Phototoxicity of Ultraviolet (UV) Radiation: Evaluation of UV-Blocking Efficiency of Intraocular Lens (IOL) Materials Using Retinal Cell Culture and *in vitro* Bioassays

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Abstract: This work involves the evaluation of UV blocking efficiency of commercially available intraocular lens (IOL) materials using a retinal cell culture and a biological *in vitro* model that was developed in a previous study, as an effort to examine the sensitivity of this *in vitro* approach for evaluating toxicity of UV radiation on the retinal pigment epithelial cells. The human retinal pigment epithelial (RPE) cell line, ARPE-19, was cultured, and cells were irradiated with broadband UVB radiations at energy levels of 0.2 and 0.4 J/cm². Some treated cells were not shielded from the radiation while others were shielded using two thicknesses (0.9 and 1.5 mm) of IOL material. After irradiation, cellular viability, mitochondrial distribution, nuclei morphology, and phagocytotic activity were analyzed using the Alamar blue assay, Rhodamine 123 staining, the Hoechst assay, and a phagocytotic activity assay. The results demonstrate that UVB radiation can cause significant decreases in RPE cell viability as well as in phagocytotic activity. Also, the results show that UVB radiation can induce the degradation of DNA and mitochondria in cultured RPE cells. However, the two different thickness IOL material sheets (0.9 and 1.5 mm) showed very effective UV blocking ability, allowing no cellular damage at all. Thus, the finding suggest that these four assays together can be used as a sensitive, and meaningful *in vitro* biomarker method for evaluating toxicity of UV radiation on RPE cells, and also for examining IOL effectiveness.

Keywords: Intraocular lens, Retinal pigment epithelium, UV radiation, Alamar blue assay, Confocal microscopy, Hoechst assay, Phagocytotic activity assay.

INTRODUCTION

The crystalline lens of the eye is the principal shield against ultraviolet (UV) radiation (between 300 nm and 400 nm), damage to the human retina [1]. Cataract removal is one of the most common operations performed in the United States [2], and generally an intraocular lens (IOL) is replaced in the lens capsule. With the possibility of extending IOL use to phakic eyes for the correction of high myopia, hyperopia, and other nonopacifying conditions, the use of IOLs may increase further still [2]. The first polymethylmethacrylate IOLs transmitted UV in addition to visible light [3]. Ultraviolet radiation is not required for vision but it can harm the retina at acute and intense exposure [4, 5]. Most IOLs incorporated UV blocking chromophores by 1986 [6]. However, spectrophotometric data show that most of the commercially available, UV-absorbing IOLs currently in use have different properties than the natural crystalline lens in absorbing UV radiation [7-9].

While there are many studies that evaluate the spectral transmission characteristics of various IOLs to verify their anti-UV efficacy [6, 7, 10], studies showing the cytotoxic effects of UV radiation on retinal cells in terms of cell biology and physiology are few in number. UV radiation can generate free radicals including oxygen-derived species [11], which are known to cause lipid peroxydation of cellular membranes [12, 13]. Also, it has been shown that UV can damage DNA, leading to DNA fragmentation [11, 14, 15], and decrease mitochondrial function [6, 16, 17].

The objective of the present study is to evaluate the UV-blocking efficacy of commercially available IOL materials using a retinal cell culture and a biological *in vitro* model that was developed in a previous study [18], as an effort to examine the sensitivity of this *in vitro* approach for evaluating toxicity of UV radiation on the RPE cells. This work involves the exposure of a RPE cell culture to broadband UVB radiation with and without the protection of IOL material flats. Cellular viability, mitochondrial damage, DNA damage, and phagocytotic activity are quantified after exposure.

MATERIALS AND METHODS

Human RPE Cell Culture

The human RPE cell line ARPE-19 was obtained from the American Type Culture Collection (ATCC). The cells were cultured in DMEM/Ham's F-12 with L-glutamine and 15mM HEPES (Mediatech, VA, USA). The medium also contained 10% fetal bovine serum (Hycolone, UT, USA), and insulin-transferrin-sodium selenite media supplement (ITS supplement) (Sigma, MO, USA) [18]. The cells were plated in T75 or T150 flasks (Falcon, NJ, USA), and allowed to grow until the cultures were confluent. The cells were grown at 37°C in a humidified incubator in an atmosphere of 95% air and 5% CO_2. Confluent cell flasks were passaged every two days using the dissociating agent Trypsin/EDTA solution (Cascade Biologics, OR, USA). The culture and

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conditioned medium were split into three flasks, and an equal volume of fresh medium was added to each flask. Subculturing was usually performed during the log (or exponential) phase when the cells are at their healthiest and are able to adapt to the new environment most efficiently [19].

UV Irradiation of Cultured RPE Cell

The IOL flats were supplied by Bausch & Lomb, Rochester New York. They consisted of yellow tinted standard silicone IOL materials (hydrosiliation cured silicone) that were manufactured by cast molding. The flats, measuring 9.0 by 7.0 cm, were large enough to cover 12 cells of a standard 24 cell multi-well plate that contained the cultured cells. Opaque black tape was applied to the periphery of the plate to ensure that only the radiation passing through the silicon flats could reach the cells. Exposure was produced by UVB fluorescence tubes (cat. No F15T8/UVB; Microlites Scientific, ON, Canada) in a custom designed UV irradiation unit with 4% CO_2 and 96% membrane-filtered air. The spectral distribution of the UVB fluorescent tubes extends from 290 nm to about 370 nm wavelengths, with a peak at around 315 nm. Since the contribution of the wavelengths within the 320 to 370 nm waveband was only 0.1%, this was ignored. Before irradiation, the irradiance of UVB source was calculated with an Instaspec II diode-array spectroradiometer (Oriel Corporation, CT, USA).

After two days of pre-incubation at 37°C to form a confluent cell monolayer, the cells were exposed with 0.2 and 0.4 J/cm² of broadband UVB. The cells were incubated for another 48 hours and assays were carried out at 24 and 48 hours after irradiation. In order to minimize absorption of the radiation by phenol red in the medium, a thin layer of medium (about 1.0 mm) was left above the cells during UV exposure. Thus, a minor phenol red effect may have influenced the results.

Exposure times for two energy levels (0.2 and 0.4 J/cm²) were calculated with the formula: $H_\lambda = t \times E_\lambda$, where H_λ is the energy level (J/cm²), t is the exposure time (second), and E_λ is the irradiance (W/cm²) [20]. The irradiance measured with a spectroradiometer was 0.000306 W/cm^2, and the exposure times were 10 min 54 sec for 0.2 J/cm² and 21 min 48 sec for 0.4 J/cm², respectively. The solar broadband UVB irradiance level (measured in June 1999 @ Waterloo, ON, Canada,) was 0.000276 W/cm^2 [21]. Thus, the irradiance produced by the UVB tubes for this study is comparable to solar spectral irradiance.

Alamar Blue Assay

The fluorescent indicator dye, Alamar Blue (MEDICORP Inc., Montreal, PQ, Canada) used to evaluate cell viability, is a commercial preparation of the dye resaruzin [22]. For experimental use, it was diluted into the culture medium (mentioned above) without serum to 8% (v/v). After two to three days of pre-incubation, the cells were transferred into a sterile flat bottom multi-well cell culture plate (BD Falcon, Franklin Lakes, NJ, USA) for fluorescence measurements. Cell density was adjusted to 1×10^5 cells/ml for 48-well plates and 5×10^4 cells in 500$\mu\ell$ of medium were added to each well [23]. After the irradiation, the culture medium was aspirated from each well, and the cells were rinsed once with culture medium with no serum. Then 100 to 150$\mu\ell$ of assay solution was added to each well containing cells, and the cells were further incubated for one hour to allow the dye to be taken up by the cells. At the end of one hour incubation, the fluorescence measurements were taken with a CytoFluor™ II fluorescence multi-well plate reader (PerSeptive Biosystems Inc., MA, USA). Before the measurements, the excitation / emission wavelengths settings were adjusted to 530 /590 nm, with the sensitivity gain set at 50.

Confocal Microscopy

Confocal scanning laser microscopy (Carl Zeiss LSM) and two fluorescent dyes (Hoechst 33342 and Rhodamine 123) (Molecular Probes, OR, USA) were used to visualize the changes of RPE cell morphologic features (nucleus and mitochondria of the cells, respectively) after UVB radiation. Hoechst 33342 is a popular cell-permeant nuclear stain that emits blue fluorescence when bound to dsDNA [24]. Rhodamine 123 is a cationic, lipophilic, water-soluble oxonium chloride salt that stains mitochondria in living cells in a membrane potential-dependent fashion [25]. Before irradiation, 1×10^5 cells in 1.5ml of culture medium were transfered into collagen coated glass bottom culture Petri dishes (MatTek Corp., MA, USA). After the UVB exposure, the cells were then stained for mitochondria and nucleic acid using Rhodamine 123 (20mM) and Hoechst 33342 (10mg/ml) for 20 min at 37ºC. A Zeiss confocal laser scanning microscope (CLSM) 510 system attached to an Axiovert 100 microscope with a 40× water-immersion C-Apochromat objective was used to visualize the effects of the two different dyes.

Hoechst 33342 Stain

Before the indicated exposure time, cells were plated into 2-well culture slides (BD Falcon, NJ, USA). After the irradiation, cultures were then fixed twice by adding an equal volume (3~4 drops) of Carnoy's fixative (methanol/glacial acetic acid, 3:1). The cells were rinsed with 1× PBS (phosphate buffered saline), and allowed to air dry. Following the initial fixation, the cell slides were stained with 0.5 μg/ml Hoechst 33342 (Riedel-de Haen Ag Seetz-Hannover) (Molecular Probes, OR, USA) for 10 min. The fluorescent nuclei were visualized using a fluorescence microscope with an ultraviolet (UV) filter (Nikon Optishot microscope, ON, Canada).

Phagocytotic Activity Assay

Phagocytosis is a critical element in the ability of RPE cells to maintain a healthy retina [26]. Confocal scanning laser microscopy (Carl Zeiss Inc., Jena, Germany) and 0.1μm diameter fluorescent carboxylate-modified microbeads (Molecular Probes, OR, USA) were used to observe the effects of UVB radiation on RPE cell phagocytotic activity. RPE cells were transfered to collagen-coated glass bottom culture Petri dishes (MatTek Corp., MA, USA) with a density of 1.3×10^5 cells in 2ml of culture medium. After incubating overnight at 37°C, 7×10^{10} microbeads (red fluorescent, excitation 580 nm/emission 605 nm) in culture medium were added at a total volume of 2ml/Petri dish.

After one day of incubation to allow the beads to be ingested by cells, the microbeads were removed and the adherent cells were washed carefully with fresh culture medium twice to remove undigested particles before confocal observation. The micobeads in the cells were visualized using the confocal microscope, and the intensity of the fluorescent was measured using the Image Analysis toolbox of Matlab 7.1 software package (MathWork Inc., MA, USA).

Statistical Analysis

Statistical calculations were completed using a two-way repeated measures analysis of variance (ANOVA). A probability value of less then or equal to 0.05 was considered significant.

RESULTS

Cell Viability

The Alamar Blue assay readings are presented as percent cell viability relative to the non-UVB exposed cells at three time points: 0-hour (before UV exposure), 24 hours (1-day after UV exposure) and 48 hours (2-day after irradiation). These time points were chosen to permit a time-related evaluation. The results for broadband UVB exposed RPE cells (without IOL protection) showed energy level and time – dependent decreases in cell viability in comparison with the control cells (Fig. **1a** and **1b**). Although the measurements for 0.2 and 0.4 J/cm²-exposed cells at the 24-hour time points presented similar loss of cell viability (64.7±1.0% and 61.2±2.0%, respectively), the Alamar blue assay results for both 0.2 and 0.4 J/cm² exposed cells at 48-hour time points clearly showed energy level-dependent decreases in cell viability (55.5±2.8% and 34.6±7.0%, respectively). The IOL covered cells showed similar viability as control cell groups during the entire culture period, showing very effective UV blocking ability for both IOL material thicknesses of 0.9 and 1.5 mm (Fig. **1a** and **1b**).

Mitochondrial Distribution

Non-irradiated cultured RPE cells (control cells) exhibited no morphological change in mitochondria (first column of Fig. (**2**), green fluorescence). Overall, increasing the energy level of UVB radiation from 0.2 to 0.4 J/cm² resulted in sparser mitochondria, indicating degradation of mitochondria (second column of Fig. (**2**), green fluorescence). In the control cells, numerous mitochondria (green fluorescence) could be seen surrounding individual nuclei (blue fluorescence), and mitochondrial lengths were longer (fiber-looking mitochondria in the first column of Fig. (**2**)). However, both 0.2 and 0.4 J/cm² UVB-exposed cells showed shorter mitochondria, fewer fiber-looking mitochondria and mostly fragmented and merged mitochondria (see second column of Fig. (**2**)). Also, the results for the 0.2 and 0.4 J/cm² exposed cells showed reduced cell populations in comparison with the control group. The results for the 0.9 mm and 1.5 mm thickness IOL covered cell groups also corresponded with the Alamar blue assay results, supporting the lack of significant morphological changes to the mitochondria (long fiber-looking mitochondria in the third and forth column of Fig. (**2**)). This inference is based on the common view that the Alamar blue assay is a measure of mitochondrial activity [27, 28].

Morphology of Nuclei

Non-irradiated cultured RPE cells (control cells) exhibited no morphological nuclear changes (first column of Fig. (**3**)). Increasing the energy level of UVB radiation from 0.2 to 0.4 J/cm² to the cells, without the protection of IOL material, reduced the number of cells attached to the slide due to cell death (second column of Fig. (**3**)). At a level of 0.2 J/cm² UVB, irradiated cell nuclei seemed expanded in size, and did not show DNA fragmentation (second column, first row of Fig. (**3**)). Also, 0.2 J/cm² UVB irradiated cells exhibited bright rings of DNA around the periphery of the nucleus (second column, first row of Fig. (**3**)). However, 0.4 J/cm² UVB irradiated cell nuclei seemed condensed and shrunken in size. They did not show DNA fragmentation (second column, second row of Fig. (**3**)). The results of 0.9 mm and 1.5 mm thickness IOL material covered cell groups also corresponded to the above Alamar blue assay and confocal microscopy results, showing no significant morphological nuclear changes and showing similar morphology and number of cells as control cell groups in both the 0.2 and 0.4 J/cm² UVB irradiation groups (third and fourth column of Fig. (**3**)).

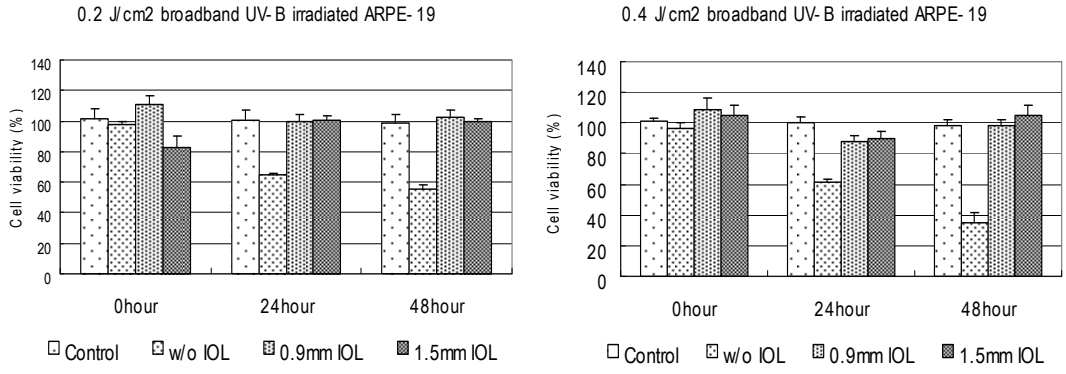

Fig. (1). Cell viability (%) is shown as a function of time (0, 24, and 48 hours) for RPE cells irradiated with broadband UV-B (Fig. (**1a**): 0.2, and Fig. (**1b**): 0.4 J/cm^2), as revealed by the Alamar blue assay. Cell viability was expressed as a percent of non-UVB exposed cells (% of control). * indicates significant differences as compared to controls ($p < 0.05$).

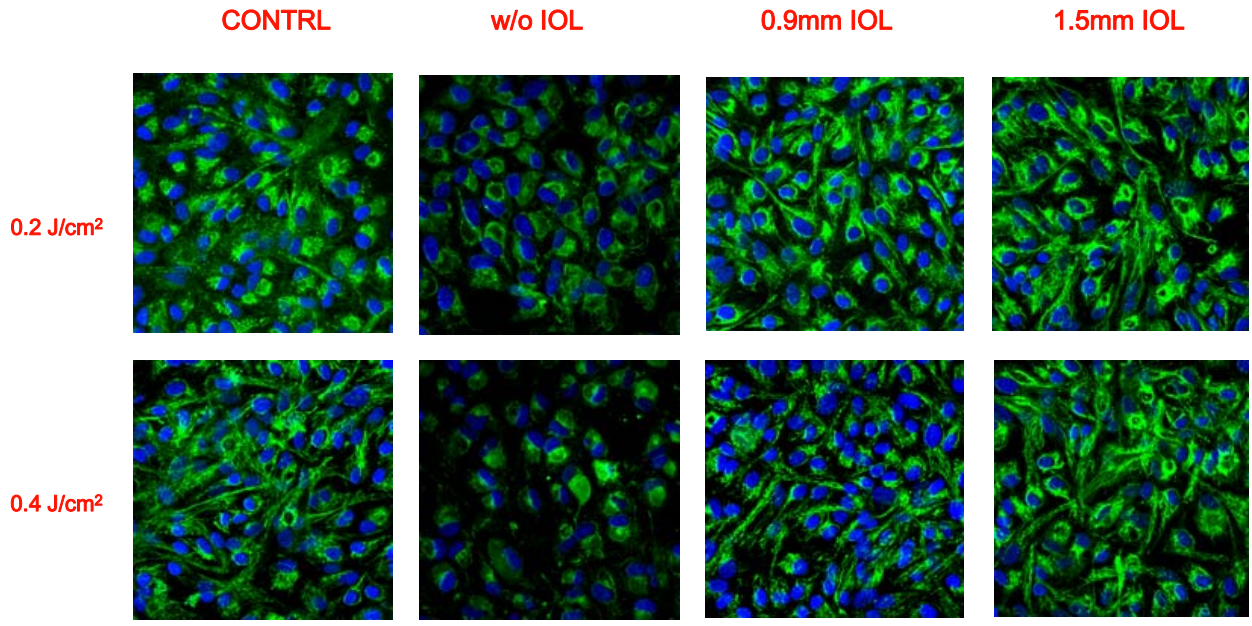

Fig. (2). Representative confocal laser scanning micrographs showing the effect of UV-B (0.2 and 0.4 J/cm^2) at 24 hours on distribution of the Rhodamine 123 stained mitochondria (green) and Hoechst 33342 stained nuclei (blue) in RPE cells. Overall, increasing the energy level of UVB radiation from 0.2 to 0.4 J/cm² resulted in sparser mitochondria, indicating degradation of mitochondria.

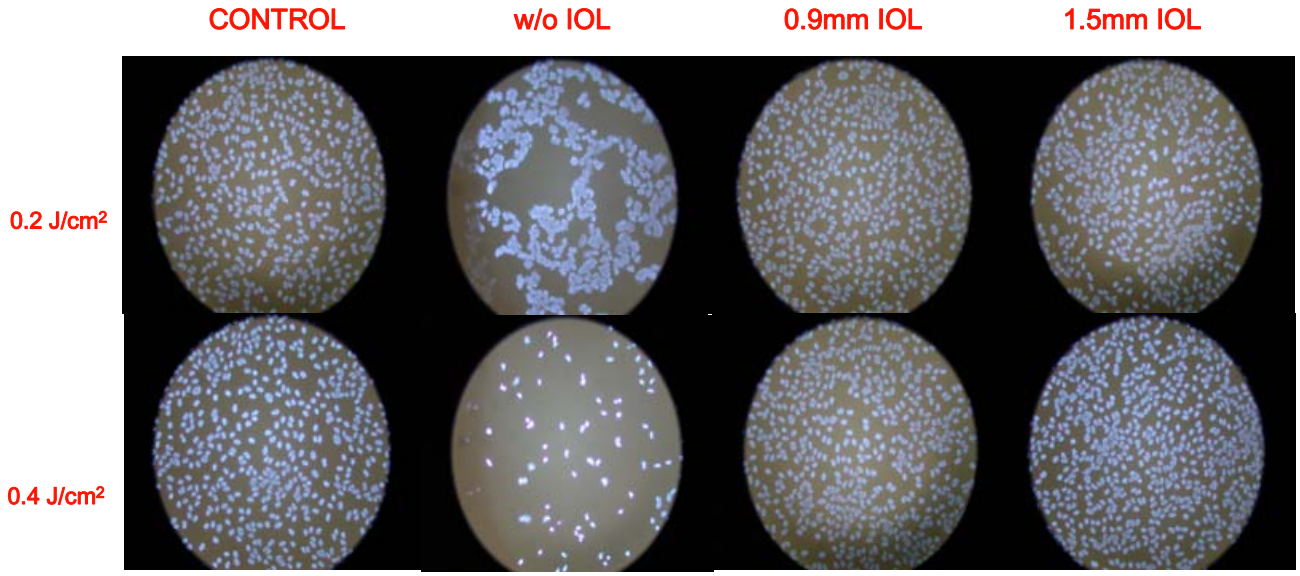

Fig. (3). Effect of 0.2 and 0.4 J/cm^2 broadband UVB exposure at 24 hours on the nuclear morphology of RPE cells (eight groups) as revealed by Hoechst 33342 staining and fluorescence microscopy). At a level of 0.2 J/cm² UVB, irradiated cell nuclei seemed expanded in size, and 0.4 J/cm² UVB irradiated cell nuclei seemed condensed and shrunken in size.

Phagocytotic Activity

Phagocytotic activity is presented as mean intensity of fluorescence of the ingested microspheres in the cells. Nine different spots of each cell plate were randomly selected, and the intensity of each spot was calculated. The final intensity value of each cell group was the average value of the 9 different spots. The ingestion of red fluorescent microbeads (580/605 nm) into RPE cells is shown in the confocal microscopic images (original magnification ×40 oil, ×2 zoom, Z stack) (Fig. **4**). The images were taken using the Z stack function of the confocal microscope and the middle Z slide image was chosen for analysis. The overall phagocytotic activity assay results are shown in Fig. (**5**). The control cells have significantly greater capacities for uptake than the 0.2 and 0.4 J/cm² UVB exposed cell groups ($p<0.05$), while the IOL material covered cell groups (both 0.9 mm and 1.5 mm thickness) showed no significant differences from the control cell groups (Fig. **5**). This phagocytotic assay result corresponds with the above 3 assay results, indicating that both IOL materials (0.9 mm and 1.5 mm) block UVB radiation very effectively.

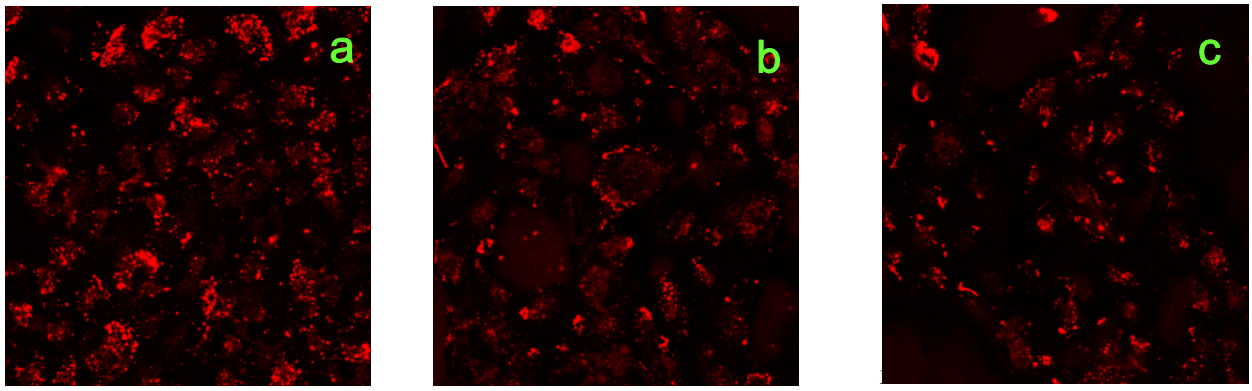

Fig. (4). Representative confocal laser scanning micrographs showing the effect of UV-B (0.2 and 0.4 J/cm^2) at 48 hours on the distribution of red fluorescent microspheres in RPE cells. Numbers on the right are the average intensity of nine different spots of each cell group.

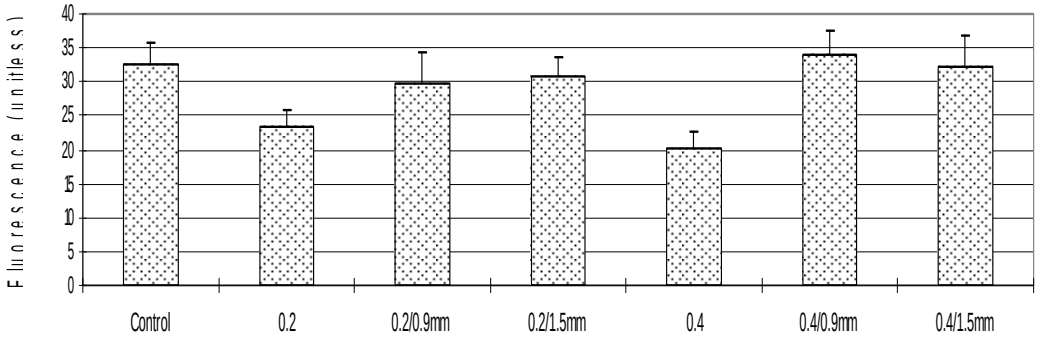

Fig. (5). Effect of 0.2 and 0.4 J/cm^2 broadband UV-B exposure at 48 hours on the phagocytotic activity of RPE cells as revealed by fluorescent microbeads (0.1 μm) and flow cytometry. Phagocytotic activity was expressed as the average intensity of nine different RPE images. * indicates significant differences as compared to controls ($p < 0.05$). In this graph, only seven cell groups are shown since two untreated control groups are combined.

DISCUSSION AND CONCLUSION

The results of this study demonstrate that broadband UVB radiation damage of RPE cells in culture can be evaluated using four assays; the Alamar blue assay, Rhodamine 123 staining, the Hoestch assay, and a phagocytotic activity assay. Also, the UV blocking efficiency of commercially available IOL materials can be tested using this biological assay model. The results clearly demonstrate that UVB radiation can cause significant decreases in RPE cell viability as well as phagocytotic activity of retinal outer segments by RPE cells. In addition, the results showed that UVB radiation can also induce the degradation of DNA and mitochondria in cultured RPE cells. However, two different thicknesses of IOL material sheets (0.9 mm and 1.5 mm) showed very effective UV blocking ability, allowing no cellular damage at all.

There have been many studies that have focused only on the spectral transmittance characteristics of various IOLs to verify their anti-UV efficacy. For example, Mainster measured the spectral transmittance (between 300 nm and 500 nm) of 16 implantable intraocular lenses from 12 manufacturers and described the importance of using ultraviolet-absorptive intraocular lenses to protect pseudophakic patients from photic retinopathy [6]. Also, Lin *et al.* analyzed the spectral transmittance (from 200 nm to 800 nm) of various lenses (spectacle lenses, contact lenses, and intraocular lenses) to clarify their ultraviolet-blocking efficacy [7]. In addition, there are clinical reports involving postoperative measurements of patients who have received intraocular implants. These include measurements of visual acuity, the occurrence of post operative sight-threatening adverse reactions, and the evaluation of long-term safety and efficacy of implanted ultraviolet absorbing intraocular lenses [29].

It is well known that ultraviolet radiation can produce photochemical lesions in the neural retina and RPE cells, including morphometric cellular changes and DNA damage [15, 30-32]. However, there are fewer studies showing the cytotoxic effects of UV radiation on retina cells in terms of cell biology and physiology. The cellular and molecular mechanisms of ultraviolet-induced retinal cell damage are important to understand how ultraviolet radiation may affect vision.

Alamar blue is a fluometric indicator of cellular metabolic activity, and it is an ideal test for *in vitro* cell proliferation and cytotoxicity [22]. In the present study, cells analyzed by the Alamar blue assay after UV iradiation showed dose and time dependent decreases in cellular

viability. A decrease in the Alamar blue fluorescence readings is a direct measure of the decline in cellular metabolic activity due to loss of appropriate cytoplasmic milieu from the photo-oxidative effect of the UV radiation [36]. Hightower and McCready explained that free radicals are often generated by UV photo-oxidation [37]. The phenomenon of photo-oxidation might have caused the impairment of metabolic activity in the cells. The Alamar blue assay, which is non-radioactive, has been used in *in vitro* photocytotoxicity studies of human RPE, cultured ocular lenses (bovine and porcine), mouse neural crest cells, and human keratinocytes [18, 36, 38-41]. Findings from these studies support the use of the Alamar blue as a sensitive assay for monitoring UV-induced changes in intracellular mitochondrial, cytoplasmic and nuclear integrity, all of which are measures of cellular viability [36].

Rhodamine 123 taken up specifically by mitochondria of living cells can be used as a supravital mitochondrial probe, discriminating between cycling and quiescent cells and having application in sorting functionally distinct cell subpopulations [42]. In the present study, cells analyzed after staining with Rhodamine 123 after UV irradiation showed that by increasing the energy level of UVB radiation, a progressive increase in morphological change, was observed, along with an increased degradation of mitochondria (mostly fragmented and merged mitochondria). Positively charged Rhodamine 123 seems to bind to the high electronegativity of the intact mitochondrial membranes [42]. Therefore, the intensity of mitochondrial staining may correlate with the activity of the oxidoreductive complexes responsible for the maintenance of the electronegativity of the mitochondrial membrane. Thus, Rhodamine 123 may be a direct marker of energy-supplying metabolic processes [43]. Mitochondria represent a primary site of phototoxicity induced by UV radiation in a variety of animal models [33-35]. Also, measurements of mitochondrial deficiencies in living cells by Rhodamine 123 fluorescent micrographs have been used in many *in vitro* photocytotoxicity studies of human corneal epithelial cells, carcinoma cells, RPE cells, and keratinocytes [16-18, 44-46]. Findings from these studies support the use of Rhodamine 123 as a sensitive mitochondrial probe for monitoring UV-induced mitochondrial changes in living cells.

Hoechst 33342 nucleic acid stain is a popular cell-permeant nuclear counter stain that emits blue fluorescence when bound to dsDNA [47]. This dye is often used to distinguish condensed pycnotic nuclei in apoptotic cells. In the present study, cells analyzed with Hoechst 33342 after UV irradiation showed progressive nuclear morphological change with size expansion for 0.2 J/cm² UVB irradiated cells, and condensation in size for 0.4 J/cm² UVB irradiated cells. Also, 0.2 J/cm² UVB irradiated cells exhibited bright rings of DNA around the periphery of expanded nuclei, which is regarded as the early stage of apoptosis [48]. Apoptosis in animal cells is characterized by chromatin condensation and DNA fragmentation [49], but in the present study there was no DNA fragmentation (data not shown) while there was chromatin condensation in the 0.4 J/cm² UVB treated cell group. Thus, more detailed studies are necessary to establish whether cell damage induced by UV radiation is apoptotic or non-apoptotic. The measurement of condensed pycnotic nuclei from Hoechst 33342 fluorescent micrographs has been used in *in vitro* photocytotoxicity studies of rat hepatocytes, Chinese hamster ovary cells, Xenopus XTC-2 cells, and B-cell hybridoma [50-53]. Findings from these studies support the use of Hoechst 33342 as a sensitive nuclui morphological marker for monitoring UV-induced cell damage.

The RPE performs several crucial functions important for maintaining the outer retina, including phagocytosis of the distal tips of outer segments, recycling of visual pigment, and transferring nutrients from the choriocapillaris to the neural retina [26, 54, 55]. *In vivo*, approximately 3%-5% of the distal tips of photoreceptor outer segments are shed daily, and proper RPE phagocytosis is necessary to maintain the health and integrity of the neural retina and choriocapillaris [26, 54, 56]. A decrease in phagocytic ability may be associated with an increase in RPE melanogenesis, and clinically, RPE hyperpigmentation is a risk factor for the development of ARMD [57, 58]. In the present study, fluorescent microspheres were used to observe the effects of UVB radiation on RPE cell phagocytotic activity. Increasing the energy level of UVB radiation induced decreased phagocytotic activity of RPE cells. Although it is not clear which molecular change is responsible for a decline in RPE phagocytosis ability, it is known that UV-induced http://www.ncbi.nlm.nih.gov/pubmed/9724002?ordinalpos=5&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSumlipofuscin accumulation in RPE cells reduces their phagocytic capacity [57]. Measurements of ingestion by RPE cells using fluorescent microspheres have been used in many *in vitro* photocytotoxicity studies of rabbit, bovine, and human RPE cells [18, 58, 59]. Findings from these studies as well as the current one indicate that the use of fluorescent microspheres may be a sensitive tool for monitoring UV-induced changes in phagocytitic activity of RPE cells.

In conclusion, the results of this study have demonstrated that the Alamar blue assay, confocal microscopy with Rhodamine123 staining, Hoechst assay, and phagocytotic activity assay showed that two thickness of an IOL material provide effective RPE cell protection from UVB radiation. These four assays together can be used as a sensitive, and meaningful *in vitro* biomarker method not only for evaluating IOL effectiveness, but also for evaluating RPE function after UV irradiation.

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ABBREVIATIONS

ACGIH = American Conference of Governmental Industrial Hygienist

ANOVA = Analysis of variance

ARMD = Age-related macula degeneration

ATCC = American Type Culture Collection

CLSM = Confocal laser scanning microscope

DMEM = Dulbecco's Modified Eagle's Medium

HEPES = N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

IOL = Intraocular lens

ITS = Insulin-transfferin-sodium selenite

PBS = Phosphate buffered saline

RPE = Retinal pigment epithelium

v/v = Volume per volume

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