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Biocompatibility enhancement of chemically etched CR-39 SSNTDs through superficial pore formation by alpha-particle irradiation

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Abstract

Alpha-particle radiobiological experiments involve irradiating cells with alpha particles and require thin biocompatible materials as substrates for cell cultures, which can record alpha-particle transversals. CR-39 SSNTDs with a thickness of about 20 μm are suitable substrates. In the present work, the biocompatibility enhancement of these thin CR-39 SSNTDs is studied through superficial pore formation by alpha-particle irradiation. HeLa cells were cultured on these detectors and the biocompatibility was assessed through the number, morphology and average cell area of the cultured cells. For short etching time (15 min) in NaOH/H_2O (with subsequent etching in NaOH/ethanol), the biocompatibility of the CR-39 detector is enhanced through superficial pore formation by alpha-particle irradiation. In contrast, for long etching time (60 min) in NaOH/H_2O(with subsequent etching in NaOH/ethanol), the biocompatibility of the CR-39 detector deteriorates through superficial pore formation. Possible reasons are discussed.
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Keywords: CR-39; Radiobiology; Biocompatibility; Alpha-particle

1. Introduction

Polyallyldiglycol carbonate (PADC) is one commonly used solid-state nuclear track detector (SSNTD). A recent review on SSNTDs and their applications has been given by Nikezic and Yu (2004). PADC is commercially available under the name CR-39. CR-39 detectors are widely used in different branches of sciences. In particular, thin CR-39 SSNTDs with a thickness of 10–20 μm have been proposed as cell-culture substrates for alpha-particle radiobiological experiments (e.g., Gaillard et al., 2005; Li et al., 2006; Chan et al., 2007a).

Biocompatibility of the substrate is a key factor to the success of such radiobiological experiments. One of the main factors affecting the cell behavior is the surface topography (e.g., Baharloo et al., 2005). It has been established that the surface topography of SSNTDs can be modified on chemical etching (Ho et al., 2003a; Baharloo et al., 2005); so in principle we can enhance the biocompatibility of the CR-39 SSNTDs through chemical etching. However, prolonged etching of thin CR-39 SSNTDs is undesirable since these SSNTDs will become too thin and fragile, and it can become very difficult to mount them onto Petri dishes. Therefore, we explore biocompatibility enhancement of chemically etched CR-39 SSNTDs through superficial pore formation by (pre-) alpha-particle irradiation.

CR-39 detectors treated differently were employed for culturing HeLa cells. The numbers of cells cultured on these detectors, the average cell area, as well as the cell morphology were then determined, which were used as indicators for the biocompatibility.

2. Methodology

2.1. Characterization of different CR-39 detectors

In the present work, “thick” CR-39 SSNTDs with a thickness of 1000 μm from Page Mouldings (Pershore) Limited, Worcestershire, were employed. Although in reality we will be using thin CR-39 SSNTDs with a thickness of 10–20 μm, their

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physical and chemical properties are the same as the thick CR-39 SSNTDs, with the latter being more convenient to handle.

CR-39 SSNTDs with a size of $2 \times 2\,cm^2$ and with different treatments have been prepared for cell cultivation. These include raw CR-39 detectors which were not etched, and both unirradiated and irradiated CR-39 detectors which were subsequently chemically etched. The irradiated CR-39 SSNTDs were prepared by irradiation with 3 MeV alpha particles from an ^{241}Am alpha-particle source for 3 h. For an irradiation time of 3 h, the average track density is 415 000 track cm^{-2}. Both unirradiated and irradiated CR-39 detectors were etched for 15, 29 and 60 min in 6.25 N aqueous NaOH at 70 °C (which is the most commonly employed condition; bulk etch rate $\sim$ 1.2 µm/h; Ho et al., 2003b), and then for 5 min in 1 N NaOH/ethanol at 40 °C (bulk etch rate $\sim$ 9.5 µm/h; Chan et al., 2007a). The etching conditions will produce superficial pores with sizes which are small compared with the cell dimensions. The SSNTDs were etched in NaOH/ethanol in the end because Li et al. (2006) found that the NaOH/ethanol-etched CR-39 SSNTDs are more biocompatible than the $NaOH/H_2O$-etched CR-39 SSNTDs.

To characterize the surface topography of the unirradiated and etched CR-39 SSNTDS, the surface roughness was measured using atomic force microscope (AFM) in terms of the root-mean-square (rms) roughness. Contact mode operation of the AFM was used. For each detector, three different areas with a size of $10 \times 10\,\mu m^2$ were scanned with a 256×256 pixel resolution to determine the rms roughness. To characterize the surface topography of the irradiated and etched CR-39 SSNTDS, the size of the superficial pores were also determined using AFM. For a comparison, the diameters of alpha-particle tracks in CR-39 SSNTDs formed by etching in $NaOH/H_2O$ alone are calculated using the computer program called TRACK_TEST (Nikezic and Yu, 2006) (also available on the webpage: http://www.cityu.edu.hk/ap/nru/test.htm). It is remarked here that the track diameters can no longer be simulated using TRACK_TEST after a further etching in NaOH/ethanol for 5 min. The lengths of tracks are actually shortened when the CR-39 detectors are etched in NaOH/ethanol, which can be explained by the insulation of the track wall from the etchant with the etched products (Chan et al., 2007b).

2.2. Cell culture and cell examination

HeLa cervix cancer cells were cultured on CR-39 SSNTDs with different treatments. These CR-39 SSNTDs were first sterilized by submerging into 75% (v/v) ethyl alcohol for 2 h. Films with similar areas ($2 \times 2\,cm^2$) 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 bovine 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 (Karlsruhe, 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 5×10^4 cell ml^{-1} for counting cell numbers and 0.5×10^4 cell ml^{-1} for studying cell morphology and area (and totally 25 ml) in 90 mm diameter Petri dish for 3 d of culture and plated out on the CR-39 SSNTDs. Each Petri dish contained detectors from all categories, one from each category, including the raw detectors.

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 (Marienfeld, Germany). Cell viability was assessed by staining with 0.2% Trypan blue (Sigma), which only entered across the membranes of dead/non-viable cells. In order to study the cell morphology, cell images are captured under the optical microscope with a magnification of 500×. Finally, the average areas for HeLa cells cultured on different CR-39 detectors were measured by the software ImageJ (Image processing and Analysis in Java version 1.29×, available on the webpage: http://rsb.info.nih.gov.ij/). An image of a calibration ruler (0.01 mm per division) was captured under the microscope and used for scale setting. 'Known Distance' and 'Unit of Length' were input to obtain the global scale. The scale 1 µm = 9.73 pixels was used.

3. Results

3.1. Characterization of CR-39 detectors

As mentioned in Section 2.1, to characterize the surface topography of the unirradiated and etched CR-39 SSNTDS, the surface roughness was measured using AFM in terms of the rms roughness. The results are shown in Table 1. It can be seen that the surface roughness in general increases with the etching time in $NaOH/H_2O$.

On the other hand, to characterize the surface topography of the irradiated and etched CR-39 SSNTDS, the sizes of the superficial pores were determined using AFM. The results are shown in Table 2. We see that both the diameters and the depths of the pores increase with the etching time in $NaOH/H_2O$. For a comparison, the diameters of alpha-particle tracks in CR-39 SSNTDs formed by etching in $NaOH/H_2O$ alone are calculated using the computer program called TRACK_TEST (Nikezic and Yu, 2006) and shown in Table 2. Apparently, the diameters of the pores increase while the depths of the pores decrease during the final etching by NaOH/ethanol (see Chan et al., 2007b).

Table 1
The rms roughness of the surface of CR-39 SSNTDs after etching in $NaOH/H_2O$ for different periods of time and with additional 5 min NaOH/ethanol in the end

Time etched in $NaOH/H_2O$ (min)	rms Roughness (Å)
15	59 ± 10
29	65 ± 11
60	86 ± 10

Table 2
The diameters and depths of the pores

Time etched in aqueous NaOH (min)	Diameter (μm)	Depth (μm)
(a) After etching by $NaOH/H_2O$ with the additional etching by NaOH/ethanol for 5 min (data are measured using AFM)		
15	0.87 ± 0.05	0.14 ± 0.03
29	1.14 ± 0.07	0.67 ± 0.15
60	1.76 ± 0.06	0.86 ± 0.06
(b) After etching by $NaOH/H_2O$ without the additional etching by NaOH/ethanol (data are calculated by TRACK_TEST)		
15	0.38	0.40
29	0.76	0.79
60	1.5	1.71

Table 3
The number of cells per cm^2 cultured on CR-39 detectors for three different culture time (in separate Petri dishes)

Code	Culture time		
	67 h	75 h	89 h
(a) Set 1			
Rw	$89\,100 \pm 5700$	$86\,600 \pm 4900$	$140\,000 \pm 1000$
15b	$84\,400 \pm 1800$	$147\,000 \pm 17\,000$	$106\,000 \pm 13\,000$
15i	$75\,900 \pm 6600$	$134\,000 \pm 16\,000$	$162\,000 \pm 9000$
29b	$93\,400 \pm 4900$	$137\,000 \pm 7000$	$112\,000 \pm 6000$
29i	$90\,900 \pm 1300$	–	$131\,000 \pm 3000$
60b	$133\,000 \pm 6000$	$156\,000 \pm 11\,000$	$139\,000 \pm 14\,000$
60i	$78\,100 \pm 1800$	$100\,000 \pm 11\,000$	–
(b) Set 2			
Rw	$114\,000 \pm 7000$	$89\,100 \pm 4000$	$118\,000 \pm 10\,000$
15b	$58\,300 \pm 8100$	$77\,200 \pm 400$	$141\,000 \pm 4000$
15i	$123\,000 \pm 13\,000$	$127\,000 \pm 10\,000$	$159\,000 \pm 1000$
29b	$122\,000 \pm 7000$	$135\,000 \pm 6000$	$134\,000 \pm 7000$
29i	$103\,000 \pm 10\,000$	$84\,400 \pm 3500$	$135\,000 \pm 8000$
60b	$106\,000 \pm 8000$	$154\,000 \pm 8000$	$189\,000 \pm 32\,000$
60i	–	$103\,000 \pm 4000$	$128\,000 \pm 8000$

For the sample codes, the readers are referred to the caption for Fig. 1.

3.2. Cell examination

The numbers of HeLa cells cultured on the detectors per cm^2 after 67, 75 and 89 h cell culture time are shown in Table 3 (two sets of data). The cell number for 60 min of $NaOH/H_2O$ etching of blank detectors appears to be larger, when compared to those for 15 and 29 min etching. When we compare the irradiated and the unirradiated detectors, which have gone through etching with the same conditions, we observe that for long etching time (60 min), the irradiated detector (60i) has a lower cell number than the corresponding blank detector (60b). On the other hand, for short etching time (15b and 15i), the number of cells increases by irradiation of the substrate. For moderate etching time, 29b and 29i, the cell numbers are quite similar.

Fig. 1 shows typical images of HeLa cells cultured on different CR-39 detectors under the optical microscope. These detectors include raw unirradiated and unetched detectors, an unirradiated detector etched only in $NaOH/H_2O$ for 1 h, and those etched in $NaOH/H_2O$ for different periods of time with or without prior alpha-particle irradiation, and subsequently etched in NaOH/ethanol for 5 min at the end. It can be observed that all the alpha-particle tracks formed on etching of the irradiated CR-39 detectors are much smaller than the cells, many of them even being invisible under the optical microscope.

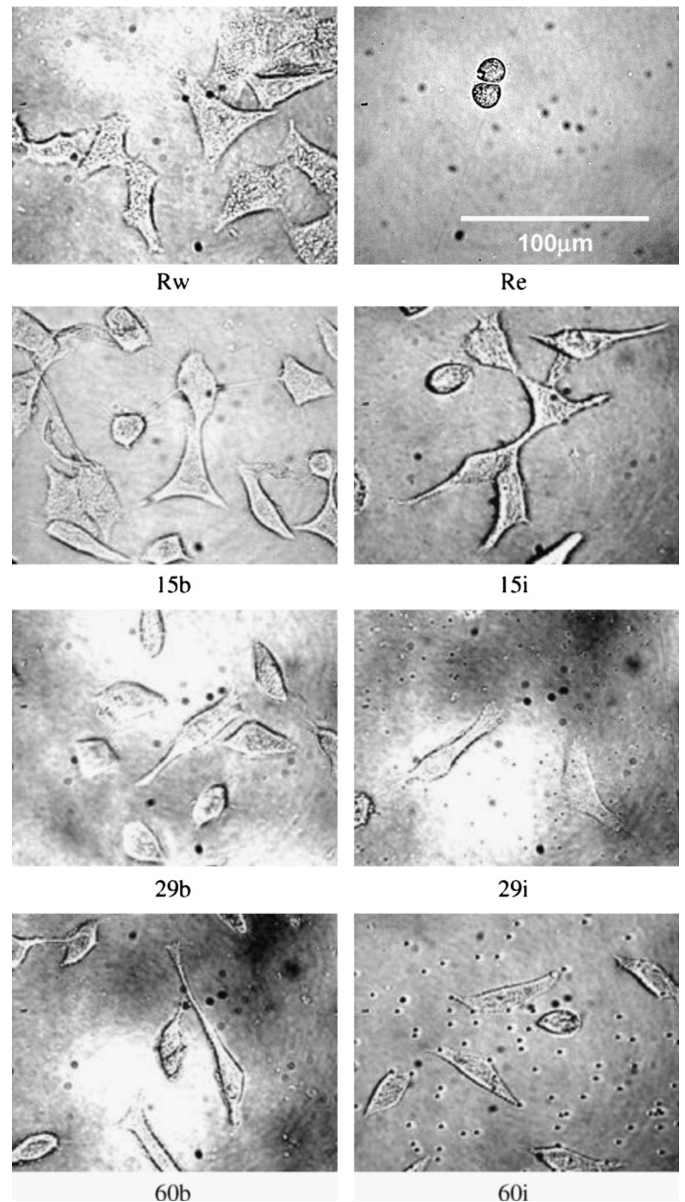

Fig. 1. Typical images of HeLa cells cultured on CR-39 detectors (captured under the optical microscope with a magnification of 500×). For the sample code, the numbers mean the time (in min) for $NaOH/H_2O$ etching; “b” means blank detectors and “i” means detectors irradiated for 3 h. Beside Rw and Re detectors, all detectors will be etched in NaOH/ethanol for 5 min at the end. The code “Rw” means raw detectors, i.e., unirradiated and unetched, while the code “Re” means an unirradiated detector etched only in $NaOH/H_2O$ for 1 h. The scale of these images is shown in the image for “Re”.

From Fig. 1, we can see that the cells cultured on the raw unirradiated and unetched detectors have the best spreading, which means the cells grow best on such detectors. Cells that do not grow well on the surface of detectors will not adhere

Table 4
The average areas measured for HeLa cells cultured on different CR-39 detectors

Code	Cell area (μm^2)
Rw1	820 ± 190
Rw2	1300 ± 540
Rw3	1500 ± 450
Rw4	1000 ± 240
15b	510 ± 83
15i	650 ± 170
29b	560 ± 110
29i	560 ± 99
60b	640 ± 170
60i	510 ± 77

The sample codes are the same as those in Table 2. A total of four different raw detectors were studied, which are denoted as Rw1, Rw2, Rw3 and Rw4. Cell culture time was 75 h.

strongly to the surface, and will be easily washed away by the phosphate buffer saline (PBS) solution, which will lead to a lower cell count. When compared to the cells cultured on unirradiated detectors etched only in NaOH/H_2O, the cells cultured on other detectors (except Rw detectors) have a higher degree of spreading and are less circular in shape. This has been explained by the very high surface energy of the NaOH/H_2O-etched CR-39 detectors (Li et al., 2006). When comparing the cells grown on the surface of "b" and "i" detectors, we see that the cells have a higher spread on "i" detectors for short NaOH/H_2O etching, and on "b" detectors for long NaOH/H_2O etching.

Table 4 shows the average areas measured for HeLa cells cultured on different CR-39 detectors measured by the software ImageJ. Among the blank detectors (15b, 29b and 60b), the average cell area increases with etching, although the differences are not very significant. An increase in the cell area means that the cells are better spread (Baharloo et al., 2005), which in turn implies better cell growth. The trend agrees with the increase in cell number for longer etching time. For short-etched detectors (15b and 15i), the average area of cells cultured on irradiated detector surfaces is larger. Again, better cell growth means better cell adhesion to the detector surface, and in turn means larger cell numbers. For long etching (60b and 60i), the average cell area decreases with alpha-particle irradiation, which implies poorer cell growth. For moderate etching time (29b and 29i), the average cell areas are similar in irradiated and unirradiated detectors. Cells cultured on raw detectors (Rw1–Rw4) in general have larger cell areas.

4. Discussions and conclusion

For short etching time (15b and 15i) in NaOH/H_2O (with subsequent etching in NaOH/ethanol), the biocompatibility of the CR-39 detector is enhanced through superficial pore formation by alpha-particle irradiation, which is evidenced through the larger cultured cell numbers (Table 3), more spreading of the cells (Fig. 1) and larger average cell area (Table 4). For such a short etching time, the depth of the pores are so small ($\sim 0.14\,\mu m$, see Table 2) that the cells probably feel them as roughness of the detector surface; so we can treat irradiated surfaces for short etching time as rougher surfaces when compared to those unirradiated surfaces. Apparently, rougher surfaces are more biocompatible. This is also supported by the results shown in Tables 1 and 2, where unirradiated surfaces which have larger surface roughness (from longer etching in NaOH/H_2O) are more biocompatible.

On the other hand, for long etching time (60b and 60i) in NaOH/H_2O (with subsequent etching in NaOH/ethanol), the biocompatibility of the CR-39 detector deteriorates through superficial pore formation, which is evidenced through the smaller cultured cell numbers (Table 3), less spreading of the cells (Fig. 1) and smaller average cell area (Table 4). For such a long etching time, the depth of the pores becomes much larger ($\sim 0.86\,\mu m$, see Table 2) so that the cells probably feel them as pits. Apparently, pits with considerable depths have deterred the growth of cells.

These findings are particularly useful since we can enhance the biocompatibility of thin CR-39 detectors through superficial pore formation by alpha-particle irradiation and then by a relatively short chemical etching. Chemical etching alone can also enhance the biocompatibility but prolonged etching of thin CR-39 SSNTDs is undesirable since these SSNTDs will become too thin and fragile.

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