in vivo *36*: 2116-2125 (2022)
doi:10.21873/invivo.12937

Comparison of UVC Sensitivity and Dectin-2 Expression Between Malignant and Non-malignant Cells

AYAKA SHINDO[1†], MIDORI KUSANO[1†], HIROSHI SAKAGAMI[2], SHIGERU AMANO[2], MEGUMI INOMATA[3], MASAYO ABE[3], MASASHI OKAZAWA[1] and TAKAFUMI OOKA[1]

[1]*Division of Feeding and Swallowing Rehabilitation, Department of Restorative and Biomaterials Sciences, Meikai University School of Dentistry, Saitama, Japan;*
[2]*Meikai University Research Institute of Odontology (M-RIO), Saitama, Japan;*
[3]*Division of Microbiology and Immunology, Department of Oral Biology and Tissue Engineering, Meikai University School of Dentistry, Saitama, Japan*

Abstract. *Background/Aim: Rapid spread of COVID-19 resulted in the revision of the value of ultraviolet C (UVC) sterilization in working spaces. This study aimed at investigating the UVC sensitivity of eighteen malignant and nonmalignant cell lines, the protective activity of sodium ascorbate against UVC, and whether Dectin-2 is involved in UVC sensitivity. Materials and Methods: Various cell lines were exposed to UVC for 3 min, and cell viability was determined using the MTT assay. Anti-UV activity was determined as the ratio of 50% cytotoxic concentration (determined with unirradiated cells) to 50% effective concentration (that restored half of the UV-induced loss of viability). Dectin-2 expression was quantified using flow cytometry. Results: The use of culture medium rather than phosphate-buffered saline is recommended as irradiation solution, since several cells are easily detached during irradiation in phosphate-buffered saline. Oral squamous cell carcinoma cell lines showed the highest UV sensitivity, followed by neuroblastoma, glioblastoma, leukemia, melanoma, lung carcinoma cells, and normal oral and dermal fibroblasts. Human dermal fibroblasts were more resistant than melanoma cell lines; however, both expressed Dectin-2. Sodium ascorbate at micromolar concentrations eliminated the cytotoxicity of UVC in these cell lines. Conclusion: Normal cells are generally UVC-resistant compared to corresponding malignant cells, which have higher growth potential. Dectin-2 protein expression itself may not be determinant of UVC sensitivity.*

In December 2019, several cases of pneumonia with unknown etiology emerged in Wuhan, China. From the throat swab sample of a patient, a novel coronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) was identified, and the disease was named coronavirus disease 2019 (COVID-19) (1). Coronavirus infects the transbronchial and alveolar epithelial cells, inducing lung damages and other organ impairments (2). The COVID-19 disease causes psychological and physical complications (3).

Environmental sodium hypochlorite cleaning and wearing masks effectively prevent infection of healthcare workers of hospitals, clinics, and schools. Also, the risk of infection in contaminated rooms can be reduced by disinfection with ultraviolet C (UVC) (4, 5). Intensive Care Units at the University Hospital use six 15 W low-vapor-pressure mercury lamps emitting 253.7 nm UVC [the average dose rate in the UVC cabinet was (2.7±0.5) mW/cm^2] (6). This value is about 27,000-fold higher than the effective and safe irradiation intensity for skin application (UVB: 0.1 μW/cm^2) (7). UVC disinfection lamps, which have strong bactericidal and virucidal power, have been solely used to improve indoor air quality in hospitals. Therefore, with increased time spent in such areas, their harmful effect on humans (such as skin cancer and cataract) may be augmented. If sterilization lamps are introduced at home, it would be necessary for us to protect ourselves from the injuring effects of UVC. It has been reported that Dectin-2 is involved in UV radiation-induced tolerance (8, 9). However, there is no report on Dectin-2 expression in human dermal fibroblast and melanoma cell lines.

†These Authors contributed equally to this study.

Correspondence to: Hiroshi Sakagami, Meikai University Research Institute of Odontology (M-RIO), 1-1 Keyakidai, Sakado, Saitama 350-0283, Japan. Tel: +81 492792758, e-mail: sakagami@dent.meikai.ac.jp

Key Words: UVC sensitivity, malignancy, dermal fibroblast, melanoma, Dectin-2, protection, sodium ascorbate.

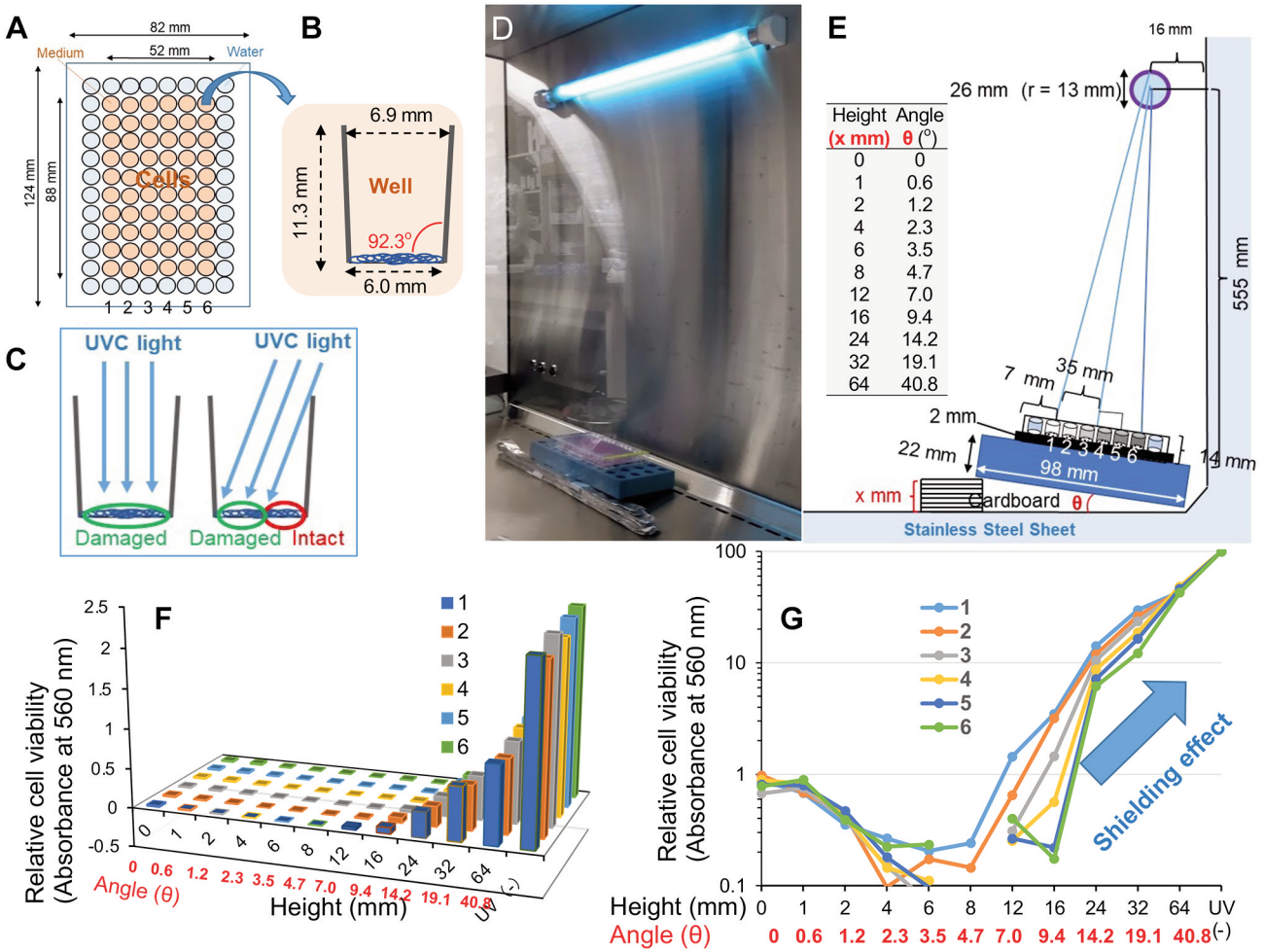

Figure 1. *UVC-shielding effect of the walls of a 96-microwell plate. The 96-microplate (A) and well (B) are shown. Diagonal UVC irradiation produced the non-irradiated area (indicated by red color) (C). HSC-2 cells (3×10^3/0.1 ml) were inoculated into the inner 60 wells of 12 microwell plates, and incubated for 48 h. The 11 plates were positioned at an angle of 0, 0.6, 1.2, 2.3, 3.5, 4.7, 7.0, 9.4, 14.2, 19.1 or 40.8° (D, E) and exposed to UVC irradiation for 8 min from a height of 555 mm. One control plate was not unirradiated. Cell viability (absorbance at 560 nm) was then determined using the MTT method (F). To visualize the cell viability more clearly especially at low angles, data were also plotted logarithmically (G). Each value represents the mean of 10 determinations.*

In the present study, UVC sensitivity of eighteen malignant and nonmalignant cells was investigated, after refinement of a previous method that used phosphate-buffered saline without calcium and magnesium [PBS(–)] as irradiation medium (10). In addition, Dectin-2 expression between UVC-insensitive human dermal fibroblasts and UVC-sensitive melanoma cell lines was compared. Furthermore, the UVC protective activity of sodium ascorbate was investigated in skin-derived cells.

Table I. *Shielding effect of various containers.*

Container	UVC strength (mW/m^2)	Shielding effect (%)
None	1.022	0
Beaker	0.02	98.0
Lid of 96-microwell plate	0	100.0
Plastic 30 cm dish	0.035	96.6

Materials and Methods

Materials. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Inc., (St. Louis, MO, USA). Phosphate-buffered saline without calcium and magnesium [PBS(–)] were obtained from Nissui Pharmaceutical Co. Ltd., (Tokyo, Japan). Dimethyl sulfoxide (DMSO) was from Wako Pure Chemical Ind., (Osaka, Japan).

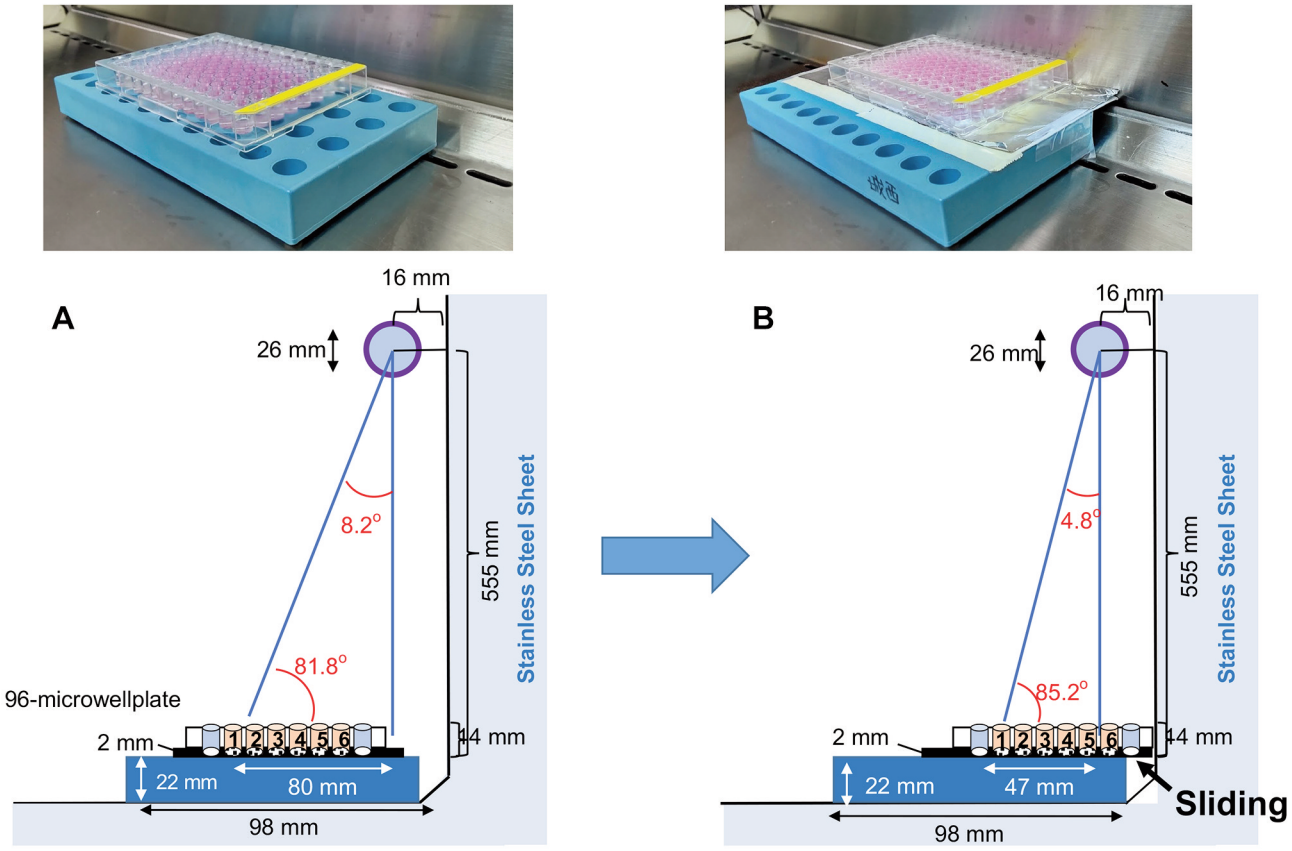

Figure 2. *Irradiation condition set up for the present study. The 96-microwell plate was placed horizontally (A) and then moved to the end of the safety cabinet (B) to allow the cells to receive the maximal intensity of UVC light.*

Sodium ascorbate was purchased from Tokyo Chemical Industry Co., Ltd., (Tokyo, Japan). 96-microwell plates were from TPP (Techno Plastic Products AG, Trasadingen, Switzerland).

Cell cultures. Human normal oral cells (HGF, HPLF, HPC) [established from the first premolar extracted tooth in the lower jaw (because of dysfunctional position or orthodontic treatment), and periodontal tissues of a twelve-year-old girl, according to the guideline of intramural Ethic Committee (No. A0808), and used in this study at 12~20 population doubling level (PDL)] (11), human dermal fibroblast adult (HDFa) used at 20~30 PDL, human oral squamous cell carcinoma (OSCC) cell lines (Ca9-22, HSC-2, HSC-3, HSC-4), human lung cancer cell lines (A549, WA-ht, LC-1/sq, A904L), human lung fibroblast (TIG-3, HFL-1) (used at 25-35 PDL), human glioblastoma (T98G) (12), neuronal cells [rat adrenal pheochromocytoma cell line (PC12), human neuroblastoma cell line (SH-SY5Y), rat Schwann cell line (LY-PPB6)] (Riken Cell Bank, Tsukuba, Japan) (13) were cultured at 37˚C in DMEM supplemented with 10% heat (56˚C, 30 min)-inactivated FBS, 100 U/ml penicillin G, and 100 μg/ml streptomycin sulfate in a humidified 5% CO_2 incubator (MCO-170 AICUVD-P, Panasonic Healthcare Co., Ltd., Gunma, Japan). Human myelogenous leukemia cell lines [promyelocytic leukemia HL-60 (14), myeloblastic leukemia ML-1 (15)] (Riken Cell Bank) were cultured in RPMI1640 medium supplemented with 10% heat-inactivated FBS and antibiotics as described above.

UVC protection assay. All attached cells except human normal oral cells (HGF, HPLF, HPC) were inoculated at 3×10^3 cells/0.1 ml in the inner 60 wells of a 96-microwell plate (indicated by orange color in Figure 1A). The surrounding 36 exterior wells were filled with sterile distilled water to minimize the evaporation of water from the culture medium. Since three human normal cells proliferate relatively slower, they were inoculated at 4×10^3/0.1 ml. Cells were then incubated for 48 h at 37˚C to allow complete cell attachment. Human myelogenous leukemia cells (HL-60, ML-1) in suspension were inoculated at 3×10^4/ml in RPMI1640/10%FBS. The 96-microwell plates that contained the cells were placed at 550 mm distance from the center of a UV lamp (germicidal lamp GL15, Toshiba Co. Ltd., Tokyo, Japan) set within a safety cabinet (MCV-B131F BioClean Bench, Panasonic) under the following two different conditions. First, since the plastic lid (Table I) and well walls (Figure 1B) of a 96-microplate block UVC light (Table I), the plate was tilted at various angles so that the cells can be uniformly hit by a UVC light (Figure 1D and E). Alternatively, a 96-microwell plate was moved to the corner of stainless steel wall, to be hit by UVC light more vertically (Figure 2B). Details are described in the section of Results. Irradiation power was

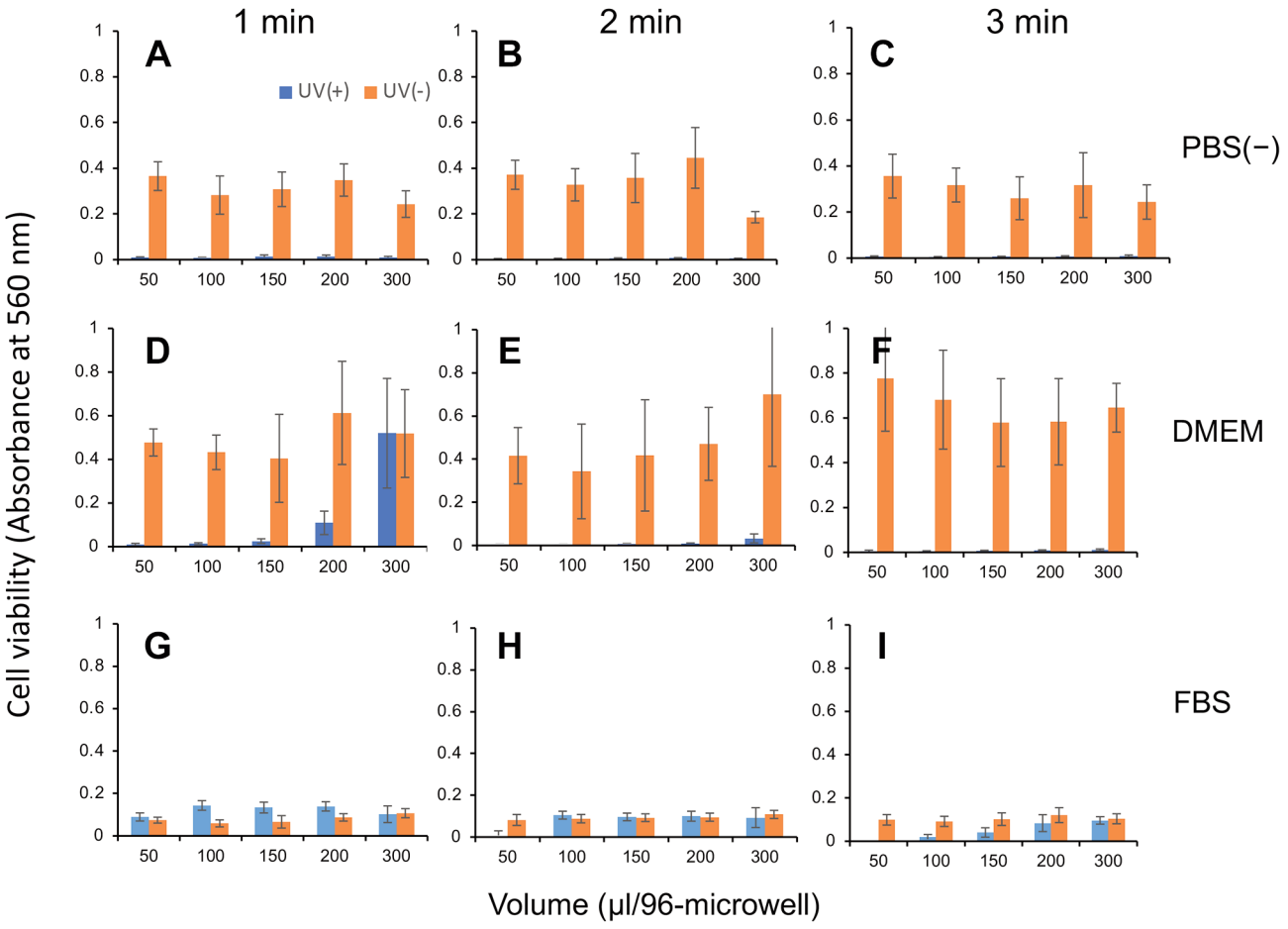

Figure 3. *Effect of type of irradiation solution and depth of solution on the sensitivity of UVC irradiation. Near-confluent HSC-2 cells received 50, 100, 150, 200 μl of PBS(–) (A~C), DMEM (D~F) or FBS (G~I), and then irradiated for 1 min (A, D, G), 2 min (B, E, H) or 3 min (C, F, I). After incubation for 48 h with 100 μl of fresh culture medium (DMEM+10%FBS), viable cell number was determined using the MTT method. Each value represents mean±S.D. (n=6).*

determined by a UVC radiometer (Gigahertz Optik GmbH, Tuerkenfeld, Germany). Cells were then irradiated (1.022 W/m^2) for various times, refed with fresh culture medium, and incubated for a further 48 h. Cell viability was then determined using the MTT method. From the dose–response curve, the 50% cytotoxic concentration (CC_{50}) and the concentration that restored half of the UV-induced decrease of viability (EC_{50}) were determined. The selectivity index (SI) was determined using the following equation: $SI=CC_{50}/EC_{50}$.

Flow cytometry. The cells (1×10^6 cells/well) were stained with mouse anti-human Dectin-2 monoclonal antibody (ab107572; Abcam, Cambridge, UK) at room temperature for 1 h. After washing with PBS(–), the cells were incubated with Alexa488-conjugated anti-mouse IgG antibody (Thermo Fisher Scientific, Waltham, MA, USA) at room temperature for 1 h. The samples were analysed using an SH800 cell sorter (SONY, Tokyo, Japan).

Statistical analysis. Each experimental value was expressed as the mean±standard deviation (SD) of 3 to 12 measurements. One-way ANOVA and Bonferroni's post-test were performed using IBM SPSS 27.0 statistics (IBM Co. Armonk, NY, USA). The significance level was set at $p<0.05$.

Results

UVC-shielding effect of culture plate. The power of UVC reaching the bottom of the safety cabinet (555 mm distance from the UVC lamp) was 1.022 W/m^2 (Table I). The UVC shielding effect of the lid of a 96-microwell plate was 100%, followed by beaker (as representative of glass) (98.0%) and a plastic 10-cm dish (96.6%) (Table I). This suggests that plastic walls of wells block the invasion of UVC light. When a 96-microwell is irradiated with UVC, cells in the shadow (not exposed to UVC) will survive, while cells in area exposed to UVC will be damaged (Figure 1C). It was therefore necessary to investigate the conditions under which UVC can uniformly hit all 60 wells.

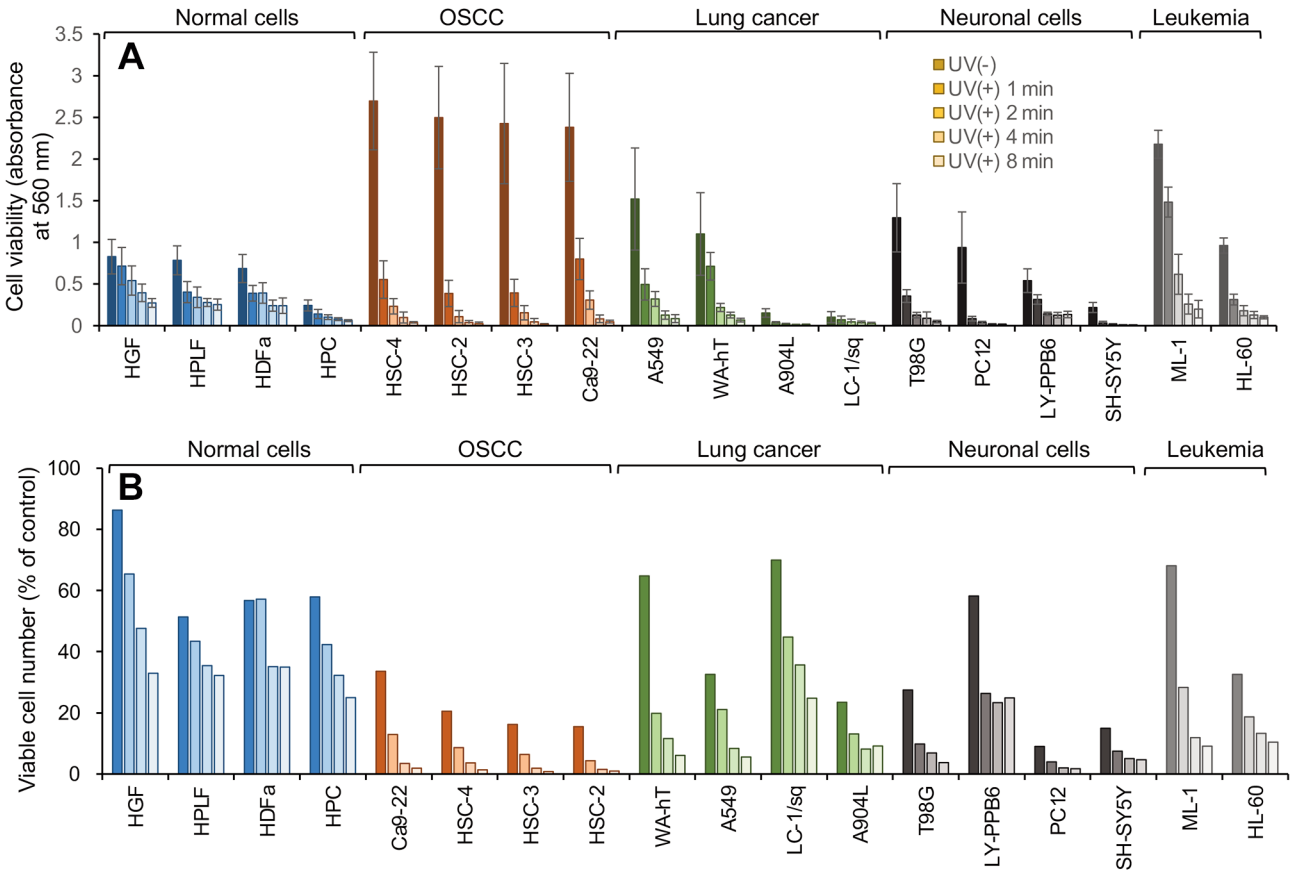

Figure 4. *Comparison of UVC sensitivity of 18 cultured cells. (A) Cells were UVC irradiated for 0 (control), 1, 2, 4, or 8 min, and then replaced with fresh culture medium to determine cell viability (absorbance at 560 mm). Each value represents mean±S.D. (n=12). (B) Data of (A) are expressed as viable cell numbers (% of control).*

Optimization of irradiation conditions. Human oral squamous carcinoma HSC-2 cells, which are highly sensitive and stable cell lines (10, 11) were used to determine the optimal conditions for UVC irradiation. Twelve 96 microwell plates that contained attached HSC-2 cells were prepared (Figure 1A). These plates were placed at various angles from 0 to 40.8˚ (Figure 1B) and irradiated for 8 minutes. Cell viability was measured 48 h thereafter using the MTT method. Each point is an average of 10 determinations. When the angle exceeds 9.4˚, a UVC shielding effect was detected (Figure 1F). When logarithmically plotted, cell viability was found to be reduced to 1%, even if the plate was placed horizontally (at an angle of 0˚). When the angle was only slightly increased (up to 3.5˚), 99.8% of cells died. On the other hand, with a further increase in the angle up to 40.8˚, cell viability exceeded 40% (Figure 1G). This result demonstrated that the plastic walls of the 96-microwell plate showed a UVC shielding effect similar to the lid (Table I).

Based on this observation, we decided to set the plate horizontally (Figure 2A), and then moved it to the corner of the stainless steel wall to allow more vertical entry of UVC light by increasing the irradiation angle from 81.8˚ to 85.2˚ (Figure 2B). This irradiation conditions were adopted in subsequent experiments.

We next investigated which is the best medium for the UVC irradiation (Figure 3). When cells were stood for 1~3 min in PBS(–), DMEM or FBS without irradiation, and further incubated for 48 in fresh regular culture medium (DMEM +10% FBS), the highest cell viability (assessed by absorbance at 560 nm) was achieved with DMEM (indicated by orange color, Figure 3D-F). When DMEM was replaced by PBS(–), the viable cell number was reduced to nearly one half (Figure 3A-B), possibly due to the detachment or adverse effect on the growth. The short exposure to FBS resulted in considerable reduction of cell viability (Figure 3G-I). When cells were incubated in PBS(–), UVC irradiation for 1 min was sufficient to kill the HSC-2 cells, regardless of the volume of PBS (50~300 μl) (indicated by blue color, Figure 3A-C). When HSC-2 cells were irradiated in DMEM, an irradiation time of 3 min was needed for

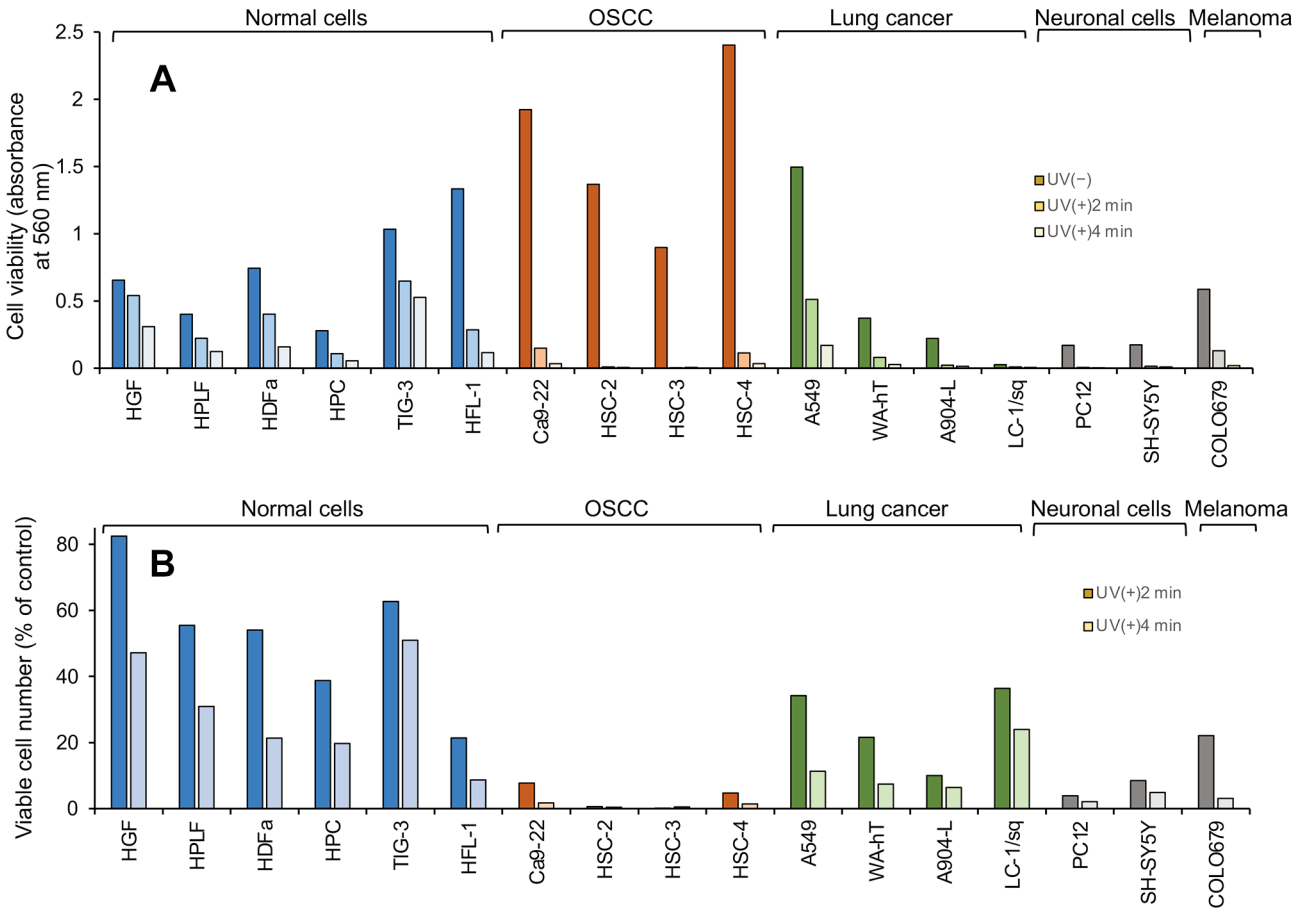

Figure 5. *Reproducibility of the results presented in Figure 4. (A) A total of 17 cell lines (T98G, LY-PPB6, ML-1 and HL-60 cell lines presented in Figure 4 were replaced by TIG-3, HFL-1, and COLO679 cell lines) were UV irradiated for 0 (control), 2, or 4 min, and then replaced with fresh culture medium to determine cell viability (absorbance at 560 mm). Each value represents mean±S.D. (n=12). (B) Data of (A) are expressed as viable cell numbers (% of control).*

killing all cells (Figure 3D-F). When the cells were incubated in FBS during UVC irradiation, the cytotoxic effect of UVC irradiation was slightly weakened (Figure 3G-I). Furthermore, LC-1/sq and PC12 cells were easily detached when washing with PBS(–). Based on these data, UVC irradiation was performed in DMEM supplemented with 10% heat-inactivated FBS in the present study.

Comparison of UVC sensitivity of malignant and non-malignant cells. When 18 types of cells were irradiated for 0, 1, 2, 4, or 8 min with UVC, the cell viability (assessed by absorbance at 560 nm) was reduced time-dependently, reaching near plateau levels between 2 to 4 min (Figure 4A). When the cell viability was expressed as % of unirradiated cells, human oral squamous cell carcinoma (OSCC) cell lines (Ca9-22, HSC-2, HSC-3, HSC-4) were found to be the most sensitive to UVC irradiation, followed by neuronal cells (PC12, SH-SY5Y), glioblastoma (T98G), lung cancer cells (A549, WA-hT, A904L, LC-1/sq), Schwan cells (LY-PPB6), leukemia cells (HL-60, ML-1), and then normal oral cells (HGF, HPLF, HPC) and dermal (HDFa) cells (Figure 4B). Repeated experiments showed reproducible results (Figure 5A). OSCC cell lines (Ca9-22, HSC-2, HSC-3, HSC-4) again showed the highest UVC sensitivity, followed by neuronal cells (PC12, SH-SY5Y) > melanoma (COLO679) > lung cancer cells (A549, WA-hT, A904-L, LC-1/sq) > human normal cells (HGF, HPLF, HDFa, HPC, TIG-3, HFL-1) (Figure 5B). These two independent experiments demonstrated that oral cancer cell lines were the most UVC-sensitive among all cell lines tested, whereas normal oral cell lines were more resistant. Similarly, melanoma cells were more UVC-sensitive than normal dermal fibroblasts. However, the difference in sensitivity between lung cancer cell lines and normal lung fibroblast was not clear.

Relationship between UVC-sensitivity and Dectin-2 expression. Figure 4B and Figure 5B show that human dermal fibroblasts

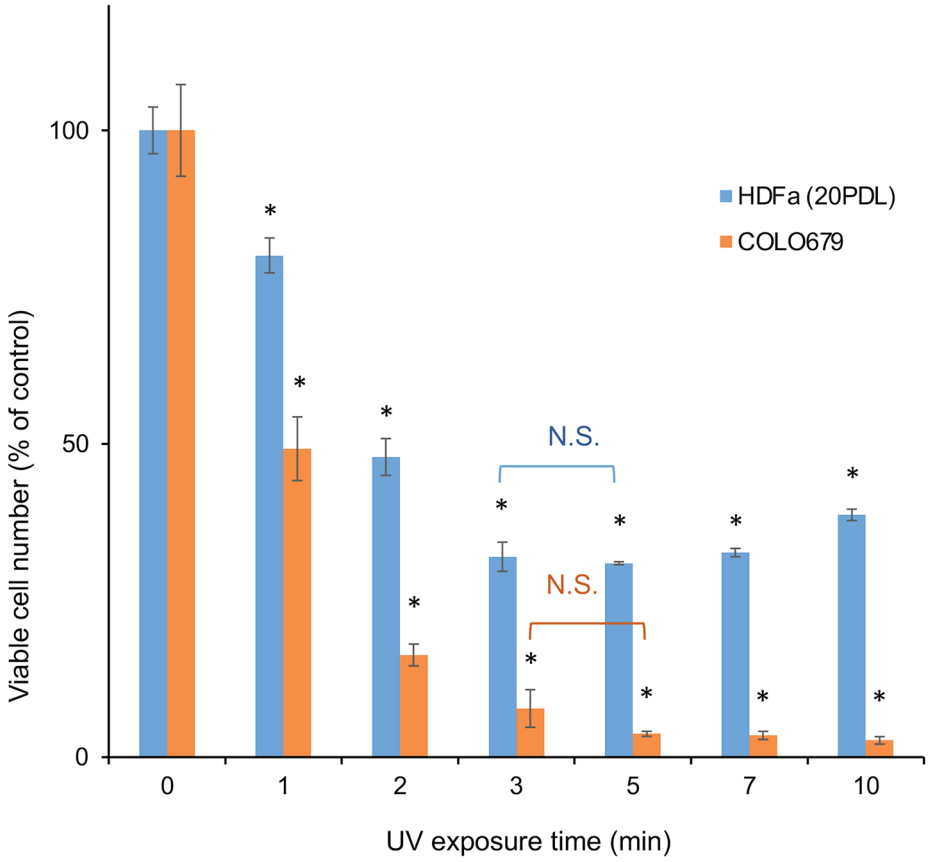

Figure 6. *Effect of exposure time to UVC. HDFa (20PDL) and COLO679 cells were exposed to UV for 0 (control), 1, 2, 3, 5, 7, or 10 min, and then cultured for 48 h in fresh culture medium. Data are plotted as viable cell numbers (% of control). Each value represents mean±S.D. (n=3). *p<0.05, ANOVA with Bonferroni post hoc. N.S.: not significant.*

(HDFa) are more resistant to UVC irradiation than COLO679 melanoma cells. This was further confirmed by additional experiments examining the effect of time (Figure 6). Although viability of both HDFa and COLO679 declined time-dependently ($p<0.05$), reaching a plateau at 3 min, a higher percentage of viable HDFa cells was maintained regardless of UVC exposure time (1~10 min). The maximum percentage of reduction in cell viability following UVC irradiation was 69.1% (HDFa) and 97.3% (COLO679) (Figure 6).

To elucidate the cell surface expression of Dectin-2 on HDFa and COLO679, flow cytometry was used. We first confirmed the cell surface expression of Dectin-2 on SH-SY5Y (positive control) (Figure 7C). It was unexpected that both HDFa and COLO679 also expressed Dectin-2 (Figure 7A and B), suggesting that the presence of Dectin-2 itself may not contribute to the sensitivity of HDFa and COLO679 to UVC.

Reconfirmation of the anti-UVC activity of sodium ascorbate. Finally, the UVC protective activity of sodium ascorbate was examined in cells irradiated while maintained in DMEM supplemented with 10% heat-inactivated FBS. When HDFa (26 PDL) cells were exposed to UVC for 3 min in the absence of sodium ascorbate, viability was declined to 42% of the control (Figure 8A). However, when sodium ascorbate was added to the irradiation medium, cells were protected in a dose-dependent manner (Figure 8A). A significant increase in cell viability was detected at a concentration as little as 4 μM, reaching a maximum level at 125-250 μM. From the dose-response curve, EC_{50} and CC_{50} were calculated to be 9.6 and >1,000 μM, respectively, yielding the SI value of >104 (Figure 8A).

When COLO679 cells were irradiated with an increasing dose of UVC, cell viability was declined to 10% of unirradiated control cells (Figure 8B). The addition of sodium ascorbate again abrogated the cytotoxicity of UVC irradiation. From the dose-response curve, EC_{50} and CC_{50} were calculated to be 12.7 and >1,000 μM, yielding an SI value of >79 (Figure 8B). These data suggest that sodium ascorbate can be used as a positive control when searching for new UVC protective substances.

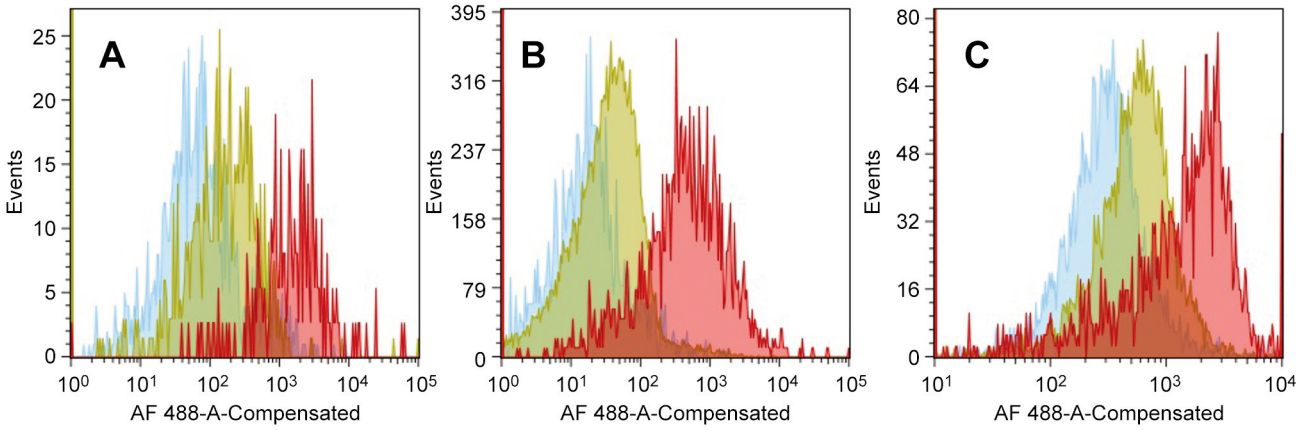

Figure 7. *Surface expression of Dectin-2 in HDFa (A), CoLo679 (B), and SH-SY5Y cells (C) was quantified using flow cytometry. Red, stained with Dectin-2 antibody; Yellow, stained with control antibody; Blue, unstained.*

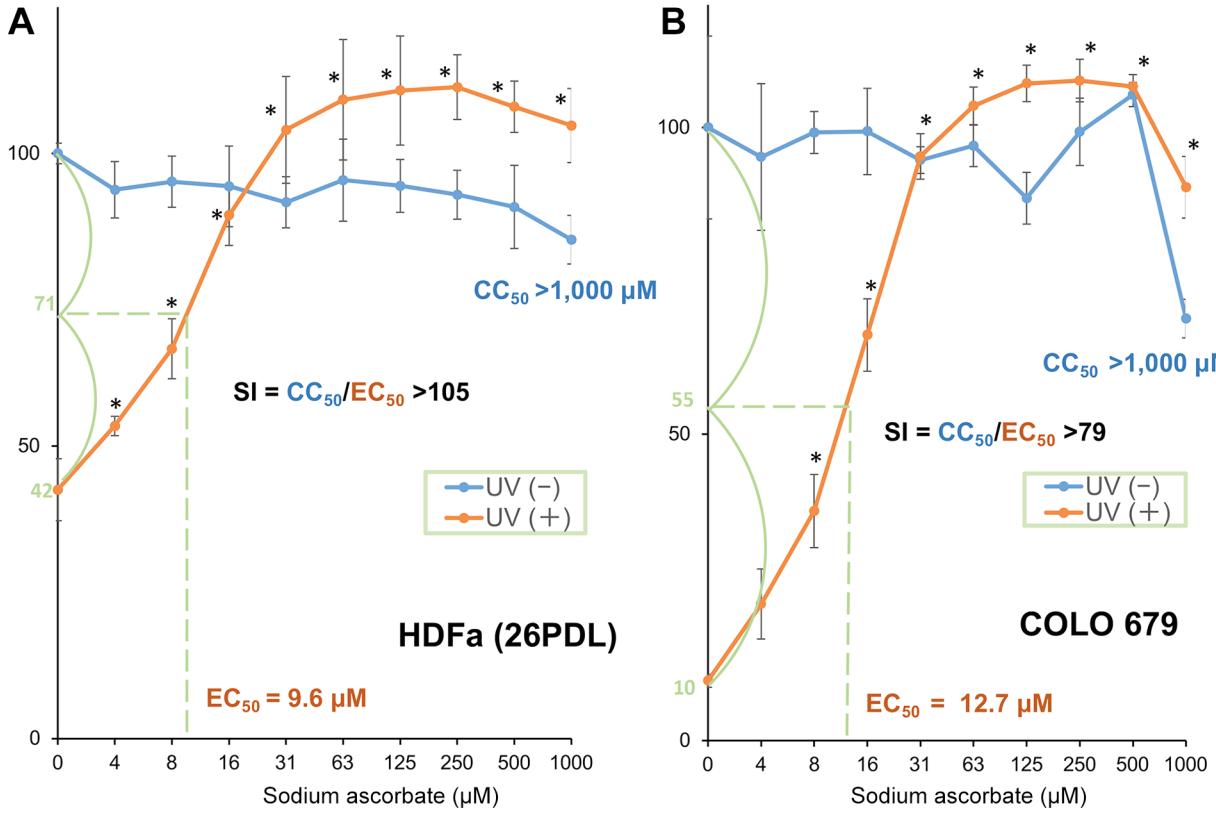

Figure 8. *Anti-UVC activity of sodium ascorbate. HDFa (26PDL) (A) and COLO679 (B) cells were exposed for 3 min with the indicated concentrations of sodium ascorbate and cultured for 48 h in fresh culture medium to determine the viable cell number. Each value represents mean±S.D. (n=3). *p<0.05, ANOVA with Bonferoni post hoc.*

Discussion

The present study demonstrated that the best irradiation medium is culture medium supplemented with 10% FBS, since the use of PBS caused detachment of some cell lines and lowered cell viability. The use of culture medium significantly diminished the cell loss during the washing process. The distance between the UV lamp and target cells was set to 555

mm, allowing near vertical irradiation of cells in the wells (Figure 2B), yielding the maximum cytotoxicity and more accurate determination of UVC-induced cytotoxicity.

Under these conditions, we could demonstrate that four human oral squamous cell lines (Ca9-22, HSC-2, HSC-3, HSC-4) were more UVC-sensitive than three human normal oral mesenchymal cells [gingival fibroblasts (HGF), periodontal ligament fibroblasts (HPLF), pulp cells (HPC)]. Similarly, COLO679 cells were more UVC-sensitive than HDFa cells (Figure 5B, Figure 6, and Figure 8). However, differences in the UVC sensitivity between human lung cancer cells (A549, WA-hT, A904-L, LC-1/sq) and human lung fibroblasts (TIG-3, HFL-1) were not observed. This may be due to the fact that normal lung fibroblasts and lung cancer cells show similar proliferation rates. These data suggest that UVC sensitivity may be related to proliferation potential. This should be examined in additional malignant and non-malignant cells.

We considered that UVC-resistant human dermal fibroblasts express higher Dectin-2 protein than UVC-sensitive COLO679 cells, whereas COLO679 cells express less or no Dectin-2. Due to the heterogeneity of carbohydrate chains (16, 17), many protein bands may appear in western blot analysis, making quantification difficult. Therefore, at the initial stage, we used flow cytometry to quantify Dectin-2. In contrast to our expectation, both cells expressed Dectin-2 (Figure 7). This finding indicates that Dectin-2 may not determine UVC sensitivity. It is very important to investigate the possible changes in Dectin-2 before and after UVC treatment.

When normal human dermal fibroblasts were subjected to UVC radiation, approximately 40-50% of cells were still viable. Subtracting this background, the EC_{50} value can be accurately determined from the dose-response curve of irradiated cells. The CC_{50} value can be determined from the dose-response curve of unirradiated cells. Using these EC_{50} and CC_{50} values, the anti-UVC activity (SI) can be calculated using the following equation: $SI=CC_{50}/EC_{50}$. The SI value of sodium ascorbate was determined to be >104, 2.5-fold higher than the value we have previously reported using PBS(–) as an irradiation medium (SI=42.4 for HSC-2 cells) (10).

Moderate doses of UV exert several favorable effects such as induction of vitamin D biosynthesis (18), hormetic response in normal oral cells (19) and plant tissues (20, 21), and increase in reproductive performance early in life (22).

However, an excessive UVC radiation absorbed by the epidermis, produces reactive oxygen species (ROS), and causes various cutaneous disorders, including photoaging and skin cancers (23). Due to the increased use of UVC for reducing COVID-19 incidence, exploration of effective UVC protective substances is urgent. The next step of our research is to determine which chemical compound exerts the most potent anti-UVC activity. We found that lignin precursors show much higher anti-UVC activity than lignin and tannin precursors (Sakagami *et al*. in preparation).

Conflicts of Interest

The Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Authors' Contributions

AS, MK, MI, SA, MA and HS performed the most experiments of the present study. HS and MI wrote the manuscript. TO and HS reviewed the manuscript. HS provided the interpretation of experimental results and edited the manuscript. All Authors read and approved the final version of the manuscript.

Acknowledgements

This work was supported by Miyata Research Fund B.

References

1 Hui DS, I Azhar E, Madani TA, Ntoumi F, Kock R, Dar O, Ippolito G, Mchugh TD, Memish ZA, Drosten C, Zumla A and Petersen E: The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - The latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis *91*: 264-266, 2020. PMID: 31953166. DOI: 10.1016/j.ijid.2020.01.009

2 Singh N, Singh P, Singh V, Krishna A and Singh S: Comprehensive study on clinical responses and socioeconomic characteristics of COVID-19 patients during outbreak. J Family Med Prim Care *10(11)*: 4002-4008, 2021. PMID: 35136759. DOI: 10.4103/jfmpc.jfmpc_579_21

3 Hosen I, Al-Mamun F and Mamun MA: Prevalence and risk factors of the symptoms of depression, anxiety, and stress during the COVID-19 pandemic in Bangladesh: a systematic review and meta-analysis. Glob Ment Health (Camb) *8*: e47, 2021. PMID: 35145709. DOI: 10.1017/gmh.2021.49

4 Su WL, Lin CP, Huang HC, Wu YK, Yang MC, Chiu SK, Peng MY, Chan MC and Chao YC: Clinical application of 222 nm wavelength ultraviolet C irradiation on SARS CoV-2 contaminated environments. J Microbiol Immunol Infect *55(1)*: 166-169, 2022. PMID: 35094944. DOI: 10.1016/j.jmii.2021.12.005

5 Harfoot R, Yung DBY, Anderson WA, Wild CEK, Coetzee N, Hernández LC, Lawley B, Pletzer D, Derraik JGB, Anderson YC and Quiñones-Mateu ME: Ultraviolet-C irradiation, heat, and storage as potential methods of inactivating SARS-CoV-2 and bacterial pathogens on filtering facepiece respirators. Pathogens *11(1)*: 83, 2022. PMID: 35056031. DOI: 10.3390/pathogens11010083

6 de Souza SO, Cardoso AA Jr, Sarmento ASC and d'Errico F: Effectiveness of a UVC air disinfection system for the HVAC of an ICU. Eur Phys J Plus *137(1)*: 37, 2022. PMID: 34956809. DOI: 10.1140/epjp/s13360-021-02240-y

7 Pattanaik P, Holderbaum W, Khandual A and Tripathy HP: UVC LED and conducting yarn-based heater for a smart germicidal face mask to protect against airborne viruses. Materials (Basel) *14(22)*: 6999, 2021. PMID: 34832400. DOI: 10.3390/ma14226999

8 Aragane Y, Maeda A, Schwarz A, Tezuka T, Ariizumi K and Schwarz T: Involvement of dectin-2 in ultraviolet radiation-induced tolerance. J Immunol *171(7)*: 3801-3807, 2003. PMID: 14500681. DOI: 10.4049/jimmunol.171.7.3801

9 Bonkobara M, Yagihara H, Yudate T, Chung JS, Washizu T, Ariizumi K and Cruz PD Jr: Ultraviolet-B radiation upregulates expression of dectin-2 on epidermal Langerhans cells by activating the gene promoter. Photochem Photobiol *81(4)*: 944-948, 2005. PMID: 15876128. DOI: 10.1562/2004-10-21-RC-349

10 Sakagami H, Sheng H, Okudaira N, Yasui T, Wakabayashi H, Jia J, Natori T, Suguro-Kitajima M, Oizumi H and Oizumi T: Prominent anti-UV activity and possible cosmetic potential of lignin-carbohydrate complex. In Vivo *30(4)*: 331-339, 2016. PMID: 27381594.

11 Kantoh K, Ono M, Nakamura Y, Nakamura Y, Hashimoto K, Sakagami H and Wakabayashi H: Hormetic and anti-radiation effects of tropolone-related compounds. In Vivo *24(6)*: 843-851, 2010. PMID: 21164042.

12 Iida M, Sunaga S, Hirota N, Kuribayashi N, Sakagami H, Takeda M and Matsumoto K: Effect of glutathione-modulating compounds on hydrogen-peroxide-induced cytotoxicity in human glioblastoma and glioma cell lines. J Cancer Res Clin Oncol *123(11-12)*: 619-622, 1997. PMID: 9620220. DOI: 10.1007/s004320050115

13 Sakagami H, Furukawa T, Satoh K, Amano S, Iijima Y, Koshikawa T, Asai D, Fukuchi K, Takemura H, Kanamoto T and Yokose S: Re-evaluation of chemotherapeutic potential of pyoktanin blue. Medicines (Basel) *8(7)*: 33, 2021. PMID: 34206186. DOI: 10.3390/medicines8070033

14 Sakagami H, Kuribayashi N, Iida M, Hagiwara T, Takahashi H, Yoshida H, Shiota F, Ohata H, Momose K and Takeda M: The requirement for and mobilization of calcium during induction by sodium ascorbate and by hydrogen peroxide of cell death. Life Sci *58(14)*: 1131-1138, 1996. PMID: 8614264. DOI: 10.1016/0024-3205(96)00071-9

15 Sakagami H, Hromchak R and Block A: Prevention of phorbol ester receptor down modulation in human myeloblastic leukemia ML-1 cells by differentiation-stimulating serum components. Cancer Res *44(8)*: 3330-3335, 1984. PMID: 6331642.

16 Vendele I, Willment JA, Silva LM, Palma AS, Chai W, Liu Y, Feizi T, Spyrou M, Stappers MHT, Brown GD and Gow NAR: Mannan detecting C-type lectin receptor probes recognise immune epitopes with diverse chemical, spatial and phylogenetic heterogeneity in fungal cell walls. PLoS Pathog *16(1)*: e1007927, 2020. PMID: 31999794. DOI: 10.1371/journal.ppat.1007927

17 Hadebe S, Brombacher F and Brown GD: C-type lectin receptors in asthma. Front Immunol *9*: 733, 2018. PMID: 29696023. DOI: 10.3389/fimmu.2018.00733

18 Barysch MJ, Hofbauer GF and Dummer R: Vitamin D, ultraviolet exposure, and skin cancer in the elderly. Gerontology *56(4)*: 410-413, 2010. PMID: 20502035. DOI: 10.1159/000315119

19 Koh T, Machino M, Murakami Y, Umemura N and Sakagami H: Cytotoxicity of dental compounds towards human oral squamous cell carcinoma and normal oral cells. In Vivo *27(1)*: 85-95, 2013. PMID: 23239856.

20 Manzocco L and Nicoli MC: Surface processing: existing and potential applications of ultraviolet light. Crit Rev Food Sci Nutr *55(4)*: 469-484, 2015. PMID: 24915377. DOI: 10.1080/10408398.2012.658460

21 Sidibé A, Charles MT, Lucier JF, Xu Y and Beaulieu C: Preharvest UV-C hormesis induces key genes associated with homeostasis, growth and defense in lettuce inoculated with *Xanthomonas campestris* pv. *vitians*. Front Plant Sci *12*: 793989, 2022. PMID: 35111177. DOI: 10.3389/fpls.2021.793989

22 Heine KB, Powers MJ, Kallenberg C, Tucker VL and Hood WR: Ultraviolet irradiation increases size of the first clutch but decreases longevity in a marine copepod. Ecol Evol *9(17)*: 9759-9767, 2019. PMID: 31534691. DOI: 10.1002/ece3.5510

23 Ridley AJ, Whiteside JR, McMillan TJ and Allinson SL: Cellular and sub-cellular responses to UVA in relation to carcinogenesis. Int J Radiat Biol *85(3)*: 177-195, 2009. PMID: 19296341. DOI: 10.1080/09553000902740150

Received April 11, 2022
Revised May 8, 2022
Accepted June 8, 2022