Simple System to Irradiate Cultured Cells with UVA Light

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We developed a simple system for the irradiation of cultured cells with ultraviolet-A (UVA) light, and used it to study the colony-forming ability of fibroblasts in culture after UVA treatment. The system requires an ordinary clean bench, and the other materials and devices needed, such as dishes with a 35-mm diameter, plate glass, and 15-W UVA lamps, are easy to obtain and assemble. Neither stirring nor cooling of cells is required, because the fan of the clean bench acts as a cooler, and cells are irradiated from the bottom after they have settled in the dish. The UVA light incident on cells after its passage through both the plate glass and the bottom part of the dish contains so little of UVB wavelengths that cell killing by pyrimidine dimers was negligible. *Key words: Colony formation; Survival; Radiation system; Hydroa vacciniforme; Xeroderma pigmentosum.*

Cell response to radiation is usually measured in terms of cell death, which is generally determined by colony-forming ability (1). Many studies have been made with UVA or monochromatic light in the UVA range. The conventional equipment for UVA irradiation requires several attached devices to cool cells and to keep them in suspension during the irradiation (2–4). We developed a simpler system to study the cytotoxicity of fibroblasts. Cells are plated into dishes at cloning densities and irradiated through the bottom of the dish. The UVA lamp is cooled by the fan of a clean bench. The equipment is complete in itself, without attached devices being needed. The apparatus remains on the clean bench during irradiation, so sterile conditions are easily maintained.

MATERIAL AND METHODS

Cells and cell culture

Fibroblast cell lines designated N22OS and HV1OS were initiated in our laboratory from the cubital skin of a healthy subject and a patient with hydroa vacciniforme (HV), respectively (5, 6). The other fibroblast cell lines used were XP33OS and XP34OS cells (complementation group A) and XP3KA cells (compl. gr. C). The cells were routinely cultured at 37°C in a humidified atmosphere (10% CO_2 in air) in Dulbecco's modified minimum essential medium (7). The medium was supplemented with 20% fetal bovine serum. All cells were between passage 12 and 29 from initiation when used. Cloning efficiency varied between 13% and 45% when 35-mm dishes were used, and 30–67% with 100-mm dishes.

Treatment of cells with 254-nm UV radiation

To develop a small experimental system for post-UVA colony formation, we first checked whether a difference in the size of the dishes used would affect the cloning efficiency of the cells or the cytotoxicity

of 254-nm UV. We used two sizes of the dish with a 35- and 100-mm diameter respectively in the two experimental methods described below.

In situ method (8). From 100 to 4000 cells were plated into 35- or 100-mm plastic dishes to give 10–50 colonies per dish. Then, 4–7 h after the plating, the cells were irradiated with 254-nm UV and incubated for 8–9 days when 35-mm dishes were used or for 12–14 days when 100-mm dishes were used. After the incubation, colonies (aggregates of 50 or more cells) were counted.

Replating method (8). Immediately after cells in exponential growth were irradiated with UV, they were treated with trypsin, counted, and plated into 35- and 100-mm dishes with the same numbers per dish at each UV dose. Then the procedure for the *in situ* experiment was followed.

Treatment of cells with UVA

Assembly of the system (Fig. 1). Plate glass 5 mm thich was set about 2 cm above a pair of 15-W black (BLB) lamps. The lamps were cooled by the fan of the clean bench during irradiation. To maintain humidity, a wet cotton cloth was placed on the glass; 35-mm dishes and the plate glass were covered with a sheet of aluminum foil. Up to 12 dishes could be irradiated with fairly uniform irradiance at one time. The temperature was maintained at between 29° and 33°C.

Spectral irradiance. Transmission spectra of plate glass 5 mm thick and of the bottom part of a 35-mm dish were obtained with a spectrophotometer (577, Hitachi, Tokyo). At each wavelength of the emission spectrum of a BLB lamp, the relative irradiance of the UVA light that passed through both the plate glass and the bottom of the dishes (UVA-GD light) used was calculated by the following product: (the relative emission of a BLB lamp) × (the transmission of the plate glass) × (the transmission of the bottom part of a 35-mm dish).

Fluence rate. The fluence rate of the light that passed through the plate glass (UVA–G light) was measured with an UV radiometer (UVR 305/365, Eisai, Tokyo) at the surface of the glass. Actual fluence rates of the light incident on the cultured cells were calculated by multiplying the fluence rates measured with the radiometer by 0.87, which was the ratio of the fluence rate of the UVA–GD light to that of the UVA–G light. During the experiments, the spatial UVA irradiance in the room was less than 1 µW/cm².
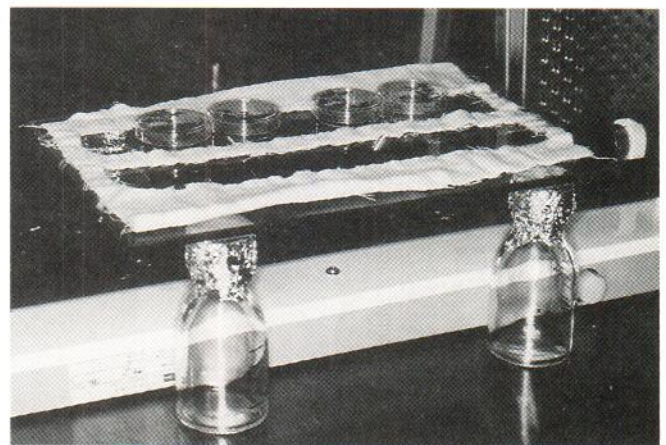

Fig. 1. Irradiation system assembled in a clean bench. Dishes 35 mm in diameter are placed on plate glass 5 mm thick. Two 15-W BLB lamps are under the glass. The damp cotton cloth on the glass is to maintain humidity when the dishes and the glass are covered with aluminum foil. Irradiance can be changed by screwing the caps of the bottle that support the glass up or down.

Table I. *Comparison of plating efficiencies*

Experiment no.	Cell line	Plating efficiency (%)			
		In situ		Replating	
		35[a]	100[a]	35[a]	100[a]
1	N22OS	28	42	26	67
	XP34OS	23	30	17	47
2	XP3KA	nd[b]	nd	22	38

[a] Dish diameter, in mm.
[b] Not done.

Survival after UVA treatment. Cells were plated into dishes one day before irradiation. The cultures were washed with 2 ml of phosphate-buffered saline without Ca^{2+} or Mg^{2+} (PBS⁻), and then 1.5 ml of glucosol (PBS with Ca^{2+}, Mg^{2+}, and glucose; PBS⁺) was added to the cultures. The pH of PBS⁺ was adjusted to 7.4 by adding sodium bicarbonate just before use. After the cells had been irradiated, the PBS⁺ was poured off and then 2 ml of medium was added to the cultures. In an experiment done to find whether a difference in the time between plating and irradiation would affect cell survival, we irradiated N22OS and HV1OS cells at 3-h intervals up to 27 h after the plating.

RESULTS

Colony-forming ability after 254-nm UV

With both plating methods, for each cell line tested, the plating efficiencies of cells plated into 100-mm dishes were better than those of cells plated into 35-mm dishes (Table I). However, the two survival curves for each cell line obtained with the replating method were very similar (Fig. 2). The survival curves for each cell line obtained in the *in situ* method were also similar (data not shown). We therefore chose 35-mm dishes for the UVA-survival experiments. In a separate experiment, the survival curves of HV1OS and XP33OS cells were
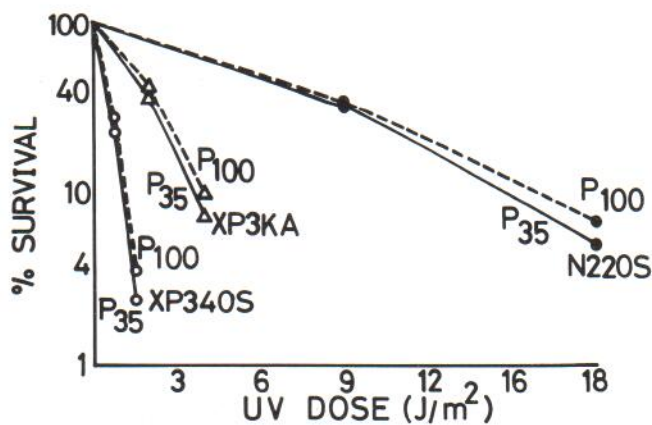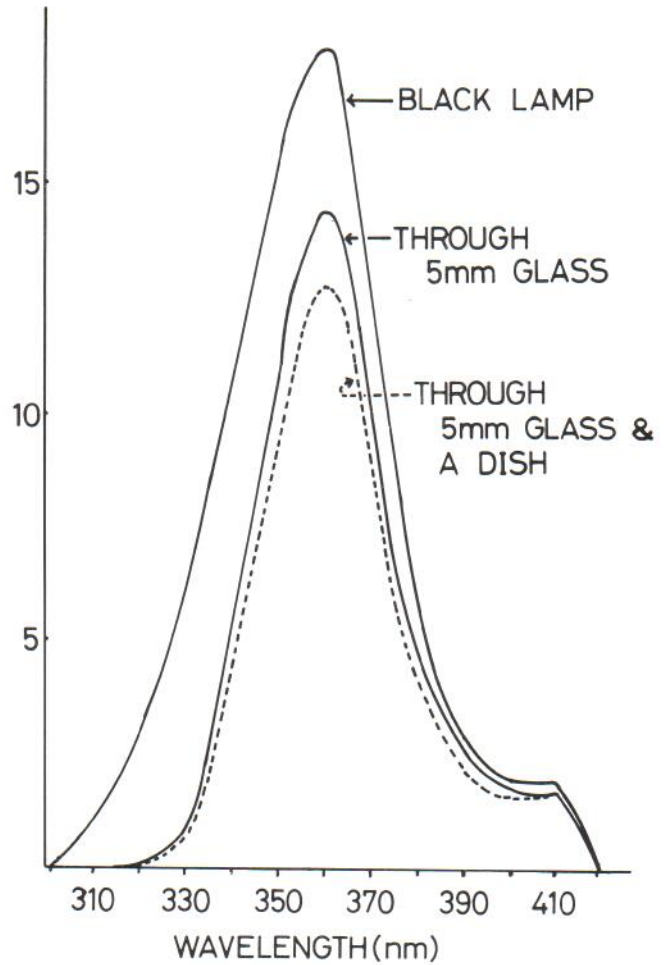

Fig. 3. Spectral irradiances. The innermost curve indicates the spectral irradiance when light has passed through both 5 mm thick plate glass and the bottom part of a culture dish (UVA–GD light). Outermost is the spectral irradiance of a BLB lamp. The middle is the irradiance of the light that has passed through plate glass 5 mm thick (UVA–G light). The units on the ordinate are arbitrary.

very similar to the curves of N22OS and XP34OS cells, respectively (data not shown).

Spectral irradiance of UVA

The spectral irradiance of the UVA light incident on the surface of cells resting on the dishes is shown in Fig. 3. The UVA–GD light had a wavelength range from 320 to 420 nm, with a peak at around 360 nm, and containing virtually no components of the UVB range. Because the patterns of spec-
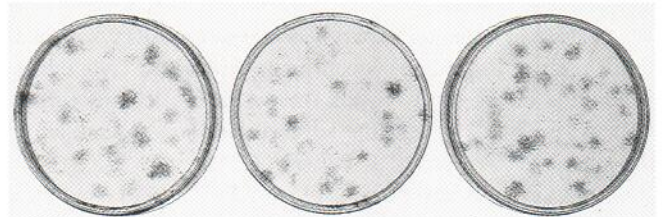

Fig. 2. Effect of dish size on the cytotoxicity of 254-nm UV when the replating method was used. Cells in exponential growth were irradiated with 254-nm UV at the designated doses, plated into 35- or 100-mm dishes, and assayed for colony-forming ability. Cell survival was evaluated by comparison of the cloning efficiency of the irradiated cells with that of control cells not irradiated. ○, XP34OS; △, XP3KA; ●, N22OS; P_{35}, 35-mm dish; P_{100}, 100-mm dish. (*N*, normals; *XP*, xeroderma pigmentosum; *OS*, Osaka; *KA*, Kanazawa.)

Fig. 4. Colonies obtained in one UVA survival experiment. From the left, exposure to UVA was for 20, 40, or 60 min. Each dish contains about 20–30 colonies. The numbers of cells seeded per dish at each dose were 200, 250, and 500.
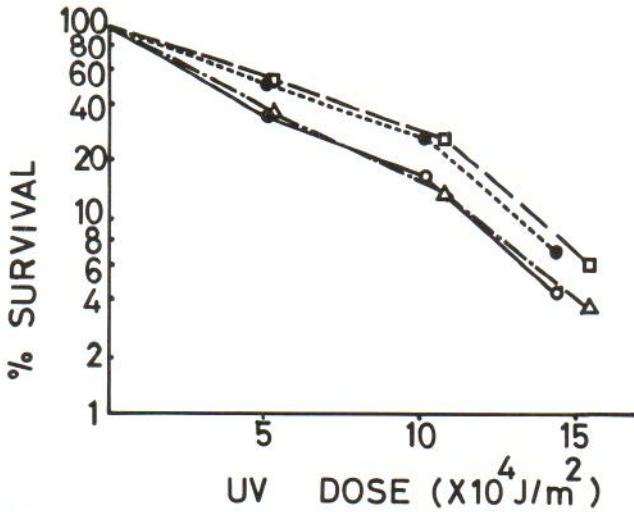
Fig. 5. Cytotoxicity of UVA toward several fibroblast cell lines. Cells were taken from cultures in exponential growth, plated at cloning densities (100–4000 cells per dish), and irradiated with UVA for 40, 80, or 120 min one day after being plated. ○, N22OS; △, XP33OS; □, XP3KA; ●, HV1OS.

tral irradiance of UVA–G and UVA–GD light were similar, irradiance was measured on the surface of the plate glass with the radiometer. It was from 2.2 to 2.6 mW/cm².

Colony formation after UVA treatment

Fig. 4 shows colonies that grew in 35-mm dishes with different periods of UVA exposure. In the UVA survival experiments, the colonies generally remained separate from each other and they could be easily counted. Survival curves of the four cell lines tested, which included those of xeroderma pigmentosum (XP) complementation groups A and C and HV patients and a healthy subject, were all similar (Fig. 5). Thus, these cell lines had similar sensitivity to UVA. The light that passed through the plate glass plus the dish did not contain light in the UVB range, because the two XP cell lines, which have differing sensitivity to 254-nm light, had similar survival rates.

Effect on cell survival of time between plating and irradiation

UVA radiation experiments sometimes require long irradiation periods, so we checked whether differences in time between plating and irradiation affected survival. The survival curves of the cell lines were close to each other (Fig. 6).

DISCUSSION

We developed a smaller experimental system for UVA irradiation than the usual one, which made stirring of cells during irradiation unnecessary. In the smaller dishes, colony formation was satisfactory and the survival curves were similar to those reported by Tyrrell & Pidoux (4).

Our system has several advantages. First, it is economical and requires no special equipment. Second, it is easy to keep the equipment and culture dishes clean. Smaller dishes require less incubation time and less space. Conventional irradiation systems require time-consuming separate exposures for differ-

ent treatments. In our system, up to 12 culture dishes can be irradiated at one time and different treatments can be done at the same time, so the total time needed is shorter. Our system also makes it possible to expose cells to chemicals short-term (for example, 1 min). Irradiation from the bottom eliminates any interference by the culture medium and allows cells to grow without being stirred and possibly injured.

The 5-mm thick plate glass almost completely eliminated wavelengths of the UVB range emitted from BLB lamps (Fig. 3). The elimination of UVB was further confirmed here by the similarity of survival curves among normal, HV, and groups A and C XP cell lines; both of the latter two kinds of cells are very sensitive to 254-nm UV. The findings indicated that pyrimidine dimers did not participate in causing cell death from UVA radiation (2). Our irradiation system could also be used for experiments that deal with photoreactivation (a DNA repair mechanism) of human cells after UVC or UVB radiation (9).
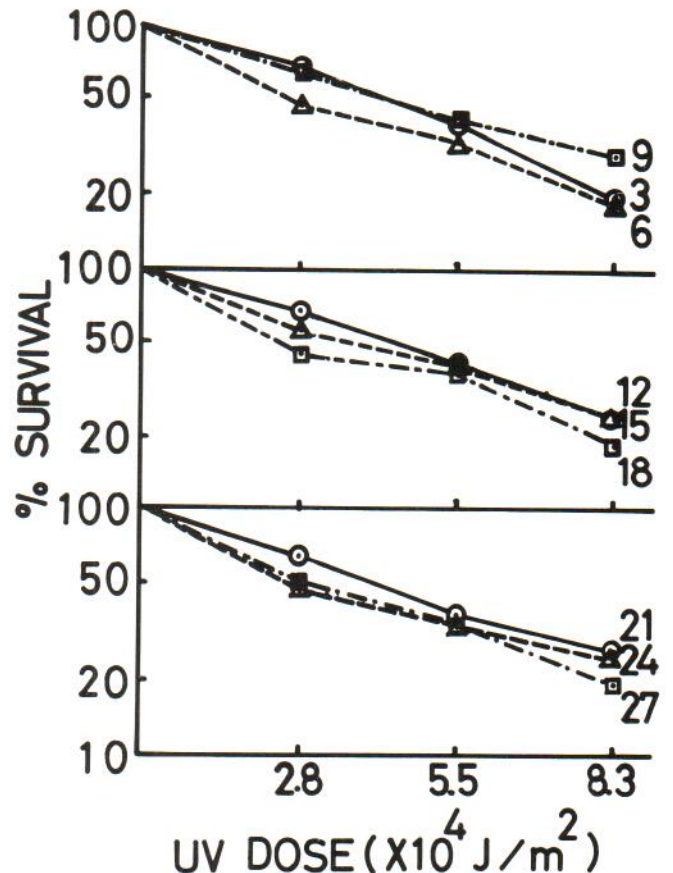
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Fig. 6. Cytotoxicity of UVA as a function of the time between plating and irradiation. HV1OS cells taken from cultures in exponential growth were plated into a series of 35-mm dishes at cloning densities. The cells were irradiated with UVA for 20, 40, or 60 min at the designated time after being plated. The numbers at the right end of each survival curve denote hours after seeding. N22OS cells (not shown) had survival curves similar to those of HV1OS cells.

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