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Construction of a secondary enclosure for Ultraviolet-B irradiation of mice

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Abbreviations: UVB, type B ultraviolet; PCV, polyvinyl chloride; MED, minimal erythemal dose; IACUC, Institutional Animal Care and Use Committee

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ABSTRACT

The use of UV irradiation is commonly used in murine models of skin cancers. Despite the popularity of using type B ultraviolet 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 demonstrates a general lack of details regarding the materials and procedures utilized in the fabrication of such enclosures. We present herein a detailed overview of the construction of a UVB exposure chamber that successfully induces lesions in hairless mice. A standardized protocol for producing a UVB enclosure may reduce methodological variation in future studies seeking to investigate photocarcinogenesis in animals.
INTRODUCTION

Type B ultraviolet (UVB) irradiation is a common method that has been used to reproduce cutaneous lesions in murine models of photodamage-induced skin cancer. While UVB irradiation has allowed researchers to investigate the pathogenesis of photocarcinogenesis and to validate novel therapies against pertinent dermatological malignancies, there is a lack of standardization in the secondary enclosures 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 pre-manufactured UV cabinets may represent a barrier for investigators. Although fabricating a dedicated enclosure may be more cost-effective and tailored for lab-specific needs, an appraisal of the literature demonstrates minimal information and standardization across the UV exposure chambers used in different studies, particularly with regards 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 herein 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.
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 450USD. The UV exposure chamber was fabricated with black polyvinyl chloride (PVC) board with the following dimensions: 60” × 18” × 18”. The decision to utilize PVC board in the construction of the enclosure was informed by the observation that certain non-porous materials are subject to degradation when exposed to commercial disinfectants. All components were secured using stainless steel screws, while the box joints were reinforced with plastic cement. Pipe cement was used to seal the joint seams of the box’s interior, and ventilation holes were drilled into all four walls to permit air flow. An additional hole was fitted with a sealing grommet and served as an outlet feeding the wire from the light fixture to the exterior. A single TL 40W12 RS SLV/25 fluorescent tube (Phillips, Amsterdam, Netherlands) was mounted on a Lithonia T12 2-light fixture (Lithonia Lighting, Atlanta, GA) 25 centimeters above the floor of the enclosure (Baek et al., 2018). S-brackets were used to mount the fixture to the ceiling of the chamber’s interior. The superior edge of one 60” × 18” wall was secured to a piano hinge to serve as a door, with a handlebar and packlockable latches on both sides of the door to enable locking, thus preventing animal escape during experiments. Four casters were secured to the underside of the enclosure for mobility, two of which were locking (Figure 1A-1D).

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)
between 6-8 weeks of age were used for this study. Four mice were housed per cage, and their skins were monitored daily for signs of injury. Prior to 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 re-calibrated 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 (MED) of these lamps identified in several independent studies) five times per week for fourteen weeks (Baek et al., 2018, Rebel et al., 2001, Rebel et al., 2012, 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 MED has been reported to be higher than SKH-1 mice, ranging from 1,200-1,900 J/m² for C57BL/6 (Memari et al., 2019, Skobowiat and Slominski, 2015, Toriyama et al., 2021), and 1,500-2,250 J/m² for BALB/c (Jeevan and Kripke, 1990, Toriyama et al., 2021). Prior to UV exposure, the factory lids and stainless-steel cage covers were removed from the cages, and a flat wire grid rack was placed on top of the open cages to prevent mice from escaping during irradiation. Of note, power output was measured under these racks as described previously to best mimic the conditions under which mice were irradiated. The positions in which cages were placed for each irradiation period were systematically rotated to ensure equal radiation administration.

*Ethical Approval*
The chamber was compliant with the Institutional Animal Care and Use Committee (IACUC) at Duke University School of Medicine (Protocol No. 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 euthanized.

RESULTS

System functionality

Given that the fluorescent bulb is four 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 5 mice, the enclosure described herein has the capacity to administer UVB radiation to up to twenty mice simultaneously. Since 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 three minutes, after which the experimental irradiation could take place. Average power output was approximately 180 $\mu$W/cm², equating to 278 seconds of exposure per irradiation period. While
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, demonstrating 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.

*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 demonstrating lesions by approximately 16 weeks (Figure 3A & 3B).

**DISCUSSION AND POTENTIAL APPLICATIONS**

The UV system described herein was built with a raw material cost of 450USD 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 IACUC at our institution included: construction with non-porous materials (for disinfection), a locking mechanism (to prevent animal escape), ventilation holes, and an alternative lid that both prevented animal 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 with minimal reflective capacity to maximize UVB administration. Knowledge of the basic requirements of IACUC 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 re-calibration of exposure time. While no power degradation was noted in our UVB source after 6 months of use, gradual temporal degradation reported in prior studies reiterate the importance of routinely checking output and adjusting exposure times (Pillon et al., 2017). Our study also demonstrated 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, while only one study noted that consistent power output was achieved when using ultraviolet LED chips (Lin et al., 2021), given that the energy is equally distributed among individual diodes rather than a single fluorescent tube. Thus, a potential method for further optimizing this enclosure may lie in the use of ultraviolet LED chips as opposed to a fluorescent bulb to maximize consistency.

These factors are of particular significance, as insufficient irradiation may prolong time to lesion formation, while 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 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 reduce variation and methodological errors in future studies seeking to investigate photocarcinogenesis in murine models.

DATA AVAILABILITY
No large dataset was generated or analyzed for this study.

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Formal Analysis: JC, ZAB

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Methodology: JC, SGK, MMK

Project Administration: CEW, SGK, MMK

Resources: MMK

Software: JC, ZAB

Supervision: CEW, SGK, MMK

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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

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CONFLICTS OF INTEREST
Dr. Shawn G. Kwatra is an advisory board member/consultant for Abbvie, Celldex Therapeutics, Galderma, Incyte Corporation, Pfizer Inc., Regeneron Pharmaceuticals, Kiniksa Pharmaceuticals, and Genzada Pharmaceuticals and has received grant funding from Galderma, Pfizer Inc., and Kiniksa Pharmaceuticals. Dr. West is an officer of and member of the Board of Directors at Genzada Pharmaceuticals.

REFERENCES


Ho YY, Sun DS, Chang HH. Silver Nanoparticles Protect Skin from Ultraviolet B-Induced Damage in Mice. Int J Mol Sci 2020;21(19).


Table 1: Select studies using UVB carcinogenesis.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Light source</th>
<th>Enclosure details/dimensions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKH-1</td>
<td>Philips TL-12/40W</td>
<td>Bulb 23-26 cm above the cage</td>
<td>(Baek et al., 2018)</td>
</tr>
<tr>
<td>SKH-1</td>
<td>Philips TL-12/40W</td>
<td>None</td>
<td>(Bertelsen et al., 2016)</td>
</tr>
<tr>
<td>SKH-1</td>
<td>Oriel solar simulators</td>
<td>None</td>
<td>(Chastkofsky et al., 2015)</td>
</tr>
<tr>
<td>SKH-1</td>
<td>FS72T12-UVB-HO</td>
<td>None</td>
<td>(Dinkova-Kostova et al., 2008)</td>
</tr>
<tr>
<td>SKH-1</td>
<td>UV6 tubes</td>
<td>None</td>
<td>(Erlendsson et al., 2016)</td>
</tr>
<tr>
<td>SKH-1</td>
<td>Daavlin Research Irradiator</td>
<td>Pre-manufactured UV cabinet</td>
<td>(Mintie et al., 2020)</td>
</tr>
<tr>
<td>SKH-1</td>
<td>F72T12 100W/12 Phillips UVB</td>
<td>None</td>
<td>(Phillips et al., 2013)</td>
</tr>
<tr>
<td>SKH-1</td>
<td>FS72T12-UVB-HO</td>
<td>None</td>
<td>(Pillon et al., 2017)</td>
</tr>
<tr>
<td>SKH-1</td>
<td>Philips TL-12/40W</td>
<td>None</td>
<td>(Rebel et al., 2001, Rebel et al., 2012)</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>Sankyo Denki UVB lamp</td>
<td>None</td>
<td>(Ho et al., 2020)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>UV-LED chips</td>
<td>3-D printed darkroom module</td>
<td>(Lin et al., 2021)</td>
</tr>
<tr>
<td>NMRI-HR-HR</td>
<td>TL12/20W</td>
<td>180 cm above the cage</td>
<td>(Boiy et al., 2011)</td>
</tr>
<tr>
<td>HRS/J</td>
<td>Philips TL-12/40W</td>
<td>1.3m x 0.43m x 0.45m box Bulb 15 cm above the cage</td>
<td>(Carrara et al., 2019, Kremer et al., 2019)</td>
</tr>
<tr>
<td>SENCAR Ptk6/-</td>
<td>FB-UVXL-1000 UV crosslinker</td>
<td>Pre-manufactured UV cabinet</td>
<td>(Chastkofsky et al., 2015)</td>
</tr>
<tr>
<td>HR-1</td>
<td>Handheld UVM-57 lamp</td>
<td>None</td>
<td>(Murata et al., 2021)</td>
</tr>
<tr>
<td>HRM-2</td>
<td>IedaBoeki UVB lamp</td>
<td>None</td>
<td>(Saba et al., 2020)</td>
</tr>
<tr>
<td>ICR-Foxn/nu</td>
<td>Bio-sun illuminator system</td>
<td>Bulb 10 cm above the cage</td>
<td>(Wang et al., 2019)</td>
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</table>
Table 2. Materials used in the construction of the enclosure

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>½” thick polyvinyl chloride board</td>
<td>User defined</td>
<td>Box structure</td>
</tr>
<tr>
<td>Philips TL 40W12 RS SLV/25 bulb</td>
<td>1</td>
<td>UVB source</td>
</tr>
<tr>
<td>Lithonia T12 2-light fixture</td>
<td>1</td>
<td>Bulb fixture</td>
</tr>
<tr>
<td>S-bracket</td>
<td>2</td>
<td>Mount fixture to the box</td>
</tr>
<tr>
<td>Handlebar</td>
<td>1</td>
<td>Handle for opening door</td>
</tr>
<tr>
<td>18-8 stainless steel screws</td>
<td>User defined</td>
<td>Securing all components</td>
</tr>
<tr>
<td>Lift-and-drop padlockable latch</td>
<td>2</td>
<td>Door lock</td>
</tr>
<tr>
<td>Pipe cement</td>
<td>User defined</td>
<td>Seal internal seams</td>
</tr>
<tr>
<td>Cement for plastic</td>
<td>User defined</td>
<td>Reinforce joints</td>
</tr>
<tr>
<td>Sealing grommet</td>
<td>1</td>
<td>Feed wire from interior to exterior</td>
</tr>
<tr>
<td>Caster (lockable)</td>
<td>4</td>
<td>Mobility</td>
</tr>
<tr>
<td>Piano hinge</td>
<td>1</td>
<td>Door functionality</td>
</tr>
</tbody>
</table>
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) ¾ aerial view. E) Representative images of the enclosure. Illustrations by Caroline Choi.

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, demonstrating diminished power output localized to the position under the lamp corresponding to cage 1.

Figure 3. The UVB enclosure and lighting system induces lesion formation in SKH-1 mice. A) Kaplan Meier analysis of tumor-free mice demonstrating 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 prior to lesion formation and after 17 weeks of UVB exposure.
UV Box (floor plan)

<table>
<thead>
<tr>
<th>Cage 1</th>
<th>Cage 2</th>
<th>Cage 3</th>
<th>Cage 4</th>
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<tr>
<td>190</td>
<td>210</td>
<td>216</td>
<td>213</td>
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Power output (mW/cm²)