Construction of a Secondary Enclosure for UVB Irradiation of Mice

Justin Choi1,2,6, Zachary A. Bordeaux1,2,6, Gabriella Braun2, Cole Davis2, Varsha Parthasarathy1, Junwen Deng1, Mathew T. Taylor3, Anusha Kambala1, Hannah Cornman1, Olusola Oladipo1, Martin P. Alphonse1, Cameron E. West3, Shawn G. Kwatra1,4 and Madan M. Kwatra2,5

UV irradiation is commonly used in murine models of skin cancers. Despite the popularity of using UV rays to model photocarcinogenesis in animals, there is a lack of standardization in the secondary enclosures used to administer radiation. An appraisal of the literature also shows a general lack of details regarding the materials and procedures utilized in the fabrication of such enclosures. We present in this study a detailed overview of the construction of a UVB exposure chamber that successfully induces lesions in hairless mice. A standardized protocol for producing a UV enclosure may reduce methodological variation in future studies seeking to investigate photocarcinogenesis in animals.

INTRODUCTION

UVB irradiation is a common method that has been used to reproduce cutaneous lesions in murine models of photodamage-induced skin cancer. Although UVB irradiation has allowed researchers to investigate the pathogenesis of photocarcinogenesis and validate novel therapies against pertinent dermatologic malignancies, there is an absence of standardization in the secondary enclosures used and UV lighting systems used to conduct these studies (Table 1).

The systems found in the existing literature fall into one of two broad categories: exposure chambers fabricated by investigators (or prefabricated enclosures that have been secondarily co-opted for this purpose [Carrara et al., 2019; Kremer et al., 2019]) and, less commonly, manufactured UV cabinets. The cost of premanufactured UV cabinets may represent a barrier for investigators. Although fabricating a dedicated enclosure may be more cost effective and tailored for laboratory-specific needs, an appraisal of the literature shows minimal information and standardization across the UV exposure chambers used in different studies, particularly with regard to the dimensions or specific materials used in their construction. This is further complicated by the variation in the specific UVB source and exposure regimens used to induce lesions.

We present in this study the materials used and steps outlining the construction of a secondary UV exposure chamber as well as an exposure regimen that produces cutaneous lesions in hairless mice. A standardized protocol for developing a UV enclosure will aid future investigators in maximizing reproducibility and minimizing methodological variation.

RESULTS

System functionality

Given that the fluorescent bulb is 4 feet long, the length of the lamp is sufficient for irradiating four standard mice cages placed side by side (Figure 2), with cages being placed directly under the light source. Given that each cage can house up to five mice, the enclosure described in this study has the capacity to administer UVB radiation to up to 20 mice simultaneously. Because cages could not have their factory default lids during irradiation, a simple wire rack is effective in ensuring that mice do not climb out of the cages when placed in the enclosure while also allowing sufficient penetration of UVB into the cages. System maintenance was not required for the chamber beyond routine disinfection.

Lamp characteristics

The time required to reach a stable power output during warm-up is approximately 3 minutes, after which the experimental irradiation could take place. The average power output was approximately 180 μW/cm², equating to 278 seconds of exposure per irradiation period. Although minor week-to-week fluctuations were noted in power output, a global decrease in power was not noted after 6 months of bulb usage. Variation in power output was also observed along the length of the lamp, showing an approximately 10% decrease at the distal end of the bulb unilaterally (Figure 2). To ensure equal UVB dosage, the positions in which cages were placed during UVB administration were systematically rotated.

© 2022 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). www.jidinnovations.org
Lesion formation
Following a regimen that irradiated mice with 500 J/m² of UVB five times per week, the time to the appearance of the first lesion was approximately 13 weeks, with all mice showing lesions by approximately 16 weeks (Figure 3a and b).

DISCUSSION AND POTENTIAL APPLICATIONS
The UV system described in this paper was built with a raw material cost of 450 United States dollars and provides a practical method for fabricating an enclosure containing a UVB source that induces lesion formation in hairless SKH-1 mice after approximately 13 weeks. Explicit requirements established by Institutional Animal Care and Use Committee at our institution included construction with nonporous materials (for disinfection), a locking mechanism (to prevent mouse escape), ventilation holes, and an alternative lid that both prevented mouse escape and allowed sufficient UVB penetration into the cage. Proper care should be taken to ensure that the fenestrations of a given alternative lid are large enough for UVB to penetrate. Future efforts should be aimed toward characterizing the use of transparent barriers

Table 1. Select Studies Using UVB Carcinogenesis

| Mouse          | Light Source          | Enclosure Details/Dimensions | Reference                      |
|----------------|-----------------------|------------------------------|--------------------------------|
| SKH-1          | Philips TL-12/40W     | Bulb 23–26 cm above the cage | Baek et al., 2018              |
| SKH-1          | Philips TL-12/40W     | None                         | Bertelsen et al., 2016         |
| SKH-1          | Oriel solar simulators| None                         | Chastkofsky et al., 2015       |
| SKH-1          | FS72T12-UVB-HO        | None                         | Dinkova-Kostova et al., 2008   |
| SKH-1          | UV6 tubes             | None                         | Eriyndsson et al., 2016        |
| SKH-1          | Daavin Research Irradiator | Premanufactured UV cabinet  | Mintie et al., 2020            |
| SKH-1          | F72T12 100W/12 Phillips UVB Broadband TL | None | Phillips et al., 2013          |
| SKH-1          | Vilber Lourmat T-40.M | Customized cabinet with 28 cage capacity | Pillon et al., 2017            |
| SKH-1          | Philips TL-12/40W     | None                         | Rebel et al., 2012, 2001       |
| C57BL/6j       | Sankyo Denki UV lamp  | None                         | Ho et al., 2020                |
| C57BL/6        | UV-LED chips          | 3D printed darkroom module   | Lin et al., 2021               |
| NMRI-HR-HR     | TL12/20W              | 180 cm above the cage        | Boiy et al., 2011              |
| HRSJ           | Philips TL-12/40W     | 1.3 × 0.43 × 0.45 m box bulb 15 cm above the cage | Carrara et al., 2019; Kremer et al., 2019 |
| SENCAR Phk6−/− | FB-UVXL-1000 UV crosslinker | Premanufactured UV cabinet | Chastkofsky et al., 2015       |
| HR-1           | Handheld UVM-57 lamp  | None                         | Murata et al., 2021            |
| HRM-2          | IedaBoeki UV lamp     | None                         | Saba et al., 2020              |
| ICR-Foxn/nu    | Bio-sun illuminator system | Bulb 10 cm above the cage  | Wang et al., 2019              |

Abbreviations: 3D, three-dimensional; LED, light emitting diode;

Figure 1. Secondary UVB exposure chamber. Blueprint schematic of the enclosure from the (a) exterior side view, (b) exterior frontal view, (c) interior side view, and (d) three quarter aerial view. (e) Representative images of the enclosure. Illustrations were by Caroline Choi.
with a minimal reflective capacity to maximize UVB administration. Knowledge of the basic requirements of the Institutional Animal Care and Use Committee at our institution may allow other investigators to proactively fulfill similar requirements established by parallel ethical committees when constructing similar enclosures, thus expediting approval for animal studies.

Our UV exposure protocol was adapted from investigators using a fixed irradiation time throughout the duration of the experiment (Baek et al., 2018); however, potential temporal degradation of power output necessitated regular internal recalibration of exposure time. Although no power degradation was noted in our UVB source after 6 months of use, gradual temporal degradation reported in previous studies reiterates the importance of routinely checking output and adjusting exposure times (Pillon et al., 2017). Our study also showed variations in power output along the length of the lamp. An appraisal of the literature did not identify any study that accounted for this factor when irradiating mice, whereas only one study noted that consistent power output was achieved when using UVB light-emitting diode chips (Lin et al., 2021), given that the energy is equally distributed among individual diodes rather than a single fluorescent tube.

These factors are of particular significance because insufficient irradiation may prolong the time to lesion formation, whereas inconsistent UVB dosage may lead to differences in time to lesion formation as well as lesion severity among mice. To account for this, cage positions were rotated on a daily basis to ensure that mice received equitable radiation dosages over the experimental period. Although this may be an individual bulb-specific phenomenon, our experiences suggest that investigators utilizing fluorescent UV tubes should measure power output along the length of the lamp to ensure that appropriate countermeasures are taken to

**Figure 2. Inconsistencies in power output along the length of the UVB bulb.**
The floorplan of the box with representative power outputs noted under each cage position is shown, showing diminished power output localized to the position under the lamp corresponding to cage 1.

**Figure 3. The UVB enclosure and lighting system induce lesion formation in SKH-1 mice.** (a) Kaplan–Meier analysis of tumor-free mice showing that lesions appeared after approximately 13 weeks of UVB irradiation, and all mice developed lesions by week 16, with no significant difference between genders. (b) Representative images of mice before lesion formation and after 17 weeks of UVB exposure.
equalize UV administration among cages and prevent undue methodological variation.

With the anticipated increase in the prevalence of sun-induced dermatologic malignancies, UV irradiation systems will continue to grow in relevance in the realm of skin cancer research and drug development. A standardized protocol for constructing a cost-effective enclosure may help to reduce variation and methodological errors in future studies seeking to investigate photocarcinogenesis in murine models.

**MATERIALS AND METHODS**

**Materials and construction of the enclosure**

All materials used to construct the enclosure are listed in Table 2 and were purchased from a hardware store at an approximate cost of 450 United States dollars. The UV exposure chamber was fabricated with a black polyvinyl chloride board with the following dimensions:

| Item                           | Quantity | Purpose                      |
|-------------------------------|----------|------------------------------|
| 1/2” thick polyvinyl chloride board | User defined | Box structure |
| Philips TL 40W12 RS SLV/25 bulb | 1        | UVB source                   |
| Lithonia T12 2-light fixture  | 1        | Bulb fixture                 |
| S-bracket                      | 2        | Mount fixture to the box     |
| Handlebar                      | 1        | Handle for opening door      |
| 18-8 stainless steel screws    | User defined | Securing all components     |
| Lift-and-drop padlockable latch | 2        | Door lock                    |
| Pipe cement                    | User defined | Seal internal seams         |
| Cement for plastic             | User defined | Reinforce joints            |
| Sealing grommet                | 1        | Feed wire from interior to exterior |
| Caster (lockable)              | 4        | Mobility                     |
| Piano hinge                    | 1        | Door functionality           |

**UV exposure regimen**

The UV exposure regimen was adopted from the protocol outlined by Baek et al. (2018). Eight male (n = 4) and female (n = 4) SKH-1 mice (Charles River Laboratories, Wilmington, MA) aged between 6 and 8 weeks were used for this study. Four mice were housed per cage, and their skins were monitored daily for signs of injury. Before UV exposure, the bulb was allowed to warm up until it reached its maximum power output, measured using a PM100D power meter and S120VC power sensor (Thor Labs, Newton, NJ) (Baek et al., 2018). Power output was calculated by measuring the power sensor in six different locations within each cage and across all cage positions within the enclosure. The final readout was calculated as the average of all power measurements and was used to determine exposure time. Exposure time was internally recalibrated on a weekly basis to account for temporal power degradation (Pillon et al., 2017). Mice were irradiated with 500 J/m² of UVB (the minimum erythemal dose of these lamps identified in several independent studies) five times per week for 14 weeks (Baek et al., 2018; Rebel et al., 2012, 2001; Voskamp et al., 2012). Of note, strains of hairy mice, such as C57BL/6 and BALB/c, are often shaven and used for models of UV-induced carcinogenesis. For these mice, the minimum erythemal dose has been reported to be higher than in SKH-1 mice, ranging from 1,200 to 1,900 J/m². A maximum erythemal dose of 2,250 J/m² for BALB/c (Jeevan and Kripke, 1990; Toriyama et al., 2021) and 1,500 to 2,250 J/m² for BALB/c (Voskamp et al., 2012) was placed on top of the open cages to prevent mice from escaping during irradiation. Of note, power output was measured under these conditions to mimicking the conditions under which mice were irradiated. The positions in which cages were placed for each irradiation period were systematically rotated to equalize UV administration among cages and prevent undue methodological variation.

**Ethical approval**

The chamber was compliant with the Institutional Animal Care and Use Committee at Duke University School of Medicine (Baltimore, MD) (protocol number A155-20-07). Mice were monitored daily for signs of deteriorating health and weighed once per week. Humane endpoints included signs of discomfort, distress or pain, reduced mobility, inactivity, abnormal posture, lack of grooming, sudden weight loss exceeding 20%, lesion ulceration or necrosis, and lesion size exceeding 10 mm in diameter (Workman et al., 2010). Mice meeting these endpoint criteria were killed.

**Data availability statement**

No large dataset was generated or analyzed for this study.

**ORCIDs**

Justin Choi: http://orcid.org/0000-0002-8388-6876  
Zachary A. Bordeaux: http://orcid.org/0000-0002-8833-6080  
Gabriella Braun: https://orcid.org/0000-0003-4496-3696  
Cole Davis: https://orcid.org/0000-0003-0253-8720  
Varsha Parthasarathy: http://orcid.org/0000-0002-1422-772X  
Junwen Deng: http://orcid.org/0000-0001-8391-8915  
Matthew T. Taylor: http://orcid.org/0000-0001-7192-7936  
Anusha Kambala: http://orcid.org/0000-0002-0350-8622  
Hannah Comman: http://orcid.org/0000-0003-1462-2479  
Olusola Oladipo: http://orcid.org/0000-0001-9498-1492  
Martin P. Alphonse: http://orcid.org/0000-0003-3447-1284  
Cameron E. West: http://orcid.org/0000-0001-9673-9165  
Shawn G. Kwatra: http://orcid.org/0000-0003-3736-1515  
Madan M. Kwatra: http://orcid.org/0000-0002-6547-8852
CONFLICT OF INTEREST
SGK is an advisory board member/consultant for Abbvie, Cellidex Therapeutics, Galderma, Incyte, Pfizer, Regeneron Pharmaceuticals, Kiniksa Pharmaceuticals, and Genzada Pharmaceuticals and has received grant funding from Galderma, Pfizer, and Kiniksa Pharmaceuticals. CEW is an officer and member of the Board of Directors at Genzada Pharmaceuticals.

ACKNOWLEDGMENTS
We thank Donald J. Pearce from Duke Health Engineering and Operations for his technical expertise in guiding the construction of the enclosure and Caroline Choi for her artwork featured in Figure 1.

AUTHOR CONTRIBUTIONS
Conceptualization: JC, CEW, SGK, MMK; Data Curation: JC, ZAB, GB, CD; Formal Analysis: JC, ZAB; Funding Acquisition: CEW, SGK, MMK; Investigation: JC, ZAB, SGK, MMK; Methodology: JC, SGK, MMK; Project Administration: CEW, SGK, MMK; Resources: MMK; Software: JC, ZAB; Supervision: CEW, SGK, MMK; Validation: JC, ZAB, SGK, MMK; Visualization: JC, ZAB; Writing – Original Draft Preparation: JC, ZAB, SGK, MMK; Writing – Review and Editing: JC, ZAB, GB, CD, VP, JD, MTT, AK, HC, OO, MPA, CEW, SGK, MMK.

REFERENCES
Baek YS, Kim J, Han G, Oh CH. Application of dynamic thermal imaging in a photocarcinogenesis mouse model. Int J Hyperthermia 2018;34:961–8.
Bertelsen M, Stahlhut M, Grue-Sørensen G, Liang X, Christensen GB, Skak K, et al. Ingenol Disoxate: A novel 4-oxazololcarboxylate ester of ingenol with improved properties for treatment of actinic keratosis and other non-melanoma skin cancers. Dermatol Ther (Heidelb) 2016;6:599–626.
Boiy A, Roelandts R, de Witte PA. Photodynamic therapy using topically applied hypericin: comparative effect with methyl-aminolevulinic acid on UV induced skin tumours. J Photochem Photobiol B 2011;102:123–31.
Carrara IM, Melo GP, Bernardes SS, Neto FS, Ramalho LNZ, Marinello PC, et al. Looking beyond the skin: cutaneous and systemic oxidative stress in UVB-induced squamous cell carcinoma in hairless mice. J Photochem Photobiol B 2019;195:17.
Chastkofsky MI, Bie W, Ball-Kell SM, He YY, Tyner AL. Protein tyrosine kinase 6 regulates UVB-induced signaling and tumorigenesis in mouse skin. J Invest Dermatol 2015;135:2492–501.
Dinkova-Kostova AT, Jenkins SN, Wehage SL, Huso DL, Benedict AL, Stephenson KK, et al. A dicyanotriterpenoid induces cytoprotective enzymes and reduces multiplicity of skin tumors in UV-irradiated mice. Biochem Biophys Res Commun 2008;367:859–65.
Erlandsson AM, Thaysen-Petersen D, Bay C, Hald A, Skak K, Zibert JR, et al. Repeated treatments with ingenol mebutate prevents progression of UV-induced photodamage in hairless mice. PLoS One 2016;11:e0162597.
Ho YY, Sun DS, Chang HH. Silver nanoparticles protect skin from ultraviolet B-induced damage in mice. Int J Mol Sci 2020;21:7082.
Jeevan A, Kripke ML. Alteration of the immune response to Mycobacterium bovis BCG in mice exposed chronically to low doses of UV radiation. Cell Immunol 1990;130:32–41.
Kremer JL, Melo GP, Marinello PC, Bordini HP, Rossaneis AC, Sábio LR, et al. Citral prevents UVB-induced skin carcinogenesis in hairless mice. J Photochem Photobiol B 2019;198:111563.
Lin MY, Lim LM, Tsai SP, Jian FX, Hwang SJ, Lin YH, et al. Low dose ultraviolet B irradiation at 308 nm with light-emitting diode device effectively increases serum levels of 25(OH)D. Sci Rep 2021;11:2583.
Memari B, Nguyen-Yamamoto L, Salehi-Tabar R, Zago M, Fritz JH, Baglole CJ, et al. Endocrine aryl hydrocarbon receptor signaling is induced by mod-erate cutaneous exposure to ultraviolet light. Sci Rep 2019;9:8486.
Mintie CA, Musarra AK, Singh CK, Ndiaye MA, Sullivan R, Eickhoff JC, et al. Protective effects of dietary grape on UVB-mediated cutaneous damages and skin tumorigenesis in SKH-1 mice. Cancers (Basel) 2020;12:1751.
Murata K, Oyama M, Ogata M, Fujita N, Takahashi R. Oral administration of Jumihaidokuto inhibits UV-induced skin damage and prostaglandin E2 production in HR-1 hairless mice. J Nat Med 2021;75:142–55.
Phillips J, Moore-Medlin T, Sonavane K, Eksheyon O, McLarty J, Nathan CA. Curcumin inhibits UV radiation-induced skin cancer in SKH-1 mice. Otolaryngol Head Neck Surg 2013;148:797–803.
Pillon A, Gomes B, Vandenberge I, Cartron V, Cépe P, Blanchet JC, et al. Actinic keratosis modelling in mice: a translational study. PLoS One 2017;12:e0179991.
Rebel H, Mosnier LO, Berg RJ, Westerman-de Vries A, van Steeg H, van Kranen HJ, et al. Early p53-positive foci as indicators of tumor risk in ultraviolet-exposed hairless mice: kinetics of induction, effects of DNA repair deficiency, and p53 heterozygosity. Cancer Res 2001;61:977–83.
Rebel HG, Bodmann CA, van de Gindt GC, de Grujil FR. UV-induced ablation of the epidermal basal layer including p53-mutant clones resets UV carcinogenesis showing squamous cell carcinomas to originate from interfollicular epidermis. Carcinogenesis 2012;33:714–20.
Saba E, Kim SH, Lee YY, Kim HK, Roh SS, Kwak YS, et al. Anti-melanogenic effects of Korean red ginseng oil in an ultraviolet B-induced hairless mouse model. Molecules 2020;25:4755.
Skobowiat C, Slominski AT. UVB activates hypothalamic-pituitary-adrenal axis in C57BL/6 mice. J Invest Dermatol 2015;135:1638–48.
Toriyama E, Masuda H, Torii K, Ikumi K, Morita A. Time kinetics of cyclo-butan pyrimidine dimer formation by narrowband and broadband UVB irradiation. J Dermatol Sci 2021;103:151–5.
Voskamp P, Bodmann CA, Rebel HG, Koehl GE, Tensen CP, Bouwes Bavinck JN, et al. Rapamycin impairs UV induction of mutant-p53 over-expressing cell clusters without affecting tumor onset. Int J Cancer 2012;131:1267–76.
Wang PW, Cheng YC, Hung YC, Lee CH, Fang JY, Li WT, et al. Red raspberry extract protects the skin against UVB-induced damage with antioxidative and anti-inflammatory properties. Oxid Med Cell Longev 2019;2019:9529676.
Workman P, Aborgye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, et al. Guidelines for the welfare and use of animals in cancer research. Br J Cancer 2010;102:1553–77.

This work is licensed under a Creative Commons Attribution 4.0 International License. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/