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ALPHA-PARTICLE RADIOBIOLOGICAL EXPERIMENTS USING THIN CR-39 DETECTORS

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The present paper studied the feasibility of applying comet assay to evaluate the DNA damage in individual HeLa cervix cancer cells after alpha-particle irradiation. We prepared thin CR-39 detectors (<20 μm) as cell-culture substrates, with UV irradiation to shorten the track formation time. After irradiation of the HeLa cells by alpha particles, the tracks on the underside of the CR-39 detector were developed by chemical etching in (while floating on) a 14 N KOH solution at 37°C. Comet assay was then applied. Diffusion of DNA out of the cells could be generally observed from the images of stained DNA. The alpha-particle tracks corresponding to the comets developed on the underside of the CR-39 detectors could also be observed by just changing the focal plane of the confocal microscope.

INTRODUCTION

Alpha particles from inhaled radon progeny are the most important source of irradiation of the human respiratory tract. It is therefore pertinent to estimate the risk by investigating the biological effects of a small number of alpha particles crossing the cell nucleus. It is generally believed that deoxyribonucleic acid double strand breaks (DNA DSBs) induced by ions with high linear energy transfer (LET) are severe due to clustering of ionisation in the DNA, so the DSBs have important biological effects. These can be studied in alpha-particle radiobiological experiments to determine the relationship between the DNA damage in individual cells and the LET. The DNA damages induced by heavy ion irradiation have been detected in individual CHO-K1 cells derived from Chinese hamster with comet assay[(1)]. In the present paper, we study the feasibility of a method to (1) use a home-made thin CR-39 detector as the cell-culture substrate to record the positions where the alpha particles hit the cultured cells and (2) apply comet assay to evaluate the DNA damage in the cells. As a demonstration of the method, HeLa cervix cancer cells will be studied.

METHODOLOGY

Preparation and use of thin CR-39 detectors

Polyallyldiglycol carbonate films (commercially known as CR-39 detectors) are one of the most commonly used solid-sate nuclear track detectors (SSNTDs). A recent review on SSNTDs can be found in Ref. (2). There are distinct advantages of using CR-39 detectors as the cell-culture substrates in alpha-particle radiobiological experiments. For example, it is transparent, relatively biocompatible, and does not dissolve in alcohol during sterilisation. For alpha-particle radiobiological experiments, it is easier to quantify the alpha energies incident on the cell nuclei if the alpha particles pass through the substrate to strike the cells from the bottom because there is always a nutritive layer with variable thickness on top of the cells. However, the substrate should then 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 is 28.77 μm. However, the thinnest commercially available CR-39 SSNTDs are ~100 μm thick and are thus not thin enough.

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. These thin CR-39 detectors (after ultraviolet irradiation, see 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. In the present work, the detectors were irradiated by ultra-violet radiation to shorten track formation time[(4,5)] to reduce the stress on the cells. Without the ultra-violet radiation, the track formation time is expected to be long for low etching temperatures.

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After irradiation of the cells with alpha particles, the tracks on the underside of the CR-39 detector were developed by chemical etching in (while floating on) a 14 N KOH solution at 37°C (to match that required for cell culture). KOH was then used as the etchant because it was more reactive than NaOH and could thus reveal tracks within a shorter time frame.

Cell cultivation and alpha-particle irradiation

The thin CR-39 cell dishes were first sterilised 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 10% fetal borine serum, 1% (v/v) penicillin/streptomycin. The cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2. All other substances were purchased from Biochrom (Berlin, Germany). The cells were trypsinised for 4 min with 0.5/0.2% (v/v) trypsin/EDTA (ethylenediamine-tetra-acetic acid; Biochrom), adjusted to a concentration of about 4×10^4 cells ml^{-1}, and plated out on the CR-39 cell dishes.

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 about 1 h to give a fluence of about 12,700 alpha particles per cm^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 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. In our experiments, the energy of the alpha particles when they enter the cells are estimated to be 3.0 (+0.1, −0.2) MeV. The corresponding LET can be determined from the SRIM program if necessary[3].

Comet assay

The alkaline comet assay procedures were adapted from those described by Siu *et al.*[6] with slight modifications. All steps described were performed under dim yellow light to prevent DNA damage from ultraviolet irradiation. The thin CR-39 film with tracks formed on the bottom and cells cultured on the top was cut out and placed on the sample area of a CometSlide (Trevigen, Gaithersburg, MD, USA), and 50 µl of 1% low melting point agarose (LMAgarose) in Ca^{2+}- and Mg^{2+}- free PBS (phosphate-buffer saline) at 42°C was immediately pipetted onto the CR-39 film on the sample area of a CometSlide. The agarose was allowed to solidify at 4°C in the dark for no longer than 10 min and the slides were then immersed into a cold, freshly prepared lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, 10% DMSO, pH 10) for at least 1 h at 4°C in a Coplin jar. Following lysis, the slides were drained to remove any residual salts from the solution, which might otherwise affect DNA electrophoretic migration and introduce variability in the results. The slides were then aligned in two rows in a horizontal electrophoresis tray and covered with an alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH > 13) for 45 min at room temperature to allow the DNA to denature. Electrophoresis was performed in the same buffer at 1 Vcm^{-1} and 300 mA for 30 min. The slides were then drained, fixed in absolute ethanol for 5 min and allowed to air-dry for storage. Prior to the analysis of comets, 50 µl of 1% SYBR Green staining solution (Molecular Probe, Eugene, OR, USA) was added to each agarose spot and the stained slides were kept in a humidified dark-box. Since laser-scanning microscopy allows for improved analysis of the comet images[7],

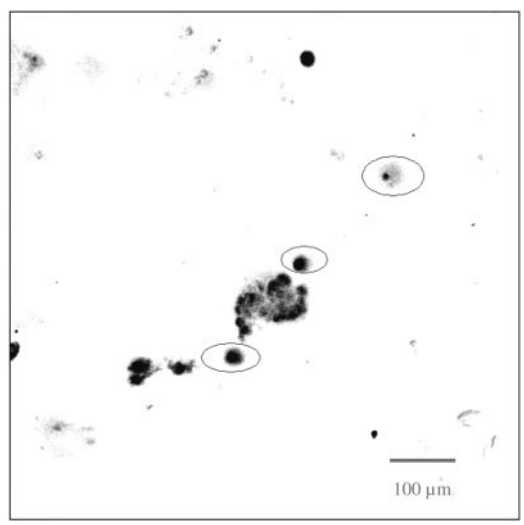

Figure 1. Images of stained DNA on a CR-39 detector. The cells were irradiated with alpha particles with a fluence of about 12,700 alpha particles per cm^2 (with an incident energy of 5.0 ± 0.5 MeV on the CR-39 detector, and 3.0(+0.1,−0.2) MeV on the cells). The three comets are circled.

the slides were analysed with an Axiovert 100M confocal microscope (Zeiss, Germany).

RESULTS AND DISCUSSION

The feasibility of applying comet assay to evaluate the DNA damage in individual HeLa cervix cancer cells after alpha-particle irradiation has been demonstrated in Figure 1 which shows the images of stained DNA. Diffusion of DNA out of the cells can be generally observed. The figure shows the images of stained DNA on a CR-39 detector with initial thickness of 15 μm and after chemical etching for 2 h 52 min. Three comet images (circled) were observed and the direction of electrophoresis was followed. Moreover, the alpha-particle tracks corresponding to the comets developed on the underside of the CR-39 detectors could also be observed by just changing the focal plane of the confocal microscope.

The captured images of stained DNA can be further analysed using the VisComet (1.5) image analysis software (Impuls, Germany). The parameters assessed will include the tail length (measured from the middle of the head to the end of the tail), tail DNA content (tail % DNA) and Olive Tail Moment. Wada *et al*.[1] also measured the tail moment from their comet assay of CHO-K1 cells irradiated by heavy ions. Analyses of the parameters for HeLa cells irradiated by alpha particles will be our next step, which will be described in a future paper.

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