which do not have the facilities or the techniques to fabricate their own CR-39 SSNTDs, it will be desirable if they can prepare thin detectors from etching the commercially available CR-39 SSNTDs, such as the 100 μm CR-39 SSNTDs from Page Mouldings (Pershore) Limited, Worcestershire [6].

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However, as mentioned at the outset, the prepared thin CR-39 SSNTDs should be transparent and biocompatible. While transparency is relative easy to check even with the naked eye, changes in the biocompatibility of the etched CR-39 SSNTDs need to be studied in more details.

In the present work, the biocompatibility of raw CR-39 SSNTDs and those etched with different etchants are studied through the abundance and morphology of HeLa cells cultured on them. The wetting properties of these detectors are also studied through analyses of the contact angles, surface energies and the polar and dispersive components.

2. Methodology

2.1. CR-39 detectors and chemical etching

In the present work, CR-39 SSNTDs with a thickness of 1000 μm (from Page Mouldings (Pershore) Limited, Worcestershire) were employed. These are more convenient to handle and their properties are the same as the thin CR-39 SSNTDs with a thickness of 100 μm (also from Page Mouldings).

Separate CR-39 SSNTDs are prepared for cell cultivation (described in Section 2.2) and for measurements of contact angles and surface energies (described in Section 2.3). From cell cultivation, the numbers as well as the morphology of cells cultured on the raw and the chemically etched CR-39 SSNTDs are to be determined.

To study the number of cells, to measure the contact angles and the surface energies, besides the raw SSNTDs, three sets of etched CR-39 SSNTDs are prepared. These include (1) CR-39 films etched for 4 h in 6.25 N aqueous NaOH at 70 °C (which is the most commonly employed conditions; bulk etch rate ~1.2 μm/h [7]), (2) CR-39 films etched for 1 h in 1 N NaOH/ethanol at 40 °C (bulk etch rate ~9.5 μm/h) [6] and (3) CR-39 films first etched for 4 h in 6.25 N aqueous NaOH at 70 °C and then for 1 h in NaOH/ethanol at 40 °C. In the determination of the bulk etch rates, epoxy was applied to partially mask the SSNTDs which were removed after chemical etching. The removed thickness by etching was obtained through the difference between the levels of the exposed and the masked parts using surface profilometry measurements. The films for cell culture had a size of $2 \times 2\ \text{cm}^2$ while those for contact angle measurements had a size of $3 \times 3\ \text{cm}^2$.

To study the morphology of the cells, two types of specially etched CR-39 SSNTDs were made (also with a size of $2 \times 2\ \text{cm}^2$). For each of these films, one half was first masked by Araldite® epoxy adhesive and the whole film was then etched for 4 h in 6.25 N NaOH at 70 °C or etched for 1 h in 1 N NaOH/ethanol at 40 °C. The epoxy was then removed to expose the raw unetched surface. Effectively, CR-39 SSNTDs half etched with aqueous NaOH or half etched with NaOH/ethanol were obtained. The entire CR-39 SSNTDs were then used for cell culture.

2.2. Cell cultivation

HeLa cervix cancer cells were cultured on the raw and chemically etched CR-39 SSNTDs. These CR-39 SSNTDs were first sterilized by submerging into 75% (v/v) ethyl alcohol for 2 h. Films with similar areas ($2 \times 2\ \text{cm}^2$) were cultured with HeLa cervix cancer cells which were obtained from American Type Culture Collection. The cell line was maintained as exponentially growing monolayers 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. 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 number of about 2×10^4 cells/ml (and totally 10 ml) in 60 mm diameter Petri dish for 3 d of culture and plated out on the CR-39 SSNTDs.

2.2.1. Determination of cultured cell numbers

In our experiments, raw and chemically etched CR-39 SSNTDs were placed inside three Petri dishes for the plate out of HeLa cells, each Petri dish containing one raw film and one etched film. Cell attachment was examined after 3 d of culture. In order to count the cell number on different CR-39 SSNTDs, the attached cells on the various films were released by digestion with trypsin–ethylenediaminetetra-acetic acid (Invitrogen) and counted using a hemocytometer (Tiefe Depth Profondeur, Marienfeld, Germany). Cell viability was assessed by staining with 0.2% Trypan blue (Sigma), which only enters across the membranes of dead/non-viable cells.

2.2.2. Determination of cell morphology

In our experiments, the specially half-etched CR-39 SSNTDs were placed inside separate Petri dishes for the plate out of HeLa cells. Images of cells were captured under the optical microscope after 3 d of culture.

2.3. Measurements of contact angles and surface energies

The wettability of raw and etched CR-39 SSNTDs were investigated using the sessile drop technique using a contact angle geniometer (JY-82, China). The accuracy of this technique is typically ±2°. The test liquids employed were doubly distilled water, glycerol and ethylene glycol. For water (or liquid phase), the surface tension γ_l, its polar component γ_l^p and its dispersive component γ_l^d are given by $\gamma_l = 72.8$, $\gamma_l^p = 51.0$ and $\gamma_l^d = 21.8\ \text{mJ/m}^2$. For glycerol, $\gamma_l = 63.4$, $\gamma_l^p = 26.4$ and $\gamma_l^d = 37\ \text{mJ/m}^2$. For ethylene glycol, $\gamma_l = 48.3$, $\gamma_l^p = 19$ and $\gamma_l^d = 29.3\ \text{mJ/m}^2$. The reported results are the mean of at least six measurements made on different positions on the same film surface. To avoid cross-contamination, a dedicated microsyringe was used for each liquid.

The work of adhesion (W_a) between a liquid and solid as given by Young [8] and Eq. (1) and the Van Oss [9] equation (Eq. (2)):

$$W_a = \gamma_l(1+\cos\theta), \tag{1}$$

$$W_a = \left(\gamma_l^p\gamma_s^p\right)^{1/2} + \left(\gamma_l^d\gamma_s^d\right)^{1/2}, \tag{2}$$

where θ is the contact angle, γ_s^p and γ_s^d are the polar and dispersive components of the solid phase. From Eqs. (1) and (2), we obtain

$$\left(\gamma_l^p\gamma_s^p\right)^{1/2} + \left(\gamma_l^d\gamma_s^d\right)^{1/2} = \gamma_l(1+\cos\theta). \tag{3}$$

In this way, measurements with different liquids with known polar and dispersive components can solve for γ_s^p and γ_s^d. Measurements for contact angles and determination of the polar component $\left(\gamma_s^p\right)$, dispersive component $\left(\gamma_s^d\right)$ and the ratio $\left(\gamma_s^p/\gamma_s^d\right)$ have been performed for raw and chemically etched CR-39 SSNTDs in order to identify the relationships with the biocompatibility of the films.

3. Results and discussion

3.1. Cultured cell numbers

The numbers of cells cultured on CR-39 SSNTD pairs in different Petri dishes after 3 d of culture are shown in Table 1. We can see that the CR-39 SSNTD etched with NaOH/ethanol has a similar biocompatibility to the raw CR-39 SSNTD, while the CR-39 SSNTD etched with aqueous NaOH has a significant drop in the biocompatibility. Therefore, if thin CR-39 SSNTDs are to be prepared from etching the commercially available thicker CR-39 SSNTDs, NaOH/ethanol is the preferred etchant. Interestingly, the CR-39 SSNTD first etched with aqueous NaOH and then with NaOH/ethanol has a similar biocompatibility to the raw CR-39 SSNTD. It is apparent that the final step is more important in determining the biocompatibility of the etched CR-39 SSNTD. Therefore, if necessary, an alternative method to fabricate thin CR-39 SSNTDs from etching CR-39 SSNTDs is to first etch the SSNTDs with aqueous NaOH and then with NaOH/ethanol.

3.2. Cell morphology and abundance

Fig. 1 shows the image of HeLa cells cultured on the CR-39 SSNTD half etched with aqueous NaOH and another half unetched. The first observation is the much smaller number of cells on the etched portion, which agrees with the results in Section 3.1. The second observation is that many of the cells adhered on the etched portion are circular in shape while the cells cultured on the unetched portion has a high degree of spreading. The cells which do not spread are not strongly adhered to the film surface and are easily washed away by the phosphate buffer saline (PBS) solution during cell counting. This also explains the small number of cells counted on the CR-39 SSNTD etched with aqueous NaOH in Section 3.1.

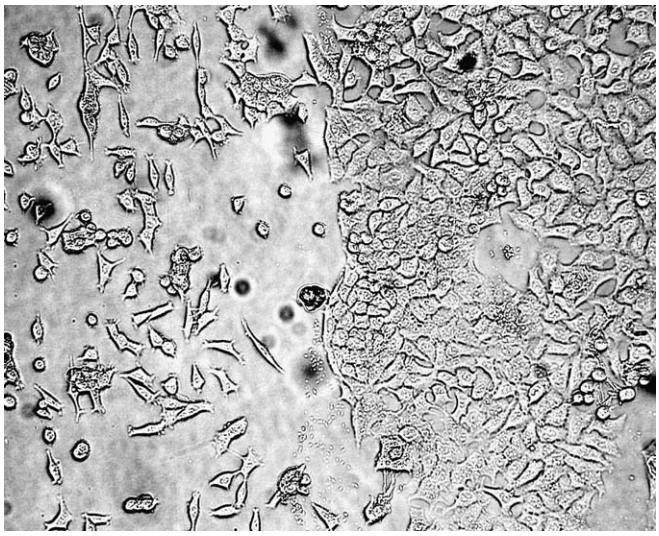

Fig. 1. The image of HeLa cells cultured on a half-etched CR-39 SSNTD. The left side was etched for 4 h with 6.25 N NaOH at 70 °C while the right side was unetched.

Fig. 2 shows the image of cells cultured on the CR-39 SSNTD half etched with NaOH/ethanol and another half unetched. The first observation is the similar number of cells on both the etched and unetched portions, which again agrees with the results in Section 3.1. The second observation is that the cells cultured on the etched side has a high degree of spreading, although there seems to be more cells circular in shape when compared to the unetched side.

From the above observations, we come to a conclusion similar to that made in Section 3.1, i.e. NaOH/ethanol is the preferred etchant to prepare thin CR-39 SSNTDs from thicker CR-39 SSNTDs.

Table 1
The number of cells cultured on CR-39 SSNTD pairs in different Petri dishes (1), (2) and (3)

	Petri dish		
	(1)	(2)	(3)
Raw CR-39 SSNTD	83800 ± 12900	65800 ± 9100	62200 ± 6100
CR-39 SSNTD etched for 4 h with 6.25 N NaOH at 70 °C	2300 ± 2000	–	–
CR-39 SSNTD etched for 1 h with 1 N NaOH/ethanol at 40 °C	–	58400 ± 8500	–
CR-39 SSNTD etched for 4 h with 6.25 N NaOH at 70 °C and then for 1 h with NaOH/ethanol at 40 °C	–	–	89400 ± 4900

Each Petri dish hosts two CR-39 SSNTDs, one of which is the raw unetched CR-39 SSNTD.

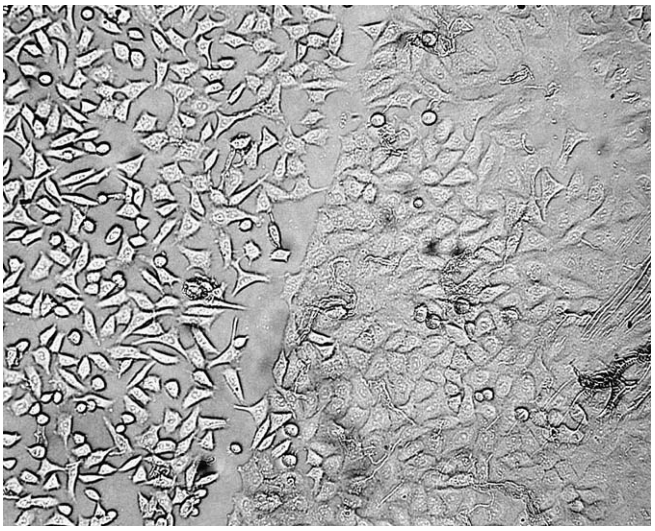

Fig. 2. The image of HeLa cells cultured on a half-etched CR-39 SSNTD. The left side was etched for 1 h with 1 N NaOH/ethanol at 40 °C while the right side was unetched.

3.3. Contact angles and surface energies

We have first performed measurements for contact angles for (1) raw CR-39 films; (2) CR-39 films etched for 4 h in 6.25 N NaOH; (3) CR-39 films etched for 1 h in 1 N NaOH/ethanol; (4) CR-39 films etched for 4 h in 6.25 N NaOH and then for 1 h in NaOH/ethanol, and then determined the surface energy (γ_s), the polar component $\left(\gamma_s^p\right)$, the dispersive component $\left(\gamma_s^d\right)$ and the ratio $\left(\gamma_s^p/\gamma_s^d\right)$. The results are shown in Table 2.

We can see that the CR-39 SSNTDs etched in aqueous NaOH are hydrophilic (water contact angle of about 39°), those etched in NaOH/ethanol (water contact angle of about 77°) or first etched in aqueous NaOH and then in NaOH/ethanol (water contact angle of about 75°) are hydrophobic, while the raw CR-39 SSNTD is in between (water contact angle of about 60°). The surface energy γ_s is similar for raw and NaOH/ethanol-etched CR-39 SSNTDs (from 30 to 40 mJ/m^2) but that for aqueous-NaOH-etched CR-39 SSNTDs are distinctly higher (~60 mJ/m^2). The polar component γ_s^p of the surface energy for aqueous-NaOH-etched CR-39 SSNTDs (~44 mJ/m^2) is also significantly higher.

From the results shown in Table 2, the moderately hydrophobic CR-39 SSNTDs are more biocompatible. Too small water contact angles, too large surface energy (γ_s) or the polar component $\left(\gamma_s^p\right)$ do not favor the cell culture. On the other hand, the dispersive component $\left(\gamma_s^d\right)$ of the surface energy and the ratio γ_s^p/γ_s^d do not seem to significantly affect the biocompatibility.

Other groups also showed that cells were more adhered, spread, and grown on moderately wettable surfaces [10–14]. The phenomenon was explained by the preferential adsorption of some serum proteins like fibronectin and vitronectin from the culture medium onto moderately wettable surfaces; these proteins played an important role for cell attachment onto substrates.

4. Conclusions

(1) Aqueous-NaOH-etched CR-39 SSNTDs are much less biocompatible than raw CR-39 SSNTDs, as judged by the number and morphology of cultured HeLa cells. These etched films are hydrophilic (water contact angle of about 39°). The surface energy γ_s is distinctly higher than those of the raw CR-39 SSNTDs as well as the NaOH/ethanol-etched CR-39 SSNTDs. The polar component γ_s^p of the surface energy for these films (~44 mJ/m^2) is also significantly higher.

(2) NaOH/ethanol-etched CR-39 SSNTDs have similar biocompatibilities as the raw CR-39 SSNTDs, as judged by the number and morphology of cultured HeLa cells. These etched films are hydrophobic (water contact angle of about 77°). The surface energy γ_s is similar to that of the raw CR-39 SSNTDs.

(3) The moderately hydrophobic CR-39 SSNTDs as well as the hydrophobic NaOH/ethanol-etched CR-39 SSNTDs are more biocompatible. Too small water contact angles (i.e. excessive hydrophilicity), too large surface energy (γ_s) or the polar component $\left(\gamma_s^p\right)$ do not favor the cell culture. On the other hand, the dispersive component $\left(\gamma_s^d\right)$ of the surface energy and the ratio γ_s^p/γ_s^d do not significantly affect the biocompatibility.

(4) If thin CR-39 SSNTDs are to be prepared from etching the commercially available thicker CR-39 SSNTDs, NaOH/ethanol is the preferred etchant since biocompatibility of the substrate is a key factor to the success of alpha-particle radiobiological experiments. An alternative method is to first etch the CR-39 SSNTDs by aqueous NaOH and then by

Table 2
The contact angles measured for different CR-39 SSNTDs using doubly distilled water (W), glycerol (G) and ethylene glycol (EG), and the determined surface energy (γ_s), the polar $\left(\gamma_s^p\right)$ and dispersive $\left(\gamma_s^d\right)$ components, and the ratio $\left(\gamma_s^p/\gamma_s^d\right)$

	W	G	EG	γ_s (mJ/m^2)	γ_s^p (mJ/m^2)	γ_s^d (mJ/m^2)	γ_s^p/γ_s^d (mJ/m^2)
1	59.9	58.3	40.1	40.9	28.8	12.1	2.38
2	39.2	35.1	10.1	57.7	44.3	13.4	3.30
3	76.5	67.9	46.0	32.7	10.0	22.7	0.44
4	75.0	68.7	52.7	30.4	14.4	16.0	0.90

(1) Raw CR-39 SSNTDs; (2) CR-39 SSNTDs etched for 4 h with 6.25 N NaOH at 70 °C; (3) CR-39 SSNTDs etched for 1 h with 1 N NaOH/ethanol at 40 °C; (4) CR-39 SSNTDs etched for 4 h with 6.25 N NaOH at 70 °C and then for 1 h with NaOH/ethanol at 40 °C.

NaOH/ethanol. The biocompatibility of the etched CR-39 SSNTDs will depend on the final step.

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