Date: Friday, March 10, 2023 12:08:33 PM

Animal Re earch Information

A. Study Title

1.0 Application type:
New Animal Use Application (AUA)

2 0 * AUA Title
Application of [redacted] for treatment of chronic tympanic membrane perforation

3.0 * Principal Investigator:
[redacted]

If the Contact Per on li ted below i not current, then you mu t complete the "Update Study Personnel" activity on the Approved AUA Workspace.

4.0 * Contact Person:
[redacted]

Objectives and Justification

B. Objectives

1.0 * Using language that can be understood by a non-scientist, explain what you are trying to accomplish with this AUA. The response should be concise, should describe the purpose of this study, and should explain why this research is significant to the general public including the importance to human or animal health, the advancement of knowledge, or the good of ociety Sample re pon e (MCW network acce required)

Tympanic membrane perforations are very commonly caused by traumatic injury or middle ear infection. Perforation causes conductive hearing loss and often requires surgical treatment. An effective alternative for treating chronic perforations is notably needed. The purpose of this study is to establish protocol to create chronic tympanic membrane perforation (TMP) and to a e the efficacy of a [redacted] repairing tympanic membrane in chinchilla model. [redacted] This study is important as it allows the testing of new potential treatment and the risk evaluation prior to its clinical application.

Objectives and Justification

C. Proposed Species

1.0 * Animal Model:
Chinchilla, Chinchilla lanigera

2 0 * Doe thi animal model include the generation or u e of genetically modified (e g tran genic) animal †?

○ Yes ● **No**

Objectives and Justification

D. Animal Acquisition

1 0 * Animal Acqui ition Will you acquire animal from any of the following ource ? Select all that apply

- [x] **BRC-Approved Commercial Vendor or Supplier**
- [ ] Non BRC-Approved Source†
- [ ] Transfer from another AUA (e.g. in-house breeding colony, other MCW Investigator)

2 0 * For other than veterinary health rea on , have any of the animal been urgically manipulated or had e perimental procedure performed on them prior to acquisition for this AUA (e.g. surgery/procedures performed under other MCW AUAs or Approved Protocols at other institutions, or surgeries conducted by a vendor†)?

○ Yes ● **No**

Objectives and Justification

E. Animal Use and Species Rationale

1.0 * Provide a rationale for the use of animals. Include reasons why non-animal systems cannot be used to meet the objectives of this AUA:

The main objective of this study is to create chronic TMP and test the efficacy of [redacted] as grafting/supportive material in repairing the tympanic membrane. The repairing process requires a biological system that can generate the new tympanic membrane and the healing process. A comprehensive literature search was completed to assess for possible alternatives to the animal model. No mathematical or non-animal models are available to substitute for the propo ed inve tigation

2.0 * Justify the appropriateness of the proposed animal species. Include the specific characteristics that make this species the best choice for this work.

We selected the chinchilla, Chinchilla lanigera, for several reasons. First, it is the most widely studied experimental animal model for chronic perforation. Second, the anatomical feature of the chinchilla middle ear and internal ear tructure bear re emblance to that of human Third, their hort traight and wide ear canal allows ease of surgical access. Last, our laboratory has significant experience in using this animal model and has pioneered many of the techniques and tools that have improved the suitability of this animal model. This research, using this animal model, is significant because it can lead to new treatments for the chronic TMP.

Description of Animal Use

F. Experimental Overview

1.0 * What type of activity best describes this AUA? Check all that apply:

- [x] **Re earch or Te ting**
- [x] **Pilot Study**

- [ ] Teaching or Training (e.g. BRC wet labs, teaching labs...)
- [ ] Animal Colony Health Surveillance (e.g. BRC sentinel programs)
- [ ] Breeding Program (i e to upport either thi AUA or other animal u e program)
- [ ] BRC Holding Protocol (to be selected by Attending Veterinarian or BRC Director only)
- [ ] Wildlife Study (i.e. in the animal's natural habitat)

2 0 * What type of procedure will be performed on the animal ? Check all that apply

- [x] **Procedure other than urgery**
- [x] **Survival Surgery**
- [ ] Non-Survival Surgery

3 0 * Provide an outline or overview of the appro imate equence and timing of all procedure performed on animal Start with acqui ition or birth, preliminary acclimation and conditioning, breeding (if applicable), completion of the studies or experiments†, and end with final disposition of the animals (e.g. euthanasia or transfer to another AUA). Do not provide details on how the procedures will be conducted because this information will be captured in later ection Sample re pon e (MCW network acce required)

A. Acquisition. Outbred chinchillas are acquired from BRC approved vendor or from collaborators at MCW.

B. Acclimation. All animals will be acclimatized for 3-7 days

C. Experimental Design
Overview of experimental procedure
1. All animals will undergo ABR threshold testing for baseline hearing level prior to the generation of tympanic membrane perforation (TMP).
2 On e perimental day 0, all animal will undergo urvival urgery to induce TMP Ba eline body weight i taken Animal are divided into 2 group and chronic TMP is induced bilaterally using two different techniques:
Group 1. Chronic TMP is created using myringotomy with topical sequential application of Mitomycin C and dexamethasone follow by ventilation tube insertion (VTi). VT is removed 2 weeks post initial myringotomy with second application of MC+Dex.
Group 2 Chronic TMP i created u ing myringotomy with topical equential application of Mitomycin C and de ametha one (MC+De) MC+De i applied on TMP for 24 hours then refresh for three more times. Antibiotic treatment is given via drinking water to prevent infection for up to 5 weeks.
3. Otoscopy will be performed up to 8 times (during the 8 weeks post initial myringotomy) until the animals enter the study on treatment day 0, to monitor the closing of TMP. Myringotomy may be repeated on those animals with closing perforations as needed to maintain patency. The period between myringotomy i 1 2 week The animal will be kept untreated for 8 week po t the initial myringotomy at which the le ion i con idered chronic TMP
4. On treatment day 0 (8 weeks post initial myringotomy), all animals that maintain bilateral chronic TMP will be tested for ABR threshold and undergo survival surgery for TMP treatment. Each animal will be given treatment ([redacted] in one ear and another ear is left untreated to serve as control for spontaneously healing.
5 On 2 week , 4 week and 6 week po t treatment Oto copy i performed on all animal

6. After 6 weeks post treatment: All are tested for ABR threshold.

D Final di po ition
1. Euthanasia. All experimental animals will be euthanized no later than 8 weeks post treatment of chronic TMP.

Document Upload Only if applicable, upload a pdf ver ion of any table( ) that illu trate the above e planation Do not upload the entire re pon e to thi question.

Name	Last Modified Date	Version
There are no items to display		

De cription of Animal U e

G. Procedures Other Than Surgery

1.0 * Describe in detail the techniques†† that will be used to perform each non-surgical procedure† outlined in F3.0. Do not include description of euthanasia here because this information will be captured in a later section. Sample response (MCW network access required)

I oflurane Ane the ia The animal i placed in a tran parent induction chamber I oflurane i delivered to the chamber via a preci ion vaporizer and compressed oxygen. For induction, the percentage of isoflurane may be as high as 5%. Once the animal is unconscious, it is removed from the chamber and placed on a warm water circulating pad. Anesthesia will be maintained by delivery through a face mask. At this point the concentration is reduced to that level which maintains the proper plane of anesthesia; typically this is between 0.5 and 3%. While anesthetized, the monitoring of anesthetic depth via a e ment of toe pinch withdrawal and re piratory rate will be demon trated and practiced The animal will be kept ane thetized during the experimental procedure. Upon completion, the animal weight will be taken prior to recovery. The animals will be placed on the warm water circulating blanket for supplemental heat during the recovery. They will be observed continuously until they can remain upright without assistance, and will be monitored frequently until deemed fully recovered from the anesthesia.

Video otoscopy
An animal is manually restrained by one of the study staff while another staff conducts the procedure. To perform otoscopy, the speculum of the video otoscope is inserted into the animal's ear canal to examine the canal and tympanic membrane for signs of inflammation. The images of tympanic membrane are collected The procedure may be performed on an ane thetized animal a well

IM injection: The animal is restrained manually on a solid surface on its dorsum. The anesthetic ketamine/xylazine injection (using a 25g needle) is given into muscle in the hind leg area. Prior to the injection, the plunger of the syringe is withdrawn to ensure the needle is not in a vessel.

Subcutaneous injection: While manually restrained, the loose skin (over the back, or area between shoulder and the neck) is lifted to form a tent. A 22 gauge or smaller needle attached to a syringe is inserted bevel up at the tent base. The needle/syringe is held parallel to the animal's body to also avoid puncturing underlying structures. Before injecting, the syringe plunger is withdrawn to ensure that the needle has not entered a blood vessel.

Ketamine/Xylazine Anesthesia: No more than 0.6 ml of ketamine/xylazine mixture is injected via IM. The unconscious animal is placed on a warm heated pad. While anesthetized, the monitoring of anesthetic depth via assessments of toe-pinch withdrawal, respiratory rate, and related observational methods will be demonstrated and practiced. The animal is kept anesthetized throughout the ABR hearing test. Upon completion of the ABR test, the animal is placed on a warm water heating pad to provide upplemental heat during recovery After the ABR te t, the animal will be admini trated with atipamezole to recover. Recovering animals will be observed continuously until they can remain upright without assistance, and will be monitored frequently until deemed fully recovered from the anesthesia.

Hearing Te t Hearing te t i conducted by recording the auditory brain tem re pon e (ABR) in chinchilla during a pre entation of acou tic timuli The anesthetized chinchilla is placed in a fixed position within an acoustic testing chamber. The chinchilla is placed on a warm pad with the head face toward

speaker, with its chin slightly elevated and cradled with a piece of supportive material to prevent movement while still allowing the chinchilla to breath easily. Apply ointment over the animal eyes to prevent drying during the procedure. The scalp area is cleaned with alcohol wipe. The active electrode (the size of 25G-30G needle) is placed into the scalp subdermally between the ears (over the vertex of the skull), with a reference electrode near one cheek and a ground electrode at the other cheek or tail. Each electrode (6mm in length) is fully inserted via direct puncture of the skin. Once electrodes are placed, acoustic stimuli is applied and ABRs will be recorded. Hearing will be tested using tone burst stimuli at octave frequencies from 4 kHz to 32 kHz. Click stimuli will also be used. The anesthetized chinchilla is kept warmed before, during, and after ABR recording using a warm pad or incandescent light. The animal conditions will be monitored throughout the procedure. Upon completion of testing, all electrodes are removed and the chinchilla is placed on a warm recovery setting (warm water circulation blanket or an incandescent lamp). The animal will be monitored frequently until deemed fully recovered from the anesthesia then placed back in it housing cage.

Fluid Administration: Lactated Ringer's Injection is given to animal with more than 5% weight loss as fluid supplement. While the animal is under isoflurane for the otoscopic exam, the animal will be administered the fluids with a 22g or smaller needle. Several 5-10 mL pockets of fluids will be administered subcutaneously at various locations along the flank areas of the chinchilla.

Description of Animal Use

G. Survival Surgery Type

2.0 * What types of survival surgery will be performed under this AUA? Check all that apply.

- [ ] Minor
- [x] **Major**

3.0 * Does this AUA include more than one survival surgery on a single animal?

(●) **Yes** ( ) No

If Yes:

3.1 Check all that apply:

- [ ] One Minor
- [ ] Multiple Minor
- [ ] One Major
- [x] **Multiple Major**

3.2 If multiple survival surgeries will be performed, provide justification† for the conduct of multiple survival surgeries (i.e. explain why survival surgical procedures cannot be conducted during a single surgical period or why the animal cannot be euthanized prior to the anesthetic recovery following the second procedure). If these surgeries are related components of the study, so state and explain.

The initial survival surgery is to generate chronic TMP, a condition that may require multiple myringotomy to maintain the opening of the perforation. In addition, the treatment of chronic TMP also require another surgery to place the [redacted] on the TMP.

3.3 If multiple survival surgeries will be performed on a single animal, what will be the rest period for the animal between surgeries?

1-2 weeks.

Description of Animal Use

G. Survival Surgery Description

4.0 * How will animals be prepared for survival surgery? Provide the details related to the survival surgical procedure(s) outlined in F3.0. Include fasting, administration of pre-operative medications including tranquilizers and analgesics, substance administration, animal prep such as shaving and skin di infection of the urgical ite, and ane thetic induction If applicable, differentiate animal preparation for major and/or minor urgery Sample re pon e (MCW network access required)

All procedures are performed under general anesthesia using inhaled isoflurane. Isoflurane anesthetic induction is as described in section G 1.0. If the no e cone that delivered i oflurane omehow interfere with acce for urgery, the animal will be ane thetized with a mi ture of ketamine/ ylazine intramuscularly. While anesthetized, the monitoring of anesthetic depth via assessments of toe-pinch withdrawal and respiratory rate will be demonstrated and practiced. Pain medication is administered subcutaneously. Surgical procedure is performed trans-ear canal. When present, ear wax is removed using a curette then the ear canal is sterilized with an iodine solution and then flashed several time with sterile 0.9% PBS.

5 0 * De cribe the urvival urgical procedure( ) Provide the detail related to the urvival urgical procedure( ) outlined in F3 0 Include urgical manipulations as well as monitoring of anesthetic depth and physiologic parameters until the animal has recovered from anesthesia. Sample response (MCW network access required):

Generation of chronic TMP Animal are ane thetized u ing inhaled i oflurane or a mi ture of ketamine/ ylazine intramu cularly whichever applicable While anesthetized, the monitoring of anesthetic depth via assessments of toe-pinch withdrawal, respiratory rate, and related observational methods will be demonstrated and practiced. Myringotomy is performed using operating microscope. The ear canal is sterilized with an iodine solution and then flashed several time with sterile 0.9% PBS. In order to otoscopically visualize the entire TM with a surgical microscope, a speculum is used to depress the bony ridge within the ear canal that partially block anterior e po ure
Group 1. Chronic TMP is created using myringotomy with topical sequential application of Mitomycin C and dexamethasone follow by ventilation tube insertion (VTi). The anterior portion of the TM will be perforation utilizing a combination of sharp dissection and cautery. Approximately 40% of the TM area will be resected to create a perforation. The undersurface of the TM mucosa will be removed with a right-angle hook. Following this, Mitomycin C will be applied to the edge of the perforation with Gelfoam for 10 minute then removed Ne t de ametha one will be applied to the edge of the perforation with Gelfoam for 10 minutes then removed. Following this, flaps will be created to fold the squamous portion of the TM under to prevent healing. Ventilation tube is inserted into TMP immediately after using a Wullstein needle. Small pieces of Gelfoam (absorbable gelatin sponge) are placed to hold the ventilation tube in place. Two weeks later, the ventilation tube will be removed using a Wullstein needle with second application of MC+Dex. If the tube fall off before the 2 week time with clo ing of TMP, myringotomy will be repeated once at 2 week po t initial myringotomy to increa e patency of TMP The additional myringotomy will be performed only once per animal and only in the animals with closure of TMP. Two week recovery period is allowed between each procedure.
Group 2. Chronic TMP is created using myringotomy with topical sequential application of Mitomycin C and dexamethasone (MC+Dex).The anterior portion of the TM will be perforation utilizing a combination of harp di ection and cautery Appro imately 40% of the TM area will be re ected to create a perforation. The undersurface of the TM mucosa will be removed with a right-angle hook. Following this, two pieces of Gelfoam soaked in a solution of Mitomycin C and dexamethasone will be applied to the edges of the perforation for 24 hours then refreshed with new pieces of Gelfoam soaked in the solution three consecutive times. On the fourth day, the last Gelfoam will be removed.
Treatment of chronic TMP Animal were ane thetized u ing inhaled i oflurane or a mi ture of ketamine/ ylazine intramu cularly whichever applicable While anesthetized, the monitoring of anesthetic depth via assessments of toe-pinch withdrawal, respiratory rate, and related observational methods will be demonstrated and practiced. The epithelial rim of each TM perforation was ablated to expose fresh tissue as would normally be performed clinically prior to patching. Ears with patent TMP are randomized to receive a [redacted] plug. A piece of Gelfoam presoaked in ofloxacin otic solution is inserted into the TMP follow by a precut [redacted] (al o pre oaked in oflo acin otic olution) i plugged into TMP Thi i facilitated by u ing a 30 degree rigid

endoscope to assist in both the placement of the plugs and image capture. Another ear is served as control for normal wound healing.
The anesthetized chinchilla is kept warmed before, during, and after survival surgery procedure using a warm pad or incandescent light. The animal condition will be monitored throughout the procedure Upon completion of the procedure, the chinchilla i placed on a warm recovery etting (warm water circulation blanket or an incandescent lamp). The animal will be monitored frequently until deemed fully recovered from the anesthesia then placed back in it housing cage.

6 0 * Describe the post-operative care activities†. Provide the details related to the survival surgical procedure(s) outlined in F3.0. Include frequency of observations, supportive care, the use of pain relieving agents and antibiotics (if any), and time of suture removal. Sample response (MCW network access required)

1. After surgery, the animal is placed back in a cage lined with absorbent pad and continuously monitored until sternal.
2. The animal cage is placed on warmed circulating water pad to prevent the development of hypothermia.
3. Analgesics is administered according to the regimen indicated in M.1.0.
4 Antibiotic treatment i given according to the regimen indicated in M 1 0

6.1 If analgesics to mitigate post-operative pain will not be provided, please explain why not.

* **6.2** Who will be responsible for post-operative care after hours, on weekends, and during holidays?

Study taff will be available to provide care after hour , on weekend and during holiday

De cription of Animal U e

G. Survival Surgery Location Type

7.0 * Will survival surgeries be conducted using aseptic technique and in accordance with the *Guide for the Care and Use of Laboratory Animals* and veterinary standards?

● **Yes** ○ No

Description of Animal Use

G. All Surgical Procedures

13.0 * Will all medical materials (e.g. sutures, indwelling catheters, medical devices and implants, etc.) be used within their expiration date?

● **Yes** ○ No

14.0 * Will neuromuscular blocking agents be used?

○ Yes ● **No**

Description of Animal Use

H. Blood Collections

1 0 * Will blood be collected from an animal as part of a **survival procedure**†?

◯ Yes ● **No**

Description of Animal Use

I. Prolonged Physical Restraint

1 0 * Will con ciou animal be e po ed to prolonged phy ical re traint (e g manually or mechanically u ing acrylic tube, chair, ling or other re traint device)?

◯ Yes ● **No**

De cription of Animal U e

J. Food and/or Fluid Restriction

1.0 * Will animals be restricted from food and/or fluid as part of the experimental design? (For non-rodent mammals, do not include overnight fasting prior to surgery).

◯ Yes ● **No**

Description of Animal Use

K. Outcomes and Endpoints

1.0 * Do these animals have any inherent behavioral or physical phenotypic abnormality that may dictate the need for special care or result in clinical signs of illne ?

◯ Yes ● **No**

2 0 * Are any induced characteristics, clinical signs† or lesions expected in the animals used on this AUA as a result of a test agent or procedure?

● **Yes** ◯ No

If Yes:

2.1 Describe the clinical signs and/or lesions. Information about monitoring and endpoints should be included in 3.0-5.0. Sample response (MCW network access required)

Eye and ear infection were reported in a subset of chinchillas after myringotomy [PMID:24865807].
Clinical signs of ear infection may include: decreased activity, head tilt, difficulty with ambulation, ataxia, and weight loss. OM may also lead to various degrees of pathological change of the tympanic membrane, fluid accumulation in middle ear cavity, and tympanic membrane perforation and discharge. Perforation cau e pain (animal will be given analge ic) o lethargy and headtilt can be e pected Drainage from the ear can al o be e pected a a re ult of infection.

Description of Animal Use

K. Outcomes and Endpoints

Responses to questions 3.0 and 4.0 pertain to all circumstances except surgical procedures. Do not include results from surgical procedures because those results were described in an earlier section.

3.0 * De cribe how the animal will be monitored to a e clinical ign , le ion , or deviation from normal health Include the frequency of ob ervation and how the observations are conducted during weekends and holidays. Sample response (MCW network access required)

Upon arrival if any animals show signs of illness prior to the study procedure, veterinary staff will be consulted for proper care and treatment or whether they deem acceptable to enter the tudy While on tudy, we will con ult with veterinary taff for any reported health concern for treatment option , including euthanasia, if appropriate.

On experimental day 0, the animal weight is first taken as a baseline. Thereafter the weight is taken at the time of otoscopy and monitored for weight loss.

After the generation of TMP, animals will be monitored by study staff twice daily for the first 24 hours post-surgery for signs of infections, then once daily for the next 5 days. After that study staff will examine the chinchillas every third day unless a clinical abnormality is noted by the BRC staff, in which case study staff will examine affected animal(s) immediately. After the first 24 hours post-surgery, animals will be weighed at each scheduled otoscopy or at lea t once a week Study taff will be available to conduct the e ob ervation during weekend and holiday

4.0 * Describe any procedures or methods designed to assure that discomfort and pain to the animals will be limited to that which is unavoidable. Include the use of non-pharmacologic measures (e.g. provisions of soft bedding, supplemental fluids) and the use of analgesic, anesthetic, and tranquilizing drugs where indicated and appropriate to minimize discomfort and pain to animals. Sample response (MCW network access required)

All procedures will be done under general anesthesia. The animals will be placed on warm water circulating blanket during the procedure and during the recovery time to provide supplemental heat.

E perimental day 0 week , all animal are given analge ic (Buprenorphine SR) for pain relief and every 72 hour thereafter All are given antibiotic via drinking water to prevent infection.

Animals with any clinical signs of illness that do not meet the endpoint criteria in K. 5.0 may be given subcutaneous fluid therapy. This will consist of admini tration of 10 20ml of 0 9% aline or lactated ringer' olution in multiple location No more than 5ml of fluid will be admini tered at any location to prevent vascular injury.

Description of Animal Use

K. Outcomes and Endpoints

Response to the following question pertains to results for <u>all</u> circumstances, including but not limited to surgical and non-surgical procedures or behavioral/physical phenotypic abnormalities.

5.0 * Animal will be euthanized when their clinical condition meet or e ceed the following

- [x] **General**
  - Severe emaciation/loss of body condition characterized by extremely prominent skeletal structure with little or no flesh cover and distinctly segmented vertebrae.
  Re piratory di tre characterized by major increa e in re piratory effort/rate and/or open mouth breathing
  - Severe neurologic signs such as seizure activity lasting more than 10 minutes continuously and/or severe ataxia i.e. loss of control of body movements
  - Severe morbidity characterized by marked depression of activity, reluctance to move upon prodding, piloerection i.e. rough hair coat, and hunched phy ical po ture
  - Moribundity characterized by a comatose state or minimal response to tactile stimulation.

- [ ] **Rodent and Rabbit Surgical Model**
  - Dehiscence of a surgical incision with exposure of a body cavity or of a previously implanted device such as a telemetry unit, vascular catheter,

etc. This does not include the portion of a catheter that is purposefully exteriorized for infusion, blood pressure monitoring, etc.

☐ **Rodent Tumor Models**
- A visible tumor more than 2 cm diameter in a mouse or more than 4 cm diameter in a rat.
- An ulcerated tumor.
- A tumor interfering with ambulation or the animal's ability to eat or drink normally.

☑ **Additional Criteria:**

An animal exhibiting any of the following signs of illness will be euthanized.
1. Unable to maintain upright posture
2. 20% weight loss compared to baseline body weight.
For all other signs of illness where endpoints are not clear, the veterinary staff will be consulted for treatment options, including euthanasia.

Description of Animal Use

L. Euthanasia

1.0 * Describe the method(s) of euthanasia to be used at the termination of the study, prior to intended study termination, or if animals are bred but not entered in the study.

- If non-physical methods of euthanasia (e.g. barbiturate overdose, CO2 asphyxiation) are used, include how death of the animal(s) will be ensured.
- If physical methods of euthanasia (e.g. cervical dislocation, decapitation) are used as the primary method, training for the method must be appropriately documented or the animals must be sedated or anesthetized during the procedure.
- If decapitation is to be performed in the absence of anesthesia, provide scientific justification for this procedure.

Following anesthesia, animals are euthanized with pentobarbital based euthanasia solution via intracardiac injection. The animal is examined for cessation of vital signs then cervical dislocation is performed to ensure death before proceeding to temporal bone harvesting in which the animal head will be removed.

Method descriptions;
Euthanasia: The animal is anesthetized by intramuscular injection with a mixture of ketamine and xylazine. Once the animal is anesthetized, pentobarbital based euthanasia solution is given by intracardiac injection through the thorasic wall using 22-gauge needle. Prior to the injection, the plunger of the syringe is withdrawn to ensure the needle is in the heart. Tissue procurement is performed after euthanasia.

Cervical Dislocation: The animal is handled by grasping the base of the tail firmly with one hand. The thumb and first finger of the other hand is against the back of the neck at the base of the skull. To produce the dislocation, the hand restraining the head is pushed forward and down while pulling backward with the hand holding the tail base. The effectiveness of dislocation is verified by feeling for a separation of cervical tissues.

Substance Administration

M. Substances

1.0 * Will you be administering any substance(s) to animals?†

● **Yes** ○ No

If Yes:
1.1 Complete the following table. Identify each substance including anesthetics (including inhalants), analgesics, tranquilizers, antibiotics, other agents, fluids (e.g. saline), experimental compounds, euthanasia agents including CO2. Enter the corresponding procedure, dose, route, volume or weight, e timated animal weight, frequency, and duration Range are acceptable

Substance	Procedure/Purpose	Dose	Route	Substance Vol or Wt	Est. Animal Wt	Frequency (#time /day)	Duration (#day / tudy)
0.3% Ofloxacin otic solution	TMP treatment	5 drop or 0.75mg	Topical	5 drop or 0.75mg	500g	1/day	1/study
Atipamezole	reverse sedative effect	1mg/kg	Subcutaneous	1mg/kg	500g	1/day	up to 10days/study
Buprenorphine-SR	Analgesia	0.4-0.6mg/kg for 72hours	Subcutaneous	0.2-0.3mg	500g	1/3days	up to 33 times/study
dexamethasone	TMP generation	5-10mg/ml	Topical	5-10mg/ml	500g	1/day	up to 4days/study
enroflaxocin	antibiotic treatment	10mg/kg/day	Dietary (feed/water)	10mg/kg/day	500g	1/day	up to 7 weeks
I oflurane	Ane the ia	1 5%	Inhalant	1 5% a needed	500g	1/day	up to 15days/study
Ketamine + ylazine	ane the ia	up to 50mg/kg Ket +10mg/kg Xyl	Intramu cular	up to 0 5ml	500g	1/day	up to 10days/study
lactated Ringer' Injection	fluid replacement	5 20ml	Subcutaneou	up to 20ml	500g	1/day	up to 15day / tudy
Mitomycin C	TMP generation	0.4-0.5mg/ml	Topical	0.4-0.5mg/ml	500g	1/day	up to 4days/study
Pentobarbital based euthanasia solution	euthanasia	120mg/kg	Other	120mg/kg	500g	1/day	1/study

If Route of Admini tration elected i "Other"
1.1.a Please describe:

Euthanasia solution is administered by intracardiac injection.

1.2 If you will be using pre-mixed drug cocktails (e.g. ketamine/xylazine cocktail), please describe the method and length of storage anticipated prior to use.

Ketamine/Xylazine will be made fresh in the amount needed for each use. We do not anticipate storage of unused amount.

1.3 If you will be administering biological products/materials (e.g. cells, antibodies, serum, etc.), have any been derived from animals?

- ○ Yes
- ○ No
- ● **N/A**

1.4 Are any of the substances DEA (Drug Enforcement Administration) controlled drugs†?

● **Yes** ○ No

Substance Administration

M. Administration Route

2.0 * Will the same route of injection be used multiple times? Check all that apply.

- ☑ **Yes**
- ☐ No
- ☐ N/A - Route of substance administration does not involve injection

If Yes:

2.1 List the method or procedure. Include number of injections and period between injections:

Analgesic buprenorphine-SR will be injected subcutaneously up to 33 times, once every 3 days.
Atipamezole will be injected subcutaneously up to 10 times, once a day.
Ketamine/xylazine mixture for anesthesia will be injected up to 10 times, once/day, at least 24 hour between injections.
Lactated Ringer's injection as fluid replacement will be given up to 5 times, once/day, at least 1 week between injections.

Substance Administration

M. Grade/Expiration

3.0 * Will all administered substances be pharmaceutical-grade?

○ Yes ● **No**

If No:

3.1 List and justify the use of each non-pharmaceutical grade substance. For non-pharmaceutical anesthetics, analgesics, euthanasia agents, antibiotics, and fluids used in survival surgery, provide a scientific or clinical justification†.

Dexamethasone and mitomycin C at the required concentration are not available in pharmaceutical grade. The specific concentration of 10mg/ml dexamethasone and 0.4mg/ml mitomycin C are required in order to reliably produced TMP lasting at least 8 weeks, at which time considered a chronic condition. It was also reported to produce chronic TMP at higher success rate [PMID: 31260172].

3.2 Describe the method of preparation and storage (including location and length of time) for each non-pharmaceutical grade substance.

A stock solution of 25mg/ml of dexamethasone is prepared in absolute ethanol and is stable at 4C for at least 30 days. The stock solution is diluted with sterile water to obtain the working concentration of 10mg/ml and store frozen as working aliquots.

A working solution of 0.4mg/ml of mitomycin C is prepared in sterile injectable water, then filter sterilized and stored in the dark at 2-8C for up to 1 week. If a precipitate forms, a fresh solution will be prepared for use.

4.0 * Will all administered substances be used before their expiration date?

- (●) **Yes, all substances will be used before their expiration date**
- ( ) No, one or more substances either will not be used before their expiration date OR do not have an expiration date

Regulated Agents, Substances, or Equipment

N. Identification

1.0 * Will you be using any of the following agents or equipment when working with live animals? Work with certain agents/equipment may require approval from a MCW Safety Committee. Check all that apply.

- [ ] Biological Agents†
- [ ] Particularly Hazardous Substances††
- [ ] Ionizing Radiation†††
- [ ] Magnetic Resonance Imaging (MRI)
- [x] **I am not working with any regulated agents, substances or equipment**

Pain and Distress

O. Category Percentages

1.0 Estimate the percentage of animals that will fall into the following pain and distress categories. The total of all four categories should equal 100%.

* 0 % Category B: animals are being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.
* 0 % Category C: animals will not experience greater than momentary or transient pain or distress such as that produced by routine injections or venipuncture.

* 100 % Category D: animals may experience greater than momentary or transient pain or distress but will receive anesthetics, analgesics or tranquilizers to prevent, alleviate, and/or minimize pain or distress.

* 0 % Category E: animals may experience greater than momentary or transient pain or distress and will not receive anesthetics, analgesics or tranquilizers.

Pain and Distress

O. Category D & E Animals

4.0 * Are the painful/distressful or potentially painful/distressful procedures included in this AUA design dictated by a federal agency (e.g. testing product safety, efficacy and/or potency for Investigative New Drug Applications)?

○ Yes ● **No**

5.0 * Are alternatives to painful or distressful procedures (Category E) or potentially painful or distressful procedure(s) (Category D) available (i.e. replacement, reduction, and refinement)?

○ Yes ● **No**

Animal Numbers

P. Estimates and Explanation

Estimate the number of animals that will be produced or used as part of this AUA during the 3 year approval period†.

1.0 * **If this AUA involves a breeding component††:**
Estimate the number of breeders plus the number of offspring that will be produced. Sample response (MCW network access required)

0

***If this AUA does not involve a breeding component or if the breeding component does not provide all the animals that will be used:**
Estimate the number of animals that will be obtained in order to meet the study objectives.

15

2.0 * Please explain how you estimated the animal number(s). Illustrate and organize your explanation using outlines and/or tables. Whenever possible, use statistical methods (e.g. power analysis) and/or literature references to justify your estimate. If this is a new or pilot study, indicate whether the numbers are based on experience with similar models in the lab or literature reports. Sample response (MCW network access required)

We need minimum of 10 animals with bilateral TMP to enter the treatment study. This number is based on literature search [PMID: 20620631/26746614/24865807]. The reported success rate of the methods use to create TMP is about 70%. So to get 10 animals (70%) to enter the study, we should start with 15 animals.

Document Upload: Only if applicable, upload a pdf version of any table(s) that illustrate the above explanation. Do not upload the entire response to this question.

Name	Last Modified Date	Version
There are no items to display		

3.0 * Will any additional animals, separate from study or breeding animals, be needed to specifically train staff to perform procedures on this AUA? (Select N/A if the sole purpose of the AUA is to provide training/teaching, and then account for all animals in 1.0).

- (●) **Yes**
- ( ) No
- ( ) N/A

If Yes:
3.1 Estimate the number of animals that will be used to train personnel during the next 3 years. Sample response (MCW network access required)

6

3.2 Explain how you estimated the number of animals. List the specific training activities (e.g. blood collection, substance administration, specific surgical procedures, euthanasia, etc.) and use outlines or bullet points to illustrate and organize your explanation. Sample response (MCW network access required)

We need to train two study staff to perform the surgery to create TMP and to repair the TM. These are difficult procedure to learn. We estimate that it will take about 6 animals for the staff to become proficient with the procedure.

Animal Numbers
P. Total

4.0 Based on your estimates in Questions P1.0 and P3.1, listed below is the total number of animals you estimate you will use during the next 3 years:

Total # of animals entered / 3 years: 15
Total # of training animals / 3 years: 6

Grand Total # of animals / 3 years: 21

Animal Husbandry
Q. Housing and Care

1.0 * Will animals be provided diet or water other than the standard available in the BRC?
( ) Yes (●) **No**

2.0 * Will animals be housed in caging (e.g. metabolism cages, wire-bottom caging for rodents, etc.) other than what is normally used for routine housing of animals†?
( ) Yes (●) **No**

3.0 * Will the caging/primary enclosures provide the minimum amount of space for the animal(s) that is recommended in the *Guide for the Care and Use of Laboratory Animals* and the *Animal Welfare Regulations*?

● **Yes** ○ No

4.0 * How will animals be housed? (check all that apply)

☐ Pair or Group

☑ **Individually**

☐ N/A†

If Individually housed:

4 1 Ju tify the need for individual hou ing††

It is the BRC standard protocol to house chinchilla individually to prevent aggresive behavior.

Animal Husbandry

Q. Environmental Enrichment

5.0 * Will animals be provided the environmental enrichment included in standard BRC animal care?

● **Yes** ○ No

Animal Husbandry

Q. Locations Outside of the BRC

9.0 * Will animals be housed or moved outside of the BRC for non-surgical procedures that do not have a surgical endpoint? (e.g. to a laboratory or area with specialized behavioral or imaging equipment, etc.)

● **Yes** ○ No

If Yes:

9.1 Explain why animals must be housed in or moved to another area or facility:

We need to move animals to B2625 for ear imaging with a more sensitive video otoscope, for delicate/precise tissue harvesting with the dissecting video microscope, and for when the BRC suite is not available.

9.2 For locations <u>within</u> the Medical College and its affiliates†: Indicate the location and continuous period of time for which the animals will be outside of the BRC for housing or non-surgical procedures.

Building Name	Floor Number / Wing	Room Number	Estimated Time Range	Maximum Time Period	Overnight Housing?
Basic Science Building (BSB)	2 - Second Floor	2625	up to 12 hours	12 hours	no

If animals will be housed outside of the BRC overnight:
9.2.a Please provide scientific justification for why overnight housing is necessary.

For locations <u>outside</u> of the Medical College and its affiliates††:

9.3.a Indicate the location(s) at which the animals will be outside of the BRC for housing or non-surgical procedures.

- ☐ Clement J. Zablocki Veterans Affairs Medical Center
- ☐ Marquette University
- ☐ University of Wisconsin - Milwaukee
- ☐ Other

9.3.b For each location, indicate the continuous period of time for which the animals will be outside of the BRC for housing or non-surgical procedures.

10.0 * Will animals be moved outside of the BRC for surgical procedures?

○ Yes ● **No**

If Yes, answer all that apply.

For locations <u>within</u> the Medical College and its affiliates†:

10.2 Survival Surgery - List building(s) and room number(s) where survival surgery will be performed:

Building Name	Floor Number	Room Number
There are no items to display		

10.3 Multiple Major Survival Surgery - List building(s) and room number(s) where multiple major survival surgery on an individual animal will be performed:

Building Name	Floor Number	Room Number
There are no items to display		

For locations <u>outside</u> of the Medical College and its affiliates††:

10.4.a Indicate the location(s) where surgery will be performed.

- ☐ Clement J. Zablocki Veterans Affairs Medical Center
- ☐ Marquette University
- ☐ University of Wisconsin - Milwaukee
- ☐ Other

10.4.b For each location, indicate the type of surgery (e.g. non-survival, survival, multiple major) that will be performed.

Animal Husbandry

Q. Animal Transportation

Animals should be transported in sanitizable, covered cages designed to prevent escape and/or injury. The time spent in public hallways or lobbies should be minimized.

11.0 * Will animals be transported according to institutional standards?

● **Yes** ○ No

12.0 * Following acquisition, will animals be transported on public roads (e.g. to/from other institutions, diagnostic or imaging centers, etc.)?

○ Yes ● **No**

13.0 * Will animals be returned to the BRC after being transported to a lab or on public roads?

○ Yes ● **No**

Consultations

R. Literature Search

This section is to determine that the investigator has made every effort to follow the following principles:

Replacement: non-animal systems, non-whole animal systems, or systems which use a lower order species
Reduction: fewer animal numbers without compromise to the statistical significance of the data
Refinement: prevention or minimization of pain or distress

1.0 * Were Literature Resources used to verify lack of redundancy in research or to seek information regarding alternatives to (1) painful/distressful procedures; or (2) animal use (i.e. replacement, reduction, and refinement)?

● **Yes** ○ No

If Yes:

1.1 Complete the following information†:

Date of Search	Time Period Covered by Search	Keywords Used	Literature Database	Other Database
5/11/2021	1953-2021	chronic tympanic membrane perforation, non animal systems, [redacted] repair	Medline/PubMed	

2.0 * Were non-literature resources used to seek information used to verify lack of redundancy in research or regarding alternatives to (1) painful/distressful procedures; or (2) animal use (i.e. replacement, reduction, and refinement)?

○ Yes ● **No**

Consultations

S. Veterinary Consultation

1.0 * Have you consulted with the Attending Veterinarian or his/her designee with regard to any aspect of the development of this AUA?

● **Yes** ○ No

If Yes:

1.1 Provide the name of the Attending Veterinarian and/or veterinary designee(s) consulted†, the approximate timeframe of the consultation and/or review, and the aspects of this AUA which have been developed in consultation and/or reviewed by the Attending Veterinarian and/or veterinary designee(s).

First Name	Last Name	Date(s) of Consultation	AUA Sections Developed in Consultation
[redacted]		4/15/2021	(G) Survival Surgery (G) Non-Survival Surgery
		5/11/2021	(M) Substance Administration

Study Personnel and Qualifications

T. Study Personnel Identification

1.0 * Identify all individuals engaged in handling live animals or entering the MRI environment (e.g. the Principal Investigator, Contact Person, Study Staff, and Vet Staff if involved with research-related duties specific to this study):

First Name	Last Name	Degree	Department	Division	Phone	Email	Location of Procedures	Handling Live Animals?	Performing Survival Surgery?	Entering MRI Environment?
[redacted]										
[redacted]							Medical College of Wisconsin	yes	yes	no

First Name	Last Name	Degree	Department	Division	Phone	Email	Location of Procedures	Handling Live Animals?	Performing Survival Surgery?	Entering MRI Environment?
[redacted]	[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	Medical College of Wisconsin	yes	yes	no

Study Personnel and Qualifications

T. Study Personnel Training and Qualifications

The information below has been automatically populated from each record in Researcher Profile for the Study Team identified as working with live animals on this AUA. The information recorded in each Researcher's Profile is updated by a Training Administrator once course confirmation is received.

2.0

Principal Investigator: [redacted]
Researcher Profile:
Core Certification Status: Certified
Orientation Component: Yes
Occupational Health and Safety Component: Yes
Continuing Education Component: Yes
Aseptic Technique Proficient: No

Technical Skills for PI and Species:

Species	Description	Completion Date
Chinchilla, Chinchilla lanigera	Injections: Subcutaneous, Intramuscular, Intravenous, Intraperitoneal	1/1/2004
Chinchilla, Chinchilla lanigera	Anesthetic Monitoring	1/1/2004
Chinchilla, Chinchilla lanigera	Anesthetic Induction: Injectable and Inhalation	1/1/2004
Chinchilla, Chinchilla lanigera	Blood Collection: Submandibular, Jugular	1/1/2004
Chinchilla, Chinchilla lanigera	Handling and Restraint	1/1/2004
Chinchilla, Chinchilla lanigera	Euthanasia: Cervical Dislocation, Carbon Dioxide	1/1/2004
Chinchilla, Chinchilla lanigera	Coered Species record keeping	8/1/2007
Chinchilla, Chinchilla lanigera	Surgical Procedure - Survival: Tympanic Membrane Perforation (TMP)	5/15/2021

3.0 **Study Personnel conducting procedures at MCW:**

First Name	Last Name	Researcher Profile	Core Certification Status	Orientation Component	Occupational Health and Safety Component	Continuing Education Component	Aseptic Technique Proficient	Technical Skills		
[redacted]		[redacted]	Certified	yes	yes	yes	no	Chinchilla, Chinchilla lanigera	Injections: Subcutaneous, Intramuscular, Intravenous, Intraperitoneal	1/1/2004
								Chinchilla, Chinchilla lanigera	Anesthetic Monitoring	1/1/2004
								Chinchilla, Chinchilla lanigera	Surgical Procedure - Survival: Tympanic Membrane Perforation (TMP)	5/15/2021
								Chinchilla, Chinchilla lanigera	Coered Species record keeping	8/1/2007
								Chinchilla, Chinchilla lanigera	Anesthetic Induction: Injectable and Inhalation	1/1/2004
								Chinchilla, Chinchilla lanigera	Blood Collection: Submandibular, Jugular	1/1/2004
								Chinchilla, Chinchilla lanigera	Handling and Restraint	1/1/2004
								Chinchilla, Chinchilla lanigera	Euthanasia: Cervical Dislocation, Carbon Dioxide	1/1/2004
[redacted]	[redacted]			yes	yes	yes	yes	Chinchilla, Chinchilla lanigera	Injections: Subcutaneous, Intramuscular, Intravenous, Intraperitoneal	1/1/2000
								Chinchilla, Chinchilla lanigera	Handling and Restraint	1/1/2000
								Chinchilla, Chinchilla lanigera	Euthanasia: Barbiturate overdose	1/1/2000

First Name	Last Name	Researcher Profile	Core Certification Status	Orientation Component	Occupational Health and Safety Component	Continuing Education Component	Aseptic Technique Proficient	Technical Skills		
								Chinchilla, Chinchilla lanigera	Injectable anesthetic induction	1/1/2000
								Chinchilla, Chinchilla lanigera	Covered species record keeping	10/23/2007
[redacted]	[redacted]		Certified	yes	yes	yes	yes	Chinchilla, Chinchilla lanigera	Anesthesia monitoring	9/1/2006
								Chinchilla, Chinchilla lanigera	Euthanasia: Barbiturate Overdose	9/1/2006
								Chinchilla, Chinchilla lanigera	Intramuscular injections	9/1/2006
								Chinchilla, Chinchilla lanigera	Temporal bone harvesting	9/1/2006
								Chinchilla, Chinchilla lanigera	Various organ collection	9/1/2006
								Chinchilla, Chinchilla lanigera	Handling and Restraint	9/1/2006
								Chinchilla, Chinchilla lanigera	Covered species record keeping	9/26/2006

Supporting Information and Study Funding

U. Supporting Information

1.0 * Are there any additional hazards/safety concerns related to the use of live animals under this AUA that were not addressed in Section N - Regulated Agents, Substances, or Equipment - Animal Use Only?

○ Yes ● **No**

2.0 Provide any additional information that you believe will help in the review of this application:

3 0 Upload upporting document in pdf format here

Name		Last Modified Date	Version
PMID31260172 2020 Animal model of chronic TMP pdf(0 01)	•••	11/16/2020 2 03 PM	0 01
PMID28726587_2018_RepairTMPerforationsBioprintedEarGraftChinModel.pdf(0.01)	•••	11/16/2020 2:03 PM	0.01
PMID28109506_2017_RatModelTMperforation.pdf(0.01)	•••	11/16/2020 2:03 PM	0.01
PMID26746614_2016_RatModelChronicTMPventilationTubeMitomycinCdex.pdf(0.01)	•••	11/16/2020 2:02 PM	0.01
PMID25455522_2014_Review TMP in animal models.pdf(0.01)	•••	11/16/2020 2:02 PM	0.01
PMID24865807_2014_ChronicTMPinChinModel.pdf(0.01)	•••	11/16/2020 2:02 PM	0.01
PMID20620631 2014 RepairChronicTMPinChin pdf(0 01)	•••	11/16/2020 2 02 PM	0 01

Supporting Information and Study Funding

V. Study Funding

1.0 * Do you have current MCW Department funding?

◯ Yes ● **No**

f Yes:

1.1 Below is a list of department funding:

Title	Cost Center	Fund	Project Number

There are no items to display

2.0 List all current and pending funding sources managed through MCW which will be used to support this AUA. Do not repeat funding already listed in V1.0 and V3.0:

	Type	Short Title	Funding Source	Prime Grantor	Grant Award Number	FP ID	FP Status	Budget ID	FP Parent State
View	Non Profit	In vivo study: Chinchilla model (tympanic membrane)	The Royal College of Surgeons in Ireland			FP00019696	Active	BU00035404	

3.0 **For funding sources not managed through MCW, list all funding sources and institutions:**

Institution	Funding Source	Title	Grant Number

There are no items to display

There are no items to display

4.0 * Have all of the animal activities described in the federally funded grants listed above been accurately described in this AUA, either by itself or together with one or more other approved AUAs?

- ○ Yes
- ○ No
- ● **N/A**