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3D-printed Wash Station with Integrated Anesthesia Delivery Manifold for High-throughput Depilation of Laboratory Mice

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Depilation (that is, hair removal) is a necessary prerequisite for many small animal surgeries and optical imaging experiments. Over-the-counter depilatory creams are widely used, owing to their efficacy, safety, and low rates of skin irritation and infection. However, the use of these creams is generally messy and time-consuming and generates considerable waste. Furthermore, the process itself varies markedly among laboratories. Here we present a 3D-printed device that simplifies the depilation procedure by integrating 3 key elements: 1) a multiple-port, self-scavenging anesthesia manifold, 2) curved animal holders with flow-through slats, and 3) a removable waste collection tray. Reflecting insights gained from an international survey about depilatory lab procedures that highlighted the lack of standardized protocols, this apparatus is designed to improve the neatness, throughput, and safety of mouse depilation, resulting in efficient and repeatable processes that bolster the welfare of both researchers and subjects.

Abbreviations: CAD, computer-aided design; MDS, mouse depilation station

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Preparing laboratory mice for surgery and optical imaging experiments often involves hair removal (that is, depilation). Preoperative removal of hair minimizes surgical site infections during the procedure and prevents hair from interfering with the procedure.[11] For example, hair poses unique challenges during several preclinical imaging techniques, including bioluminescence, fluorescence, and Cerenkov luminescence imaging, because hair both absorbs and scatters light, resulting in poor acquisition sensitivity and output resolution.[1,5,13,14] Although several hairless mouse models exist, they are often limited in experimental application because the nude trait is often accompanied by other genetic mutations, such as defects in immune competence.[3,9] As a result, many small animal models require depilation before use in a laboratory setting.

There are several different ways to remove hair from rodents prior to a given experiment, including trimming with electric clippers, shaving with a razor, or depilating with specialized creams.[9] Each of these methods has associated strengths and weaknesses. For example, shaving can produce microscopic defects in the skin that may result in an increased risk for infection.[7,8] Although clipping represents an alternative method to shaving, it often fails to achieve complete hair removal and therefore is often used in conjunction with other depilation methods. As a result, the preferred approach for hair removal is through the application of depilatory cream, often preceded by clipping. The chemical agents in depilatory creams break down the keratin in the hair but leave an intact, functional follicle for future growth.[6] As such, the hair removal accomplished through depilatories is far more thorough than can be achieved with other methods. An additional advantage of the use of depilatory creams is the avoidance of sharps, thus reducing the potential of injury to a lab worker or small animal from razors or clippers. Furthermore, these cream-based agents minimize skin irritation, have a low occurrence of surgical site infection, save time, and allow for straightforward hair removal in areas difficult to shave.[6,8,11,12]

The procedure for using a depilatory cream is often done in accordance with manufacturer's instructions. However, human products are used in the vast majority of cases, and the directions are optimized for humans, not animals. Regardless, the procedure is initiated by placing a layer of product on an area of the animal for a designated period. Traditionally, the depilatory cream and hair are removed subsequently by using a water-moistened gauze or cloth or a jet of water from a spray bottle. Although it yields the desired end-state, this method of depilation is often messy, time-consuming, and subject to variability between research groups and animal anatomic locations. Because rodents often are under general anesthesia, it is imperative that the depilation procedure is as brief as possible, because prolonged exposure increases the risk of hypothermia, reduced cardiovascular function, and other physiologic alterations that may affect experimental outcomes.[2,4] Furthermore, research personnel are often exposed to waste anesthesia gas from benchtop nose cones, because the physical manipulation of each animal compromises the latex seal of these breathing circuits.[10]

Here we present a 3D-printed device that is designed to improve the cleanliness and throughput of mouse depilation and increase the safety and efficiency of anesthesia delivery. This apparatus simplifies mouse hair removal by integrating

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3 critical elements: 1) a self-scavenging, 3-port, mouse manifold to improve anesthesia delivery; 2) curved animal holders with flow-through slats to minimize animal handling and enable waste pooling; and 3) a removable waste collection tray to simplify cleanup. The manifold maintains steady delivery of anesthesia for as many as 3 animals to increase throughput yet prevent waste gas exposure through an active scavenging system. The curved slats offer an ergonomic surface on which animals can be rinsed without extensive manipulation, and the tray collects waste solutions for disposal. Patent and literature searches indicated that this type of device is not currently available to the veterinary and preclinical research communities. The lack of standardized depilation conditions across academic and industry labs was demonstrated by the results, reported herein, of an anonymous questionnaire sent to preclinical animal researchers. The implementation of the depilation station may serve as a platform to increase the efficiency of hair removal procedures by allowing for parallel processing of multiple animals as well as accommodating customized protocols depending on experimental conditions, such as the type of mice used and the anatomic region of interest.

Materials and Methods

All animal studies were performed at the Freimann Life Sciences Center at the University of Notre Dame (Notre Dame, IN). All animal procedures were approved by the IACUC of the University of Notre Dame.

Mouse depilation procedures survey. An anonymous online questionnaire was written and distributed through Google Forms (Google, Mountain View, CA) to elucidate the procedures used with depilatory creams to remove mouse fur. The survey comprised 13 questions to reveal multiple aspects of depilation protocols, including the products are used, duration of application, removal method, number of applications, anesthesia conditions, purpose for depilation, other steps taken for fur removal, the region of interest on the mice, whether mice were treated individually or in parallel, the number of researchers participating in the depilation process, and number of mice depilated. Questions were short-answer or multiple-choice, allowing for the selection of multiple answers where applicable. A total of 27 researchers representing 19 institutions in academia and industry responded. Five respondents did not use depilatory creams, leaving survey results from 22 researchers from 16 academic and industry settings. Responses were collected, recorded, and analyzed to identify prevalent procedural trends. Average times were calculated for the massage (application) and incubation times by taking the mean of the shortest and longest times for the selected range.

Mouse rinse station design and assembly. The Mouse Depilation Station (MDS) was designed to integrate several important features to make it a robust hair-removal platform. First, the MDS featured legs to facilitate the placement of a pan underneath, to catch and remove waste. The rinse station was designed to have oval-shaped beds, in which the mice could be held and positioned. The slats of the oval bed were spaced to allow removed fur and cream to drain easily into the removable pan during the wash step. Because mice often are sedated prior to depilation, the MDS was designed to hold a removable anesthesia manifold. The manifold was attached to the rinse station by using magnets, which secured the device in place during its operation, through a metal strip inlaid on the bottom side of the flat MDS shelf. The manifold delivered and scavenged the isoflurane gas to prevent exposure of researchers to waste emissions and to provide continuous anesthesia during the depilation process. The overall pitch of the wash station was such that heads of the mice and the anesthesia manifold were oriented away from the direction of the water and fur flush from the animals.

An Equaflow 3XL manifold (In Vivo Concepts, Granger, IN) was acquired to serve as the anesthesia delivery mechanism. An aluminum, removable waste-collection tray (WebstaurantStore, Lancaster, PA [webstaurantstore.com]) was powder-coated in flat black paint. Three sets of curved slat elements, on which the mice would be placed, were created by using Inventor computer-aided design (CAD) software (Autodesk, San Rafael, CA). These slats were aligned side by side and elevated by using corner legs for suspension over the waste tray. Furthermore, a 'shelf' was included to integrate and interface the manifold with the curved slat area (Figure 1 A). The output design was saved as an STL file and uploaded to a third-party 3D printing company (Shapeways, New York, NY) to create a model from fine polyamide (PA 2200) material. This resin was chosen for its strength, flexibility, and chemical inertness to bleach. The complete assembly of manifold, suspended slats, and waste tray form the MDS (Figure 1 B). The CAD files for the stage are available for free download (http://www.thingiverse.com/thing:2229283). This file can be uploaded at third-party 3D printing companies to acquire the part at cost. The stage was designed to integrate with a commercially available anesthesia manifold (Equaflow 3XL, Somni Scientific, Pittsburgh, PA [www.somniscientific.com] or Patterson Scientific, Waukesha, WI [https://pattersonscientific.com/]). Alternatively, researchers can use an injectable anesthetic in conjunction with the stage if their standard process does not include inhalant anesthesia.

Mouse depilation. The materials needed to complete the hair removal process included depilatory cream, a cotton-tipped applicator, distilled water, an isoflurane vaporizer, a vacuum pump, and the MDS. All materials were sprayed with 70% ethanol, wiped down, placed in a laminar flow hood, and sterilized for 30 min by using UV light prior to use. Thirty mice (*Mus musculus*; male and female; age, 5 to 8 wk; weight, 17 to 25 g) were randomly selected from C57BL/6 and BALB/c backgrounds and separated into 8 groups of 3 mice each for processing through the MDS system, and one cohort of 6 to be depilated sequentially. The groups of 3 mice for the MDS system were given general anesthesia through short-term exposure (2.5% isoflurane in O_2 at 2 L/min for 2 min) in a mouse induction chamber. Once anesthetized, the group of 3 mice was transferred to the MDS in a supine position, and isoflurane was delivered continually through the manifold (Figure 2 A). The manifold was connected to an evacuation pump system (available from Patterson Scientific or Somni Scientific) to enable active scavenging of waste gases; alternatively, gas scavenging can be achieved through connection to a house vacuum system. Depilatory cream (1.5 g; Nair, Church and Dwight, Trenton, NJ) was applied to the abdomen of each mouse and worked into the fur for 30 s by using a cotton-tipped applicator, followed by an incubation period of 80 s (Figure 2 B). The cream was removed by using a stream of distilled water from a rinse bottle for 20 s. The depilatory process was repeated to remove any remaining hair, by using a 45-s application and 30-s cream incubation period, followed by a 10-s rinse (Figure 2 C). The shorter times for the second application were due to the small amount of hair remaining after the first procedure. After rinsing, the mice were gently blotted with gauze to remove excess cream, hair, and water before being returned to their housing. All steps were done in parallel. All 8 groups of mice for the MDS underwent the same procedure.

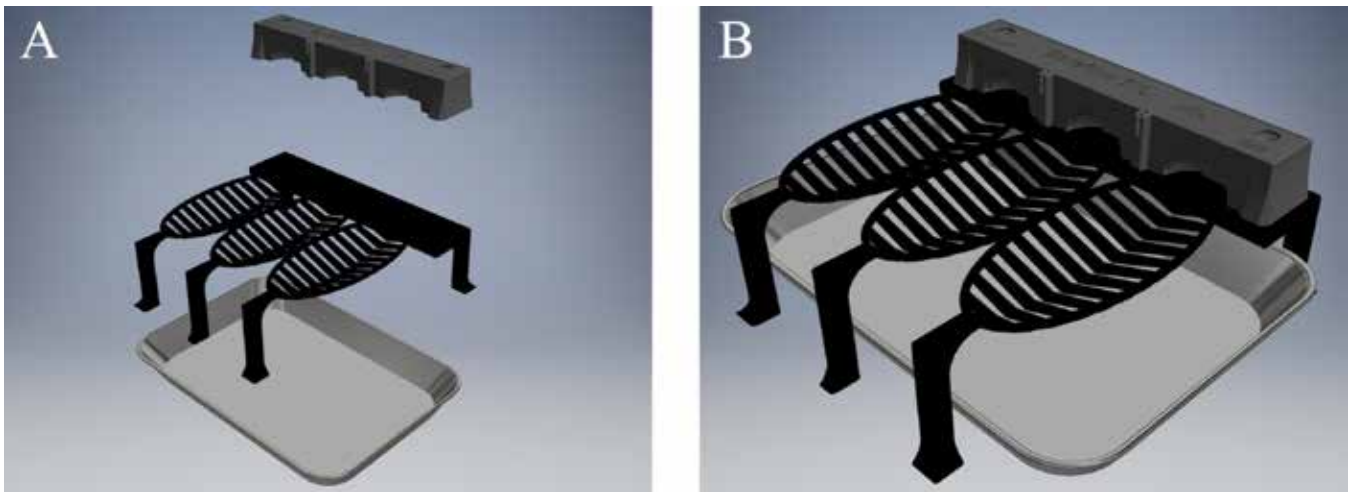

Figure 1. CAD design of Mouse Depilation Station. (A) CAD drawings of components of the MDS, including the 3D-printed slat staging area, anesthesia manifold, and waste collection tray, which is shown in uncoated aluminum for increased visualization. (B) CAD representation of the complete assembly.

Figure 2. Visualization of the mouse depilation procedure and setup. (A) Mice in supine position in the MDS, with noses placed in the anesthesia ports of the anesthesia manifold. (B) Incubation of the depilatory cream on the mouse abdominal area after 30 s of application. (C) Depilated mice abdominal region after the second incubation and removal of the depilatory cream through water jetting.

For sequential depilation, the same protocol was followed as for the MDS, except that mice were treated individually. All materials were sprayed with 70% ethanol, wiped down, placed in a laminar flow hood, and sterilized for 30 min by using UV light prior to use. A single mouse was anesthetized through short-term exposure to isoflurane prior to being moved to the MDS. The mouse was then depilated on the MDS by using the same procedure before being returned to its housing. This process was repeated for the final 5 mice of the group, for a total of 6 time measurements for the sequential cohort.

All mice were SPF for the following pathogens: *Aspiculuris tetraptera*, *Syphacia muris*, *Syphacia obvelata*, *Myocoptes*, *Radifordia* or *Myobia*, *Spironucleus muris*, *Entamoeba muris*, mouse hepatitis virus, minute virus of mice, mouse parvovirus, Theiler murine encephalomyelitis virus, epizootic diarrhea of infant mice, *Mycoplasma pulmonis*, *Ectromelia*, lymphocytic choriomeningitis virus, pneumonia virus of mice, reovirus type 3, and Sendai virus. The animals were housed in groups of 3 to 5 mice per cage in IVC (Allentown Caging, Allentown, NJ), with automated watering in an AAALAC-accredited nonbarrier vivarium of the University of Notre Dame. As part of standard husbandry practices, room temperatures are maintained at 69.0 ± 1.1 °F (20.6 ± 0.5 °C), with a 12:12-h light:dark cycle and 10 to 15 room-air changes hourly. The mice were fed a commercial rodent chow (diet 2918, Envigo Teklad, Madison, WI). The animals were housed on corncob bedding (Bed-o'Cobs, The Andersons, Maumee, OH) and provided with paper huts (Shepherd Specialty Papers, Watertown, TN). All listed procedure times are approximate and depended on the specific animal. The procedures were timed by using a stopwatch, with time points taken after anesthesia induction, each application of cream, each incubation of cream, each rinse, return of animals to housing, and cleanup of the work area. All hair removal was completed by using depilatory cream only, without shaving or clipping.

Statistical analysis. Statistical analysis was used to compare the MDS depilation procedure with the sequential method. The overall procedure times for the groups of 3 mice (n = 8) depilated on the MDS were divided by 3 to get a per-mouse time. The procedure time for each mouse from the sequential procedure (n = 6) was logged also. The average time per mouse was compared by using an unpaired, parametric, 2-tailed t test with Welch correction. The same methodology was used to compare procedure times for groups of 3 mice (n = 8) on the MDS with the summed time of 3 individual mice with the sequential method (n = 2). Statistical analyses were done by using Prism software (version 7, GraphPad, La Jolla, CA).

Results

Mouse depilation questionnaire. An online, 13-question survey (Table 1) was distributed to researchers in academia and industry to understand mouse depilation procedures. Results from 22 respondents, representing 16 distinct companies and academic institutions, were recorded. Nair (Church and

Table 1. Questionnaire responses regarding laboratory depilation procedures

Question	Option 1 (%)	Option 2 (%)	Option 3 (%)	Option 4 (%)	Option 5 (%)
Which depilatory cream do you use?	Nair	Veet	Magic Shave	Other	
	59%	27%	5%	9%	
How long do you massage in the cream?	<30 s	30–60 s	>1 min	not applicable	
	45%	36%	5%	14%	
How long do you leave the cream on the mouse?	<30 s	30–60 s	1-2 min	>2 min	not applicable
	5%	36%	36%	23%	0%
How do you remove the cream?[a]	Wash (water)	Wash (other)	Wipe (dry)	Wipe (wet)	Other
	34%	0%	21%	45%	0%
How many times do you do your process per mouse per session?	1	2	3+		
	86%	14%	0%		
Do you keep mice under continuous anesthesia the entire time?	Yes	No			
	91%	9%			
What is your typical reason for depilation?	Imaging	Surgery	Both	Other	
	59%	9%	27%	5%	
Do you take any other steps to remove fur?[b]	Clip	No	Wax or pluck		
	59%	36%	5%		
What part of the mouse do you typically depilate?[c]	Hindquarters	Abdomen	Thorax and neck	Back	Entire body
	26%	37%	15%	19%	4%
Do you typically depilate mice individually or in parallel?	One at a time	In parallel			
	55%	45%			
How many people typically are needed to perform the depilation process?	1	2	3+		
	73%	18%	9%		
How many mice do you depilate on average?	3 or fewer	4–10	10–20	21 or more	
	5%	50%	27%	18%	

Each question allowed the respondent to select the range that was most applicable to their experimental method. Percentages reflect the number of respondents who gave the indicated answer (total responses, 22).
[a]Multiple selections were allowed (total responses, 29).
[b]Respondents were allowed to enter their own answers when they used additional steps.
[c]Respondents were allowed to enter their own answers and provide multiple regions, totaling 27 anatomic regions.

Dwight, Ewing Township, NJ) is the most commonly used depilatory cream (59% of respondents), followed by Veet (Reckitt Benckiser, Slough, Berkshire, United Kingdom; 27%). Most (81%) researchers massage the cream into mice for 60 s or less. Incubation times primarily ranged from 30 to 60 s (34%) and 60 to 120 s (34%). Approximately 45% of respondents remove fur and cream by using a wet wipe; 34% performed a water rinse. The vast majority (86%) of researchers apply cream only once per animal, whereas 14% repeat the process. However, 64% of respondents use creams in conjunction with another depilation method, such as clipping or waxing. The abdominal regions and hindquarters are the regions most commonly depilated, with 4 researchers reporting that they commonly dehair multiple anatomic regions. For more than half of respondents, a single person depilates mice in a sequential fashion during a given experiment, which included 4 or more mice 95% of the time.

Mouse depilation. The abdomens of 8 groups of 3 mice each were depilated by using the MDS. We followed our standard method for removing abdominal fur from BALB/c and C57BL/6 mice, with all procedure time guidelines approximated and dependent on the specific animals. The mice underwent anesthesia for 2 min prior to transfer to the MDS. Depilatory cream was applied for 30 s per mouse, followed by an 80-s incubation and 20 s of jetted water for removal of hair and cream (Figure 2). The first animal was ready for rinsing approximately 20 s after cream was applied to the last mouse, while the other mice continued their cream incubation. The second and third mice each required an additional 80 s of wait time only, even though the entire protocol for the first application was 130 s per mouse, due to parallel steps. After each mouse was rinsed, a second round of cream application and rinsing was conducted to remove any residual hair.

Table 2. Using a sequential method of depilation compared with the Mouse Depilation Station

	Sequential method			Mouse Depilation Station		
	1 mouse	3 mice	24 mice[a]	1 mouse	3 mice	24 mice
Total time (s)	359	1077	8616	337	695	5560
Time (s) per mouse	359	359	359	337	232	232
Total time saved (s)	—	—	—	22	382	3056

[a]The time for 24 mice to be depilated by using the sequential technique was extrapolated from the time to depilate 6 mice by using that technique.

Overall, the average total procedure time for depilating a group of 3 mice was 695 s (approximately 11.6 min), with a standard deviation of 62 s, for an average of 231 s (3.9 min) per mouse. These findings compared favorably with those for a sequential depilation procedure that used the same time parameters for anesthesia induction, cream application, cream incubation, water rinse time, and clean up. Six mice were depilated sequentially by using this procedure, with an average procedure time of 359 s (5.98 min) and a standard deviation of 40 s. On a per-mouse basis, the sequential procedure took 55% longer than using the MDS ($P < 0.0005$). In addition, the procedure time for 3 mice on the MDS was faster ($P < 0.005$) than the summed time for 3 mice depilated by using the sequential protocol. The total time to depilate all 24 mice by using the MDS was just under 93 min, whereas depilating 24 mice by using the sequential process was projected to take more than 143 min, on the basis of the total time to clean 6 mice. Using the MDS for 3 and 24 mice resulted in time savings of 6.4 and 50.9 min, respectively, relative to the time required for a sequential procedure for the same number of animals (Table 2).

None of the 30 animals tested experienced complications before, during, or after depilation. No mice showed any signs of discomfort or stress during the procedure, including the rinsing steps, which might cause fluctuations in body temperature.

Discussion

For many surgical procedures and in vivo optical imaging experiments, the hair of the animal under study must be removed for easier access, improved resolution, and decreased chance of infection. To address the inefficiency and messiness of using depilatory creams, we created the Mouse Depilation Station to achieve benefits relative to conventional benchtop practices. The results of the survey revealed that although benchtop depilation may be widely used, methodology is inconsistent between researchers. For example, most researchers massage in creams for 60 s or less and incubate for just under 90 s, the total time of cream exposure (the sum of application and incubation times) can vary widely: 45% of respondents used less than 1 min of total exposure time, whereas 32% of respondents used at least 2 min of total exposure time. This variation likely reflects the use of different brands of hair removal cream, which may differ in effectiveness based on variations in the composition of active ingredients, and the types of, sizes of, and areas of interest on the animals. Maximum exposure times might be less than 1 min or exceed 6 min, depending on the researcher. In addition, the bias toward researchers using mice for imaging rather than surgery may be due to the respondent population, which was connected to the authors through the medical imaging community.

Overall, cream exposure time varied by the anatomic region of interest, brand of agent used, and preclipping steps. On average, cream was applied for 96 s on the hindquarters, 105 s on the back, 116 s on the thoracic region, and 145 s on the abdomen. The total average exposure time was 105 s for Veet (Reckitt Benckiser) compared with 138 s for Nair (Church and Dwight). This difference may imply that Veet works more quickly than Nair; this hypothesis is further supported by comparing the total cream exposure time for procedures that do not use any other depilation technique. When Nair was used exclusively, the average exposure time was 186 s, compared with 110 s for Veet only. In addition, Nair is used by all 3 respondents who typically repeat their depilation procedure, although this association may merely reflect the small sample size. The combination of creams with preliminary clipping decreased the total cream exposure time from an average of 157 s to 101 s. The total time needed for clipping was not assessed in this survey; thus, the relative efficiency of a clipping–cream compared with a cream–cream process could not be analyzed directly. However, clipping does take time and must be done sequentially. Perhaps a cream–cream process, done in parallel, saves time overall. Furthermore, one underlying rationale for the clipping–cream process is to avoid or limit the messy cream steps. The MDS was designed to decrease obstacles in the cream process and might encourage researchers to move to a process with multiple cream steps, thereby reducing reliance on clippers and their associated complications, such as specimen abrasion and clipper maintenance. Nevertheless, researchers who choose a clipping–cream strategy likely will benefit from the time savings of doing the procedure on 3 mice in parallel on the MDS.

One potential benefit of the MDS is amount of time saved during depilation. The tested mouse depilation procedure was a 6-step process comprising 2 rounds each of cream application, incubation, and jetted-water rinsing, along with initial anesthesia induction and final cleanup. Researchers completed 3 mice in less than 12 min (average of 3 min 52 s per mouse) by using the MDS; a similar task would require nearly 18 min by using the sequential method. In a typical study involving 24 mice, the use of the MDS would save more than 50 min relative to the sequential method (Table 2), reducing the total procedure time by more than 33%. The MDS procedure time for the 24 mice was confirmed through experimentation, whereas the sequential time was extrapolated from the average time to depilate the 6 mice in the cohort, given that in that procedure, depilation of a single mouse was repeated a total of 6 times. By grouping the mice into cohorts, the total number of mice subject to depilation was decreased from 48 to 30. Conceivably, the time savings from the MDS procedure would be even greater when compared with a conventional, benchtop depilation procedure, because the MDS facilitates easy and rapid cleanup relative to the use of disposable, absorbent pads. These pads can leak, require frequent replacing, and can be difficult to secure to the work surface. The application, incubation, and rinse times described were used for the abdominal area of BALB/c and C57BL/6 mice; thus these parameters likely vary with mouse strain, size, and anatomic site of interest. The entire procedure was conducted in a laminar flow hood to emphasize the compatibility of the MDS with immunocompromised mice strains, including NOD and NSG.

The use of the MDS provided several advantages in addition to time savings relative to sequential hair removal. First, the overall cleanliness of the process decreased time, inconvenience, and waste during the procedure. For example, some labs rinsed animals over absorbent pads, which must be changed between mice. Other groups used wet paper towels to remove cream from each animal—a process that is inefficient and generates waste. When used with a water jet from a squeeze bottle, the waste collection pan of the MDS took only 30 s to empty and rinse before the next 3 mice were loaded. Another benefit of the MDS was the integrated anesthesia manifold, which facilitated efficient anesthetic delivery and waste gas scavenging, virtually eliminating exposure of personnel to anesthetic gas while keeping the animals under constant and consistent anesthesia. Because the manifold created a pocket of anesthetic gas within each nose cone, mice can experience slight movement due to the application of cream or rinsing without concern of breaking the seal and causing waste gas exposure, which might occur in traditional breathing circuits.

The MDS enabled clean, efficient, and safe hair removal by providing a platform to customize and batch-process mouse depilation. The integrated anesthesia manifold enhanced the safety of anesthetic gas delivery to the murine subjects, and the parallel processing of 3 mice saved valuable time relative to benchtop strategies that prepare mice individually. The waste collection system improved the cleanliness of the process, ultimately decreasing paper refuse and researcher inconvenience. The survey results demonstrated the variability in depilation procedures, including reagents, techniques, and timing, between researchers. Therefore, the MDS may provide a convenient platform for which depilation protocols could be established and reproduced across research labs and fields of study.

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