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The Effectiveness of Hot Bead Sterilization in Maintaining Sterile Surgical Instrument Tips across Sequential Mouse Surgeries

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One strategy commonly employed for rodent surgeries is a "tips-only" surgical technique, which restricts the surgeon to using only the sterile working ends of the surgical instruments to manipulate the surgical field and sterilizes instrument tips with a hot bead sterilizer between consecutive rodents. Despite the common use of the "tips-only" technique, research is lacking on the number of sequential surgeries for which the same set of hot bead-sterilized instruments can be used before introducing bacterial contamination. We performed serial mouse surgeries using the "tips-only" technique under 3 different conditions (aseptic, fur contamination, or cecal contamination) and assessed aerobic bacterial growth before and after each round of hot bead sterilization. Instrument tips showed an increasing probability of contamination of at least one instrument in a series of consecutive surgeries. The probability that all surgical instrument tips in the series were sterile after hot bead sterilization fell by 4% for each surgery involving inadvertent or fur contamination and by 11.5% for each surgery with contamination for all surgical types combined (including entering the gastrointestinal tract). Based on our results, hot bead sterilization is not adequate for surgeries associated with gross contamination. Under our experimental conditions and assuming independence of outcomes between consecutive surgeries, up to 5 surgeries associated with minor or inadvertent contamination could be performed in series with a probability higher than 80% that all instrument tips were sterile for all surgeries. A case-by-case risk assessment should be conducted to derive institutional guidelines for the maximal number of surgeries that can be performed in sequence using the "tips-only" technique with hot bead sterilization of the same set of surgical instruments between surgeries. Full sterilization of instruments after every surgery provides the greatest confidence in maintaining sterility.

Abbreviations: CDC, Centers for Disease Control and Prevention; COVID-19, Coronavirus 2019; RLU, relative light units; SOP, Standard Operating Procedure; TNTC, too numerous to count

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The purpose of aseptic technique in rodent surgery is to reduce microbial contamination to the lowest possible level and prevent infection.[1] To achieve asepsis, the subsequent steps are followed: sterilization of surgical instruments and materials, reduction of bacterial load on skin with antiseptics, creation of a sterile working field around the surgical incision, preparation of the surgeon with proper personal protective equipment, and sanitization of the procedure area.[3] The *Guide for the Care and Use of Laboratory Animals*[28] emphasizes the importance of aseptic technique, stating that "inadequate or improper technique may lead to subclinical infections that can cause adverse physiologic and behavioral responses affecting surgical success, animal well-being, and research results." While the importance of asepsis is universally understood, the exact practices that constitute aseptic technique in rodent surgeries are not as clear.[8,19] In the research setting, rodent surgeries of brief duration are often performed in series by one person with minimal supplies. Sterilizing instruments between surgeries can pose a challenge because traditional methods of sterilization such as autoclaving or ethylene oxide are time consuming and inefficient for serial rodent surgeries.

As an alternative to traditional sterilization methods, hot bead sterilizers offer a fast and safe method of sterilization between rodent surgeries when using the "tips-only" technique.[1] Hot bead sterilizers use dry heat, act only on the tips of instruments between surgeries, and should therefore not be used as an initial means of sterilization.[3] The "tips-only" technique restricts surgeons to using only the sterile ends of surgical instruments to manipulate the surgical field. The surgeon can therefore wear clean, nonsterile exam gloves[16] and directly manipulate nonsterile objects during surgery. This technique is useful when working alone and manipulation of nonsterile objects is necessary.[16] Many institutions[6,7,22,24,29,30,34] have Standard Operating Procedures (SOPs) that provide guidance to research staff using the "tips-only" technique. While some of these SOPs recommend a maximum number of surgeries (for example up to 5) allowed in series using the same set of initially autoclaved instruments, to our knowledge no published studies have evaluated the efficacy of the hot bead sterilizer for use in sequential rodent surgeries with regard to preventing aerobic bacterial growth in the clinical setting.

Limited regulatory guidance exists on surgical instrument sterilization between surgeries in rodent research. Historic use of hot bead sterilizers has been previously reported in the

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dental profession.[12,14,31] The CDC's Guideline for Disinfection and Sterilization in Healthcare Facilities,[37] however, cautions against their use due to the risk of infection from the potential failure to sterilize dental instruments when using hot bead sterilizers. From the *Guide*: "Bead or dry heat sterilizers are an effective and convenient means of rapidly sterilizing the working surfaces of surgical instruments, but care should be taken to ensure that the instrument surfaces have cooled sufficiently before touching animal tissues to minimize the risk of burns."[28] Regulatory guidance does not address the number of surgeries that can be performed sequentially or the type of rodent surgeries that hot bead sterilization is best suited for.

The hot bead sterilizer has been validated as an effective means of sterilizing the tips of rodent surgical instruments after inoculation with common bacteria found on murine skin.[4] This method of sterilization has yet to be tested in a clinical setting in which contamination of the instrument tips with organic debris is likely to occur. Based on current SOPs and institutional policies found through an internet search,[6,7,22,24,29,30,34] we hypothesized that hot bead sterilization would be effective at eliminating aerobic bacterial growth on the tips of contaminated surgical instruments for up to 5 rodent surgeries. The present study aims to provide evidence-based guidance on the decontamination of rodent surgical instruments when using the "tips-only" technique with hot bead sterilization between sequential surgeries.

Materials and Methods

Animals. This study used 104 male and female mice that had been previously identified as cull animals and are representative of the strain, age, and genetic diversity in the vivarium setting. Mice were housed in an AAALAC International-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals*[28] and the Public Health Service Policy on Humane Care and Use of Laboratory Animals.[26] Colony health was monitored by use of a quarterly dirty-bedding sentinel system; these sentinels remained negative for the following infectious agents during the time period during which the study was conducted: Mouse Hepatitis Virus (MHV), Mouse Parvovirus (MPV), Minute Virus of Mice (MVM), Lymphocytic Choriomeningitis Virus (LCMV), Sendai Virus (SV), Pneumonia Virus of Mice (PVM), Epizootic Diarrhea of Infant Mice (EDIM), Theiler Mouse Encephalomyelitis Virus (TMEV), Ectromelia Virus (Mouse Pox), Mouse Adenovirus (MadV), Mouse Reovirus (Reo), *Mycoplasma pulmonis*, endoparasites (pinworms), and ectoparasites (fur mites). Prior to being identified for this study, cull mice were housed based on institutional policy at a density of 1 to 5 adult mice per IVC (Lab Products Item # 75031-GAM, Seaford, DE) on a paper-based bedding (The Andersons ALPHA-dri + PLUS, Maumee, OH).

The housing rooms were maintained under controlled conditions (room temperature: 68 to 76 °F [20 to 24°C]; relative humidity: 30% to 70%; 12:12-h light:dark cycle; 10 to 15 room air changes per hour) and received a commercial pelleted laboratory rodent diet, 5LOD or 5LJ5 (Lab Diet, St Louis, MO), and access to water ad libitum. Mice were euthanized prior to surgery following the IACUC-approved SOP on euthanasia. The study was performed in accordance with institutional policy, which does not require IACUC approval for postmortem animal use.

Study design. We evaluated hot bead sterilization of surgical instruments when performing serial laparotomies under 3 different conditions: strict aseptic technique, a deliberate break in aseptic technique by running the tips of instruments through fur after skin closure, or a deliberate break in aseptic technique by dipping the tips of instruments into cecal contents during surgery. Surgeries were performed on 14 or 15 mice per series, using a new autoclaved set of surgical instruments (forceps, scissors, and needle holders) at the beginning of each series. Seven series of surgeries (104 total surgeries) were performed, with 3 series (45 surgeries) using strict aseptic technique, 3 series (45 surgeries) with deliberate fur contamination (Figure 1 A), and one series (14 surgeries) with deliberate cecal contamination. Some series had culture data for fewer than 15 mice due to COVID-19-related supply shortages (flocked swabs were not available for purchase).

Surgical space. All surgical procedures were performed on a clean exam table in a dedicated animal procedure room. Surgical preparation was divided into 2 locations: one procedure room for euthanasia and shaving, and an adjacent animal procedure room for aseptic preparation and surgery. Each preparation station was covered with a clean absorbent pad. The rooms were reserved solely for this study and free of any traffic flow for the duration of each series.

Surgical materials and procedure. Surgical instruments and supplies were steam autoclaved (STERIS V120, sterilization cycle at 250-260 °F [121-127°C]) before the start of each series of surgeries. Sterilization indicators (Propper Smalstrip chemical indicators, Long Island City, NY) were included in each pack of autoclaved instruments along with autoclave color-indicator tape, and autoclave sterilization was verified monthly using STERIS Verify Biologic Indicators. Mice were euthanized according to the 2020 AVMA Guidelines for the Euthanasia of Animals[21] by using 100% CO_2 gas followed by cervical dislocation immediately before undergoing the surgical procedure. The surgical site was clipped and aseptically prepped by an assistant with 3 alternating applications of 4% chlorhexidine scrub and 70% isopropyl alcohol wipes, starting in the center of the site and working outwards in a circular pattern. The surgeon wore hair tied back, a surgical mask and a gown, and a new pair of clean exam gloves donned prior to each surgery.[16] The mouse was then placed on a clean, dry, absorbent pad in dorsal recumbency in front of the surgeon. Clear plastic wrap (Glad® Press'n Seal®) (Figure 1 B) was used as a sterile surgical drape and a fenestration was made in the center of the prepared area of the animal.[11] For the aseptic and fur contamination series, a 2-centimeter incision was made along the ventral midline of the mouse with scissors, first through the skin and subcutis, then through the abdominal wall. The spleen was exteriorized, and the gastrosplenic ligament broken with a cotton tipped applicator. The spleen was gently grasped with forceps while the splenic artery was cauterized using a fine tip cautery pen (Jorgensen Laboratories, Bovie Medical Corporation, Clearwater, FL). The abdominal wall was sutured closed and wound clips applied to the skin. For the cecal contamination series, a 2-centimeter ventral midline incision was made with surgical scissors, first through the skin and subcutis, then through the abdominal wall. The cecum was exteriorized, and a small incision made on the antimesenteric surface. The tips of the grasping surfaces of the forceps and needle drivers were exposed to the cecal contents and removed. The abdominal wall was sutured closed and wound clips applied to the skin. The average surgery time was approximately 10 min for all surgeries. The same suture pack (synthetic monofilament, 3-0 or 4-0) was used until depleted, with 3 to 4 packs used per series. The same surgeon performed all surgeries throughout the study.

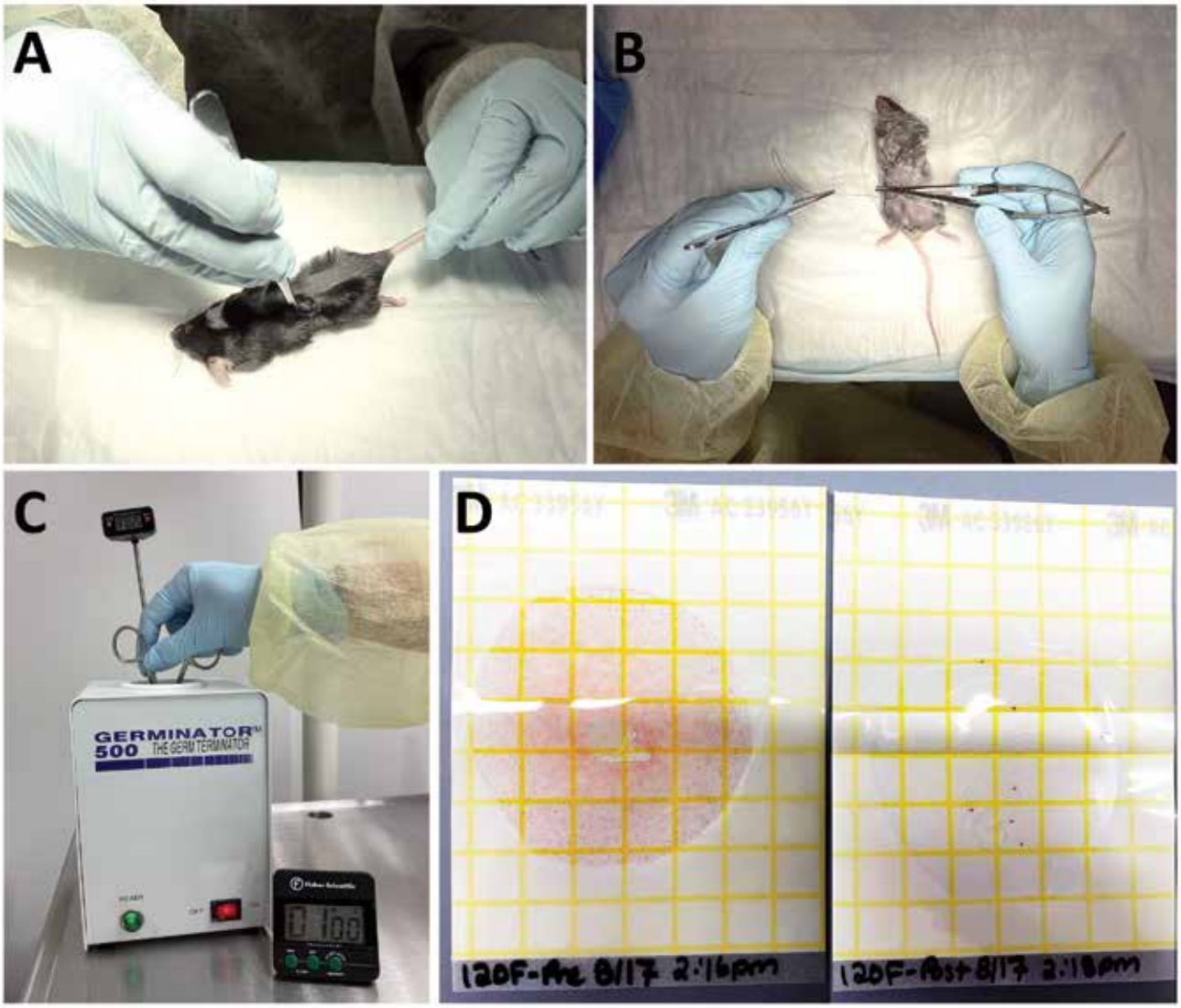

Figure 1. (A) Surgeon demonstrating fur contamination technique. (B) Animal is draped with Glad Press'n Seal while the surgeon closes the abdominal cavity using the "tips-only" technique. (C) Surgical dressing forceps and Castroviejo needle holders (D) Germinator 500 with forceps and needle holders being sterilized. (E) 3M Petrifilm plates both pre- (left plate) and post- (right plate) sterilization. Plate on the left shows culture colony counts described as TNTC.

Sampling methods. Separate swabs (BD Liquid Amies Elution Swab (ESwab) Collection and Transport System, Becton-Dickson, Spark, MD) were used for each sample obtained. Instrument tips were swabbed by rolling one swab up and down the inner surface going against the serrations on one designated side of the instrument twice, followed by sliding the swab along the serrated surface. Dressing forceps and Castroviejo needle holders (Figure 1 C) were swabbed for culture immediately after opening the sterile pack to confirm the effectiveness of autoclaving prior to beginning each series of surgeries. For the first 2 of the 7 series (one aseptic series and one fur contamination series), instrument tips were swabbed only after exposure to hot bead sterilization; for each instrument, one grasping surface was swabbed for bacterial culture and the other surface was swabbed for ATP analysis (Neogen AccuPoint ATP Sanitation Monitoring System, Lansing MI). For the remaining 5 series, upon completion of each surgery, one tip of each instrument was swabbed for bacterial culture before hot bead sterilization and the other tip of each instrument was swabbed for bacterial culture after hot bead sterilization. The tip of each instrument that was sampled before and after hot bead sterilization was consistent for all surgeries. All samples were swabbed by the same individual, who was blind to the surgical technique being performed.

Hot bead sterilization. A new glass bead refill pack (GER 5289, Braintree Scientific, Braintree MA) was placed before beginning the study and used throughout. Based on manufacturer recommendations for hot bead sterilization, after each surgery (and after the pre-sterilization swab sample collection), the instruments were cleaned of visible debris using sterile saline, placed in the hot bead sterilizer (Germinator 500) for one minute at a depth of 1.5 in into the glass beads, and allowed to cool for 30 s (Figure 1 D). Bead temperature was monitored throughout all procedures with a digital thermometer (Fisherbrand Traceable Digital Thermometers with Stainless-Steel Stem catalog number: 15-077-54, Pittsburg, PA) to confirm that temperature was maintained at 500 °F ± 50 °F (260 ± 10°C).

Culture method. Bacterial culture swabs were vortexed for approximately 5 s in one mL of liquid Amies media was then pipetted onto aerobic count plates (3M Petrifilm Aerobic Count Plates, Saint Paul, MN). The plates were placed in an incubator at 37 °C for 48 h. At the end of the incubation period, the total colony forming units (cfu) were counted for each plate. Any bacterial growth (cfu > 0) was considered nonsterile. Any bacterial growth over 500 cfu (Figure 1 E) was considered too numerous to count (TNTC) and a value of 500 was used for analysis.

Statistical analysis. To assess the risk of failure of hot bead sterilization to sterilize a contaminated instrument over sequential surgeries, χ^2 tests of proportions were calculated with a Wilson confidence interval for all series combined and for all series combined except cecal contamination. Hot bead sterilization failure was defined as failure to eliminate all bacterial growth after sterilization if pre-sterilization bacterial growth (cfu > 0) had been identified for an instrument. Only positive pre-sterilization cultures were used in the analysis, and surgeries in which the pre-sterilization value was zero were not included in proportion analyses. We assumed that the culture history of any given instrument tip and the extent of contamination would not affect subsequent culture results in the series. As such, each sterilization of a contaminated instrument was treated as an independent event. Under this assumption, p was the probability that a contaminated instrument would remain contaminated after hot bead sterilization. Suppose that k consecutive surgeries are performed with an instrument that was contaminated prior to hot bead sterilization. Let p_k denote the probability that this instrument was sterile before each of these k surgeries. Then the independence assumption permits us to calculate the probability of sterility as $p_k = (1 - p)^k$. χ^2 tests of proportions were used to determine whether culture data could be combined across instrument types and/or surgical technique. Descriptive statistics and a posthoc unpaired t test to compare ATP data between surgical instrument types were performed in GraphPad Prism software (GraphPad, Version 9.0.0, San Diego, CA). P values less than 0.05 were considered statistically significant.

A repeated measures analysis of the hot bead sterilization was performed to compare cecal contaminated surgeries with aseptic or fur-contaminated procedures, with no assumptions made about the correlation structure of outcomes in the same series. The results after each sterilization procedure were dichotomized as contaminated or sterile. We then ran a generalized estimating equation analysis of these results using a logistic link function and a binomial random component.[38] The Huber–White (robust) sandwich estimator was used to estimate the variance-covariance matrix of outcomes within the same series of experiments.[36] The preceding statistical analyses were performed with Stata software (Version 17.0 StataCorp LLC, 2021, College Station, TX).

Results

Across all experimental groups, no bacterial growth was found on autoclaved instruments before the start of each series. Culture data are shown in Table 1. The 2 series that included measurement of ATP showed no apparent relationship between ATP and culture data when both were measured after sterilization (Table 2). ATP measurement was therefore not assessed in subsequent series in favor of including both pre- and post-sterilization bacterial culture.

Risk of hot bead sterilization failure over time. No significant difference was found in the proportion of hot bead sterilization

Table 1. Individual culture results for all groups with cultures performed before and after sterilization. Summary of aerobic bacterial cultures reported in cfu for all series performed with before and after sterilization. TNTC= too numerous to count (cfu > 500).

		No. of aerobic bacteria colonies obtained (cfu)			
		Before Sterilization		After Sterilization	
Group	Mouse no.	Forceps	Needle Drivers	Forceps	Needle Drivers
Aseptic technique	1	1	6	0	1
(2 series)	2	1	3	0	0
	3	1	0	0	0
	4	0	0	0	0
	5	1	0	0	0
	6	0	0	0	0
	7	0	1	0	0
	8	1	2	0	0
	9	0	0	0	0
	10	0	15	0	0
	11	0	0	0	0
	12	0	0	0	0
	13	0	0	0	0
	14	0	2	0	0
	15	0	0	0	0
	16	0	0	0	0
	17	0	0	0	0
	18	0	0	0	0
	19	0	0	0	0
	20	0	0	0	0
	21	0	0	0	0
	22	0	0	0	0
	23	0	0	0	0
	24	0	0	0	0
	25	0	0	0	0
	26	0	0	0	0
	27	0	1	0	0
	28	0	0	0	0
	29	0	0	0	0
	30	0	0	0	0
Fur contamination	1	2	0	0	0
(2 series)	2	0	0	0	0
	3	1	0	0	0
	4	TNTC	168	0	0
	5	TNTC	TNTC	7	0
	6	396	8	0	0
	7	6	1	0	0
	8	6	0	0	0
	9	2	0	0	0
	10	3	1	0	0
	11	4	0	0	0
	12	0	16	0	0
	13	4	2	0	0
	14	26	1	0	0
	15	3	2	0	0
	16	0	0	0	0
	17	10	0	0	0
	18	0	0	0	0

Table 1. Continued

		No. of aerobic bacteria colonies obtained (cfu)			
		Before Sterilization		After Sterilization	
Group	Mouse no.	Forceps	Needle Drivers	Forceps	Needle Drivers
	19	44	11	0	0
	20	0	0	0	0
	21	TNTC	252	0	0
	22	197	123	0	0
	23	70	3	0	0
	24	134	4	0	0
	25	0	0	0	0
	26	1	0	0	0
	27	1	21	0	0
	28	0	0	0	0
	29	59	0	0	0
	30	1	0	0	0
Cecal contamination	1	TNTC	134	0	2
	2	TNTC	5	0	0
	3	TNTC	11	0	0
	4	TNTC	127	0	0
	5	41	5	0	1
	6	179	106	0	0
	7	TNTC	4	0	0
	8	TNTC	224	3	0
	9	TNTC	142	0	0
	10	TNTC	65	0	0
	11	TNTC	161	1	0
	12	TNTC	89	0	0
	13	89	21	0	2
	14	TNTC	112	1	1

failures between the forceps and needle drivers ($\chi^2(1) = 0.36$; $P = 0.55$), and subsequent analyses combined both instruments. Although we found no significant difference in the proportion of hot bead sterilization failures between surgical techniques ($\chi^2(1) = 1.46$; $P = 0.23$), we analyzed the data for all series combined and for all series combined excluding cecal contamination. For all surgical techniques combined, the probability that all contaminated instrument tips in the series were effectively sterilized by hot bead sterilization decreased by 11.5% (95% CI [6% to 20%]) after each surgery in series (Figure 2). For aseptic and fur contamination series combined, with the cecal contamination series excluded, the probability that all contaminated instrument tips in the series were effectively sterilized by hot bead sterilization fell by 4% (95% CI [3% to 13%]) after each surgery in series (Figure 3). In a repeated measures analysis, the odds of having nonsterile instrument tips after hot bead sterilization was 21.8 times higher in the cecal series as compared with the other series (95% CI [7 to 68]; $P < 0.0005$).

Surgical technique. Surgeries performed with either strict aseptic technique or fur contamination had similar proportions of nonsterile instrument tips after hot bead sterilization, with 3.3% (3 out of 90) and 1.2% (1 out of 85) of all instrument tips culturing positive for at least one cfu, respectively. The proportion of nonsterile instrument tips after hot bead sterilization for the cecal contamination series was 25% (7 out of 28 instrument tips). When considering clinical outcomes in terms of the number of surgeries starting with contamination of one or both instruments, 3 out of the 45 aseptic technique surgeries (6.7%) and 1 out of the 42 fur contamination surgeries (2.4%) had a positive bacterial culture for either instrument after sterilization. In contrast, 6 out of the 14 surgeries (42.9%) performed in the cecal contamination series had a positive bacterial culture of the forceps and/or needle driver after sterilization (Figure 4).

Surgical instruments. For the 2 series that we collected ATP data (Table 2), forceps (154 Relative Light Units (RLU) ± 42 SEM) had higher ATP measurements than needle drivers (54 RLU ± 27

Table 2. ATP and bacterial culture for each instrument after sterilization.

	Aseptic technique series				Fur contamination series			
	Forceps		Needle Drivers		Forceps		Needle drivers	
Animal	ATP (RLU)	Bacteria culture (cfu)	ATP (RLU)	Bacteria culture (cfu)	ATP (RLU)	Bacteria culture (cfu)	ATP (RLU)	Bacteria culture (cfu)
1	0	0	0	0	0	0	0	0
2	7	2	1	0	571	0	26	0
3	458	0	0	0	127	0	10	0
4	0	0	0	0	232	0	25	0
5	5	0	0	0	0	0	52	0
6	70	0	8	0	113	0	11	0
7	0	0	0	0	9	0	0	0
8	278	0	136	0	0	0	0	0
9	553	0	3	0	136	0	20	0
10	0	0	16	0	0	0	0	0
11	46	0	9	0	0	0	0	0
12	862	0	0	0	193	0	54	0
13	2	1	0	0	343	0	92	*
14	28	0	0	0	548	*	511	*
15	0	0	648	0	46	*	5	*

Summary of data collected from initial aseptic technique and fur contamination series (30 animals). Data reported includes aerobic bacterial culture (reported as cfu) and ATP (expressed as relative light units [RLU]) post hot bead sterilization for the surgical forceps and needle drivers. Data is shown for each animal in series (1-15).
* indicates cultures not taken due to supply shortages.

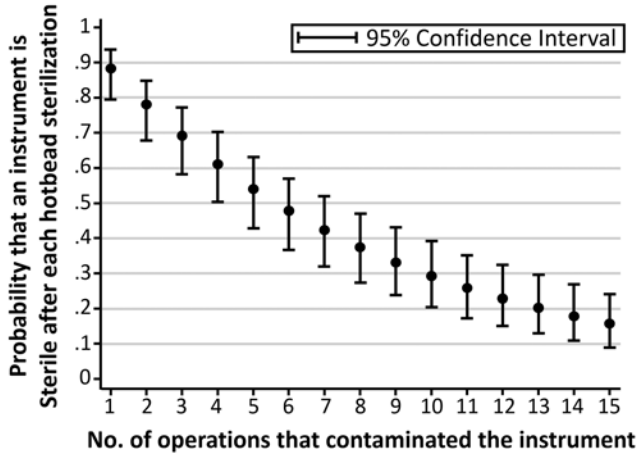

Figure 2. Risk of instrument tip contamination over a series of 15 rodent surgeries with the cecal contamination series included. The probability that all instrument tips were sterile after hot bead sterilization for all surgeries decreased by 11.5% per surgery when all surgical techniques were included (aseptic, fur contamination, cecal contamination). Probabilities for each surgery in series shown with 95% confidence interval. The 2 series lacking pre-sterilization culture data (shown in Table 2) were excluded from this analysis.

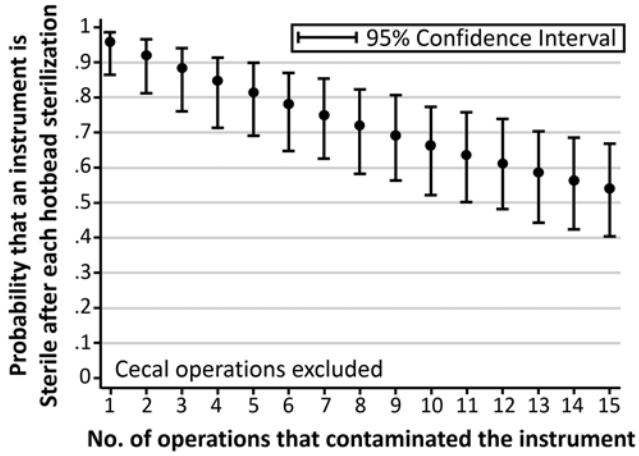

Figure 3. Risk of instrument tip contamination over a series of 15 rodent surgeries (cecal contamination series excluded). The probability that all instrument tips were sterile after hot bead sterilization for all surgeries decreased by 4% per surgery when only aseptic technique and fur contamination series were included. Probabilities for each surgery in series shown with 95% confidence interval. The 2 series without pre-sterilization culture data (shown in Table 2) were excluded from this analysis.

SEM; $P < 0.05$). However, the proportion of nonsterile instrument tips after sterilization across all series as measured by bacterial culture was similar between instrument types, with bacterial growth (cfu > 0) from 5.8% (6 of 103) of forceps tips and 4.9% (5 of 102) of needle driver tips, respectively.

Discussion

The objective of this study was to evaluate hot bead sterilization of contaminated surgical instrument tips between serial rodent surgeries and provide guidance on the maximum number of sequential "tips-only" surgeries that should be performed before sterilization of the entire instrument is necessary (for example, by autoclave). Based on current SOPs and institutional policies found through an internet search,[6,7,22,24,29,30,34] we hypothesized that hot bead sterilization would be effective at

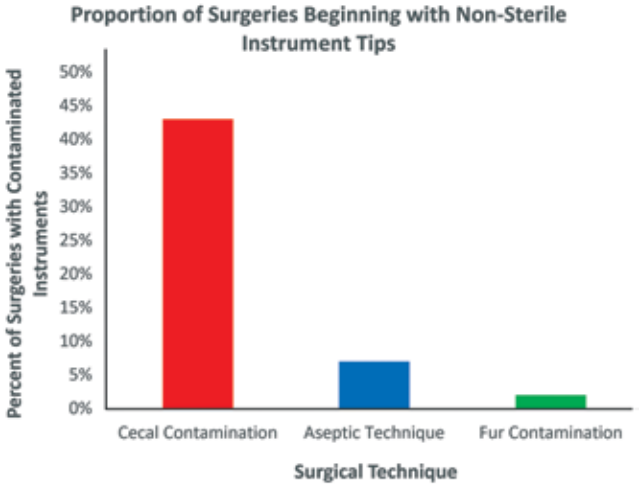

Figure 4. Proportion of surgeries beginning with nonsterile instrument tips (CFU > 0 for forceps and/or needle drivers) after hot bead sterilization across all 7 series.

eliminating aerobic bacterial growth on the tips of contaminated surgical instruments for up to 5 rodent surgeries. We found that our hypothesis was true only under some surgical conditions. While strict surgical asepsis is expected, most surgical infections are due to common skin commensals.[8,15,20] To encompass different levels of contamination, we evaluated the efficacy of the hot bead sterilizer between surgeries that followed 3 techniques: splenectomy using strict aseptic technique, splenectomy followed by a deliberate break in asepsis produced by running the instruments through fur, or a laparotomy with cecal puncture and contamination of the instrument tips with cecal contents. We found that we could perform up to 5 sequential surgeries associated with inadvertent or fur contamination with a higher than 80% probability that all surgical instrument tips were sterile after hot bead sterilization. However, when we included data from the cecal contamination series, the hot bead sterilizer had an 11.5% failure rate, such that no more than one surgery could be performed before the probability fell below 80% that both instrument tips were sterile. Hot bead sterilization did not reliably eliminate aerobic bacterial growth when the instrument tips were contaminated with cecal contents, as 6 of 14 surgeries (43%) were performed with nonsterile instrument tips. We therefore do not recommend the hot bead sterilizer for use in sequential surgeries with a high risk of heavy contamination, such as those involving entry into the gastrointestinal tract.

When using hot bead sterilization between surgeries performed with the "tips only" technique, our results indicate that with each additional surgery performed in series, the risk increases that at least one surgery in the series will be performed using a contaminated instrument tip, and therefore the probability that each sequential surgery begins with sterile instruments is lower. The purpose of achieving and maintaining asepsis during surgical procedures is to reduce the risk of postoperative infection.[8] Infections decrease animal welfare and can lead to surgical site dehiscence, which in turn can negatively affect research parameters of interest by influencing the animal's physiology.[9,28] Because no published studies have tested the efficacy of hot bead sterilization to provide guidance on its use in sequential rodent surgeries, this study provides the first data to inform evidence-based decisions about the maximum number of rodents that can undergo surgery safely per pack of autoclaved instruments with hot bead sterilization. Based on

the 4% failure rate of the hot bead sterilizer for surgeries with minor or inadvertent contamination, the use of no more than 5 animals in series with the same autoclaved pack will maintain a probability above 80% that all instrument tips are sterile for all 5 surgeries. This 80% probability assumed independence in contamination of surgeries in the same series. If this assumption is incorrect, the correlation between contamination in the same series remains positive, and the probability of 5 consecutive surgeries with sterile instruments will be less than 80%. Therefore, the 80% estimate is a best-case scenario. While some institutional policies either allow more than 5 sequential surgeries[17] or do not specify a maximum number,[2,18] our recommendation of allowing up to 5 surgeries in series is consistent with the majority of institutional policies currently available online, which specify no more than 4 to 6 animals be used per sterilized pack.[6,7,22,24,29,30] If instruments must be sterile with 100% certainty, then hot bead sterilization should not be used, and instruments should be autoclaved before each surgery, particularly if the surgery involves a high risk of inadvertent or fur contamination.

We recommend performing a case-by-case risk assessment when determining the maximum number of "tips-only" surgeries to perform in series using hot bead sterilization. This risk assessment should include, but not be limited to, surgical procedure classification (major or minor), duration of surgery, and the surgeon's level of experience. A risk assessment of contaminated surgical instruments by the World Health Organization (WHO) lists 3 factors involved in transferring microorganisms from instruments: the type of procedure performed (invasive or noninvasive), the presence of microorganisms, their number, and their virulence, and the body site at which the instrument will be used.[20] All of the surgeries performed in our study were considered major or invasive procedures due to the entry into a body cavity.[28] With regard to surgical time, previous publications on surgical instruments in human medicine have found positive correlations of surgical time with bacterial load[27] and risk of surgical site infection.[5,13,25,35] Our study was performed by a single surgeon trained in aseptic technique but new to rodent surgery. We observed a noticeable decline in the number of positive pre-sterilization cultures between the first and second series using aseptic technique. This difference was attributed to a previously described learning curve in which increasing surgical case volume and years of practice are associated with improved performance and a decrease in complication rates.[23,32] When considering how many sequential rodent surgeries to perform using a hot bead sterilizer, conducting a risk assessment using the factors described above will indicate the risk of contamination on a case-by-case context. For example, an experienced surgeon with impeccable aseptic technique performing a brief minor surgery might consider performing many more surgeries in series without significantly compromising surgical instrument tip asepsis, while a less experienced surgeon performing a major surgery would have a much higher risk of contamination and should therefore err on the side of caution. We recommend that initial risk assessment include bacterial culture data for instrument tips when performing surgeries in series using the 'tips only' technique to evaluate the likelihood of hot bead sterilization failure in the context of the procedure being performed.

Current published literature on rodent surgery includes modifications to surgical materials and procedures to improve cost efficiency, allow higher throughput, and be feasible for a single surgeon. Some of these modifications include using plastic food wrap for sterile draping,[11] the "tips-only" technique,[16] and clean (rather than sterile) exam gloves for each surgery.[16] While these modifications have been evaluated individually and become accepted practice for rodent surgeries, no previous study has combined these techniques to evaluate the efficacy of the hot bead sterilizer in successful sterilization of instruments in a clinical setting with risk of contamination. Although modifications in aseptic technique can be allowable for rodent surgery, the *Guide* and AAALAC accredited institutions generally continue to require strict asepsis for rodent surgeries, as this is the undisputed best practice.[28]

Animal research facilities often use ATP monitoring as a means of assessing environmental contamination.[10,11,33] However, the 2 surgical series we performed using ATP data had no obvious relationship to culture results obtained after sterilization (Table 2). ATP-based bioluminescence systems have been developed to monitor the sanitization of equipment and can detect organic contaminants (for example, feces, dead cells, etc.)[11] that may not be directly pathogenic but provide an environment in which opportunistic bacteria can live and grow. The limitation of this detection method is that a positive ATP reading does not equate to the infectious potential of a sample.[33] We did detect a significant difference in post-sterilization ATP readings between the forceps and the needle drivers. We suspect that the forceps are more likely to trap organic debris within their deeper grooves. However, given that the proportion of nonsterile instrument tips was similar after hot bead sterilization as measured by cfu, the difference between the 2 surgical instruments used here was not clinically significant for our surgical conditions.

The current study had several potential limitations. First, supply chain shortages of flocked nylon swabs precipitated by the COVID-19 pandemic was the major limitation of data acquisition in this study. In addition, the surgical instruments we tested had 2 sides used for grasping and therefore provided only 2 surfaces for swabbing, limiting the data collected (ATP compared with pre- and post-sterilization cultures) without swabbing the same surface more than once per surgery. In this study of 5 series of surgeries, we had limited power to explore the correlation structure of contamination events within the same series of surgeries. The risk of post-sterilization contamination was so much greater in the cecal contaminated surgeries than the other surgeries that we were able to show a significant odds ratio for contamination in the cecal series compared with the aseptic or fur-contaminated series. Another potential limitation of our study was that we found unexpected contamination when using strict aseptic technique (Table 1, Aseptic Series 1). As discussed above, aseptic technique has a learning curve, and improved performance occurs with increasing experience.[23,32] All surgeries in this study were performed by a single surgeon, and results may differ for other surgeons. In addition, the probability of hot bead sterilization failure is affected by the extent of pre-sterilization contamination and the number of surgeries that result in contamination of the instrument. However, our study lacked the power to determine whether either of these factors affected the probability of sterilization. Other contributing factors leading to positive bacterial growth could have included using an open benchtop for sample collection and plating of culture media and/or use of the same suture pack across surgeries (despite maintaining suture within the sterile field).

Future studies of aseptic rodent surgery could evaluate the reuse of suture material for multiple series. Cull mice representative of our mouse colony were used for this study, so our results may not be generalizable to all rodents. Future studies evaluating the efficacy of hot bead sterilization could be expanded to include other species (for example, rats) or specific

strains (for example, nude mice) or ages to determine if species, strain, immune status, or the presence of fur affects the likelihood of contamination. Although the mice used in this study were euthanized immediately before surgery, future studies could also include the use of live mice to monitor recovery after surgery. This could provide insight on the clinical significance of a positive post-sterilization culture (cfu count greater than 0) as related to the likelihood of an animal developing a surgical site infection.

This study contributes the first published data on the effectiveness of the hot bead sterilizer in decontaminating surgical instruments between sequential rodent surgeries in various clinical settings. Our findings support the use of hot bead sterilization between rodent surgeries for at most 5 sequential "tips-only" surgeries with little to no risk of contamination. Hot bead sterilization may provide a cost-effective method of sterilizing surgical instrument tips for rodent surgeries when performed by trained surgeons adhering to strict aseptic technique. In contrast, hot bead sterilization is not recommended for surgeries that enter the gastrointestinal tract, where the risk of contamination outweighs the benefit of rapid throughput with hot bead sterilization. We recommend surgeons and research staff conduct their own risk assessments when determining the number of rodent surgeries to perform in series based on surgical procedure classification, duration of surgery, and the surgeon's level of experience. Adherence to strict aseptic technique is always advised when performing survival surgery in any species,[1,28] and verification of sterilization of instruments between surgeries provides the greatest confidence in minimizing risk of contamination.

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