animal research at DRDC Downsview: a hidden history
DRDC Downsview is one of eight Federal military research facilities. A brief overview is here:

https://en.wikipedia.org/wiki/DRDC_Toronto

Apparently the Downsview location no longer conducts animal tests. I wrote them in April 2018, hoping to arrange the transfer of “spent” lab animals from Downsview to Ontario animal sanctuaries. The Armed Forces Public Inquiry Desk wrote me back on May 1, claiming that “the DRDC Toronto lab in Downsview no longer has an animal facility/capability.”

DRDC animal facilities in Suffield [Alberta] and the Maritimes are still active.

So the appended docs - secured by Liz White and Animal Alliance Canada thru an ATIP request - offer a peek into the historic practices at Downsview [2004 to 2007], and indicate, perhaps, some of on-going research at other DRDC sites in Canada.

The docs reveal:

- “yearly basic trauma-related procedures” : practicing on pigs “different surgical procedures”, “performed under various combinations of stressors”
- Tests on a “proprietary gel” they’ve developed for treating burn wounds, on 130 rats
- Testing another “proprietary wound care agent using a porcine model of partial thickness wounds” on 17 Yorkshire pigs
- “Evaluation of hemostatic agents and dressing materials using an acute rat model of moderate liver hemorrhage” : on 600 rats
- A “partial-thickness burn wounds” study, again, for a “proprietary gel”: on 260 rats. They note that “This scald model has been used in previous DRDC experiments”. Their method of “Euthanasia” is “Cervical Dislocation under anaesthesia”. They also assure the CCAC that “Animals that will lose more than 15% WB, show signs of withdrawal, abnormal breathing rate, undue pain or distress will be sacrificed. The animals will be humanely euthanized within 24 h following burn injury if the nature of the signs of illness (hunched position, reduced muscle tone, lack of planar reflex), their rate of onset and a marked hypothermia (<33C) strongly suggest impending death.”
- A pilot study on 215 New Zealand rabbits “to assess the feasibility of establishing a reliable rabbit model of liver hemorrhage; and 2) determine the hemostatic efficacy of various hemostatic agents”
- A study “to establish a non-lethal model of contaminated open wounds in pigs” on up to 21 Yorkshire pigs. Their justification of species choice: “The pig is used extensively in animal models of wound healing since pig skin is very similar to that of humans.”

DRDC Downsview once maintained an in-house rat colony. They brought in rabbits, pigs, and other rats for a variety of experiments.

DRDC is a voluntary member of the Canadian Council on Animal Care, which means they file overviews of their research protocols with the CCAC to get the “humane” seal of approval. It’s these CCAC applications which Liz managed to get through her ATIP request. They took three years to send her anything, and some of it is blanked out, e.g. names of researchers & suppliers.
some of it is, surprisingly, not blanked out: room numbers in the building where they kept the animals, and room numbers where the experiments took place.

many of these studies involved experiments at the CCAC’s Category D: which is their second-highest category, second-most “invasive”. note that even Category B studies [“Experiments which cause little or no discomfort or distress”] can involve

domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intra-muscular, intraperitoneal, or oral, but not intrathoracic or intracardiac (Category C); acute non-survival studies in which the animals are completely anesthetized and do not regain consciousness; approved methods of euthanasia following rapid unconsciousness, such as anesthetic overdose, or decapitation preceded by sedation or light anesthesia; short periods of food and/or water deprivation equivalent to periods of abstinence in nature.

[from CCAC Policy Statement]

the DRDC may tell you their research is to protect Canadians and help “our peacekeepers” around the globe.
the Defence Corporate Secretary, Isabelle Daoust, wrote me on behalf of our Minister of Defence on May 15:

DND precisely adheres to the CCAC guidelines and internationally accepted protocols to ensure the ethical treatment of animals and that the use of animals is weighed against the acknowledged benefits to the Canadian Armed Forces.

regarding the 2005 study of liver hemorrhage, on 600 rats, the DRDC notes: “Operations Enduring Freedom [the U.S. term for their global ‘war on terrorism’] and Iraqi Freedom [the U.S. term for the second Iraq War] have recommended further study to develop solutions for treatment of non-compressible hemorrhage.” the links between peacekeeping and the interests of U.S. empire seem close, here.

Animal Alliance has an ongoing campaign to end the use of animals in Canadian military research, focused on the use of pigs in trauma training:

https://www.animalalliance.ca/campaigns/other-campaigns/military-trauma-training/
from the CRDC medic-training program, held annually.
appendix: the Downsview docs
form A [2006-2007]

trauma training for medics/medical officers, using pigs. a study in Ottawa, replicating field work in Toronto. DRDC Toronto was asked by [blanked out] to organize a similar field test at [blanked out] in Ottawa

“yearly basic trauma-related procedures” for medics and medical officers wers already held at DRDC Toronto that year [i.e. 2006?]

here’s the protocol overview:

“Up to two male Yorkshire pigs (70-75 kg) will be obtained from [blank], 4-6 times a year. Large pigs are considered the best alternative to using primates when performing trauma-related procedures. The surgical procedures [...] will be performed within 3-6 h of the animals being delivered at [blanked out] The animals will be: fasting for 24 h (instructions will be provided to the supplier); have free access to water until their pre-anaesthesia; and, temporarily, housed in a room that will provide 2-4 m2 of pen space per animal, part of the floor being covered with straw. Following pre-anaesthesia with ketamine (15 mg/kg body weight, i.m.) and acepromazine (0.5 mg/kg body weight, i.m.), the animals will be lifted on a trolley, covered with a blanket, and, wheeled outside the building to a secluded area. The different surgical procedures will then be performed under various combinations of stressors”
ANIMAL USE PROTOCOL

PROTOCOL NO. 12/00
FOR OFFICE USE ONLY

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Surname, First Name, 인(inal)</td>
</tr>
<tr>
<td>2</td>
<td>Title: Establishment of a non-lethal model of full-thickness contaminated wound in pigs.</td>
</tr>
<tr>
<td>3</td>
<td>Proposed start date of research: Day 01, Month Aug, Year 2001</td>
</tr>
<tr>
<td>4</td>
<td>Expected date of completion: Day 310, Month Apr, Year 2002</td>
</tr>
<tr>
<td>5</td>
<td>Type of experiment: Surgical [x], Research [x], Testing</td>
</tr>
<tr>
<td>6</td>
<td>Funding source number: Identifying code (THB) 2001</td>
</tr>
</tbody>
</table>

The experimental procedures described herein represent standards protocols in the TDL's manual (TDL-COT, 1997). If protocol is submitted between scheduled ACC meetings, it may be given "provisional" approval by the Chair, Section Head, Veterinarian, and Community Member Collectively. A copy, however, must be submitted to all members of the committee at that time for formal approval at the next meeting.
1. Make up to 6 lacerations on the animal and suture of laceration(s)
2. Suture of laceration(s)/intravenous cannulation for administration of saline or other clinically-approved fluid (e.g., HSD)
3. Intravenous cannulation for administration of fluids (maybe)
4. Needle decompression of the chest
5. Chest tube insertion/intravenous/intravenous cannulation for administration of saline or other clinically-approved fluid (e.g., HSD)
   Needle-and-surgical cricothyroidotomy
6. Femoral venous or arterial injury, followed by:
   a. Application of hemostatic agents such as OR along with pressure dressing;
   b. Placement of tourniquet
7. Needle and surgical cricothyroidotomy (note: this procedure is listed last as it can be performed once the animal has been euthanized)
   Application of hemostatic agents such as
   OR along with pressure dressing;
   Placement of tourniquet

Laceration & suturing (see Annex C; 60 min):
   Intravenous puncture (see Annex F; 30 min)—(maybe)
   Venous catheter (see Annex B; 60 min)
   Central venous puncture (see Annex A; 30 min)
   Needle thoracostomy (see Annex D; 30 min)
   Chest tube insertion (see Annex F; 60 min)
   Femoral venous or arterial injury
   Needle-and-surgical cricothyroidotomy; see Annex G; 60 min
   Thoracotomy
   Laparotomy

After completion of all surgical procedures (i.e., approximately 5 h), the animals will be humanely euthanized using 10% (i.v.) staff will then disposed of the animal carcasses according to DRDC standard operating procedures.

See Annex A.

Staff Involved

will travel to-er to provide animal care and performing all pre-operative procedures (e.g., anesthesia induction; analgesic administration).

(a surgeon of 1 Cdn Field Hospital) will be responsible for supervising all experimental procedures.

Yearly, up to medical assistants at will be performing the different experimental procedures.

Reference
DESCRIPTION OF PROJECT AND PROCEDURES. Describe in DETAIL all procedures, techniques to be used; emphaizing those performed on animals. Apped additional page(s) as necessary.

Introduction
As part of the medics and medical officers need to must practice yearly basic trauma-related procedures that constitute the standard of care in modern trauma management in order to maintain their surgical skills at the desired level. While all this training was successfully held at DRDC Toronto in the past year under protocol ACC 1 ns has recently asked DRDC to organize a similar animal laboratory practicum on-site at Ottawa, ON, where they can effectively reproduce some of the stressors encountered under which they work in the field. The experiment described in the following protocol will be carried out under the Thrust 20 cf.

Objective
The objective of the present protocol is to allow the medical assistants at the medical practice and maintain their surgical skills under the environmental conditions that they experience during overseas described in published surgical manuals [1, 2].

Methods
Up to three 6 male Yorkshire pigs (70-75 kg) will be obtained from 4-6 lives a year. Human-sized-Large pigs are considered the best alternative to using primate when performing trauma-related procedures. The medical assistants will undergo the surgical procedures (see list below) will be performed within a few hours-3-5 h of the primate being delivered at. The fasting animals will thus be: fasting for 24 h (instructions will be provided to the supplier); housed in a room, and have free access to water until their pre-anesthesia; and, temporarily housed in a room that will provide 2 to 4 m² of pen space per animal, part of the floor being covered with straw. Following pre-anesthesia with ketamine (15 mg/kg body weight, i.m.) and acepromazine (0.5 mg/kg body weight, i.m.), the animal will be lifted on a trolley, covered with a blanket, and wheeled outside the building to a secluded area. The different surgical procedures will then be performed under various combinations of stressors:

Each practicum will be conducted in accordance with the guidelines from the Canadian Council on Animal Care (CCAC) under the supervision of the DRDC Toronto Senior Animal Technician. It is noteworthy that the Principal Investigator and the Senior Animal Technician will visit facilities prior to undertaking the first practicum to ensure that all conditions are favorable for holding animals and performing surgical procedures in compliance with the guidelines from the Canadian Council on Animal Care (CCAC).

Up to medical assistants will be trained weekly in groups of four students. Each pair of medical assistants will use one pig to perform the seven different experimental procedures.

Surgical Procedures
Immediately prior to the scheduled surgical procedures, two pigs will be pre-anesthetized with ketamine (15 mg/kg body weight, i.m.) and acepromazine (0.5 mg/kg body weight, i.m.) followed by gas inhalation (oxygen: 1-2% isoflurane). The experimental protocols to be used are fully described in the ATLS manual [1]. More specifically, the essential steps to be practiced sequentially on each live animal are:
12 SPECIAL ANIMAL CARE REQUIREMENTS. Specify if any special housing, nutritional, temperature, lighting, post-operative care or other facilities or conditions that are or may be required.

The animal will be housed within a 24-hr cycle (12-h light/12-h dark) for at least 7 days before or after surgery. Animals will be fasting, housed individually, and will have free access to water during the study until pre-anesthesia.

Daily injection of long-lasting analgesics for 21-d or as required.

SPECIFY ANIMAL HOUSING ROOM (IF ANY), be determined when visit from DRC Recoding Staff.

SPECIFY EXPERIMENTAL SITE ROOM

13 DRUGS FOR ANAESTHESIA/ANALGESIA

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Pre-Anesthesia</td>
<td>Ketamine</td>
<td>15 mg/kg BW</td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>0.015 mg/kg BW</td>
</tr>
<tr>
<td>B. Anesthesia</td>
<td>Ketamine/Atropine/2% Eutectol</td>
<td>2-4mg/kg BW</td>
</tr>
<tr>
<td></td>
<td>Thorazine</td>
<td>3mg/kg BW</td>
</tr>
<tr>
<td>C. Post-Anesthesia</td>
<td>Metoclopramide</td>
<td>0.1mg/kg BW</td>
</tr>
<tr>
<td></td>
<td>Butorphanol</td>
<td>0.1mg/kg BW</td>
</tr>
<tr>
<td>D. Other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

POST-OPERATIVE CARE needs defined in Section 12 are fully detailed in Section 6 para. - Anesthesia Post-op, NOT APPLICABLE.

14 EUTHANASIA

- Physical euthanasia can be used only with pre-anesthesia.

- Cervical Dislocation

- Exsanguination (under Anesthesia)

- Stunng

- Carbon Dioxide

- NOT APPLICABLE

HAZARDOUS AGENTS AND PRECAUTIONS

Specify each Agent and Potential Hazard (include amount, route, and frequency of admin, precautions OR indicate if included in Section 6). Not Applicable

- Biological: Pe-staphylococcal, Staph. epidermidis, Pe-streptococcal

- Chemical

- Carcinogen

- Radioisotope/Radiation (include RIA Permit No. and expiration date)

- NOT APPLICABLE

ENDPOINTS

Specify Endpoints and Criteria (in detail):

All animals will be closely examined and weighed daily. Animals that lose more than 10% of their BW, show signs of withdrawal, abnormal breathing rate, fever or undue pain or distress will be euthanized.

NOT APPLICABLE.
IDENTIFY THE PRIMARY OBJECTIVE(S) OF THE PROJECT. In layman's terms, please summarize the primary objective(s) and benefit(s) expected from the study.

The objective of the project is to train the medical assistants at the Veterans Affairs hospitals in the VA System to practice and maintain their surgical skills under different stressful environments, and to develop new surgical techniques that can be used in actual surgeries. This model will be useful in future studies to evaluate the efficacy of wound-care agents, including dressings, antibiotics, and growth factors.

RESEARCH STAFF

<table>
<thead>
<tr>
<th>Name</th>
<th>Pref</th>
<th>Tech</th>
<th>PO</th>
<th>Grad</th>
<th>UnderGrad</th>
<th>Terms</th>
<th>Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>From</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>— Valentinian Yehl (VDC)</td>
<td></td>
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<tr>
<td>— Medical Assistants at</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Note: University one-day course; at least one per year required for all personnel, including students, post-doctoral fellows, and RA's. Check if the individual has completed this or equivalent training.

ANIMALS

<table>
<thead>
<tr>
<th>Animal Species (Common Name)</th>
<th>Total Number of Animals</th>
<th>Source of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yorkshire Pig</td>
<td>Up to 10 per project</td>
<td></td>
</tr>
</tbody>
</table>

JUSTIFICATION FOR: A. Species (see Note 1) and B. Number of Animals Used (See Note 2)

A. Pigs are considered the best alternative to using primates when performing trauma-related studies. The pig is used extensively in animal models of wound healing since pigs are very similar to that of humans.

B. Each pair of medical assistants will use one pig. The number of anesthetics should yield sufficient data for completing statistical analysis and performing the required experimental procedures. Only one pig will be used to allow the participant to operate on one animal in case any animal dies or requires additional complications during the procedure. An extra pig will be reserved if needed to replace the pig being operated under this protocol. The extra animal will be used for experiments planned under AC-2004.

Note 1: Is the choice of animal the most scientifically appropriate one, and of the lowest level of sentient consistency with the Objectives? Justified in Section B, App. 4.1, 4.2, 4.3, 4.4, 4.5.1.2, 4.5.1.3, 4.5.2.

Note 2: Is the number of animals based on a plan incorporating statistical considerations that ensure sufficient, but not excessive, number for drawing reliable conclusions? Justified in Section B, App. 4.1, 4.2, 4.3, 4.4, 4.5.1.2, 4.5.1.3, 4.5.2.

ALTERNATIVES: Are non-animal alternatives available for this project? □ YES □ NO
DECLARATION

All animals in this research/teaching proposal will be maintained and used in accordance with the recommendations of the Canadian Council on Animal Care, the requirements under the Animals for Research Act, R.S.O. 1990, and the DCEM Animal Care Policies and Guidelines, and other applicable DCEM policies and procedures.

Principal Investigator __________________________ Date __________

MANAGEMENT APPROVAL

Section or Group Head __________________________ Date __________

ANIMAL CARE COMMITTEE APPROVAL

Chair, Animal Care Committee __________________________ Date __________

DCEM Consultant Veterinarian __________________________ Date __________

OBSERVATIONS, RESTRICTIONS OR CONDITIONS (as applicable), INCLUDE THE NEED FOR (and details of) EXTERNAL PEER REVIEW.

THIS PROTOCOL IS APPROVED FOR THE PERIOD SPECIFIED IN SECTION 2. IT WILL BE REVIEWED SEMI-ANNUALLY AT SCHEDULED DCEM ACC MEETINGS. IF AN EXTENSION IS REQUIRED, A FORMAL AMENDMENT TO THE PROTOCOL MUST BE SUBMITTED FOR APPROVAL BY THE SECTION HEAD, CHAIR AND VETERINARIAN.
protocols for a series of wound studies involving rats and pigs. from what i can tell, over a thousand rats and 44 pigs.

e.g. study “of full thickness, contaminated wounds in pigs” on 21 Yorkshire pigs

tests on a “proprietary gel” they've developed for treating burn wounds, on 130 rats. possible incorporation of gel into field first aid kits for CF soldiers.

testing another “proprietary wound care agent using a porcine model of partial thickness wounds”: 17 Yorkshire pigs.

on 600 rats: “Evaluation of hemostatic agents and dressing materials using an acute rat model of moderate liver hemorrhage.”
<table>
<thead>
<tr>
<th>Protocol No.</th>
<th>CI</th>
<th>Investigator</th>
<th>Protocol Description</th>
<th>PAU*</th>
<th>Species</th>
<th>AA³</th>
<th>AU/YR²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/01</td>
<td>B</td>
<td></td>
<td>Health surveillance program. One or two rats in each room will be identified as sentinels and not used for experimental purposes. Under general anaesthesia, a blood sample for serological testing will be obtained by cardiac puncture. Serum samples will be prepared and tested in-house using the Murine Immunocomb Kit and, if necessary, serology profiles will be performed.</td>
<td>2</td>
<td>Sprague-Dawley Rat</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>1/04</td>
<td>D</td>
<td></td>
<td>Evaluation of the wound-healing properties of a proprietary gel in a rat model of uncontaminated, partial thickness burn wounds. The unique combination of properties in the gel, if confirmed experimentally, would make the product a valuable candidate for incorporation into the DRDC Toronto biomaterial for use in field first aid kits for CF soldiers.</td>
<td>2</td>
<td>Sprague-Dawley Rat</td>
<td>130</td>
<td>0</td>
</tr>
<tr>
<td>2/04</td>
<td>D</td>
<td></td>
<td>Establishment of non-lethal model of full thickness, contaminated wounds in pigs. Delays in providing adequate therapy for open wounds continues to result in high infection rates and infectious complications. The trauma model developed will be used in future studies to evaluate the efficacy of wound-care agents.</td>
<td>2</td>
<td>Yorkshire Pig</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>2/04</td>
<td>D</td>
<td></td>
<td>Standard operating procedures in rats for the evaluation of wound-care agents, including dressings, formulations and antimicrobial agents. The standard operating procedure developed will be used in future studies to determine the bactericidal efficacy. Preliminary testing will be carried out using a four-wound model of infection. Final testing of the compound/dressing under study will be performed using a modification of the rat model developed under ACC 1/98.</td>
<td>2</td>
<td>Sprague-Dawley Rat</td>
<td>450</td>
<td>375</td>
</tr>
<tr>
<td>3/04</td>
<td>D</td>
<td></td>
<td>Evaluation of the wound-healing properties of a proprietary wound care agent using a porcine model of partial thickness wounds. In vitro experiments performed previously confirmed the bactericidal properties of a proprietary gel formulation. This protocol will assess the wound healing properties of the gel in a porcine model. If the results are conclusive, the wound-healing of the DRDC Toronto biomaterial loaded with the active ingredients of the gel will be assessed.</td>
<td>2</td>
<td>Yorkshire Pig</td>
<td>17</td>
<td>4 (3R)</td>
</tr>
<tr>
<td>4/04</td>
<td>D</td>
<td></td>
<td>Evaluation of hemostatic agents and dressing materials using an acute rat model of moderate liver hemorrhage. Uncontrolled bleeding remains a major cause of death in combat. First responders still have limited means to stop truncal hemorrhage. Hemostatic dressings have proven useful where it is possible to mechanically suppress bleeding and they are expensive. Hemostatic dressings have proven useful where it is possible to mechanically suppress bleeding and they are expensive. has caused concerns related to high temperatures generated during the chemical process leading to hemostasis so it is not recommended for soft internal tissues. Operations Enduring Freedom and Iraqi Freedom have recommended further study to develop solutions for treatment of non-compressible hemorrhage.</td>
<td>2</td>
<td>Sprague-Dawley Rat</td>
<td>600</td>
<td>0</td>
</tr>
<tr>
<td>5/04</td>
<td>B</td>
<td></td>
<td></td>
<td>5</td>
<td>Yorkshire Pig</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

A protocol may contain more than one purpose, species and/or level of invasiveness - each should be listed separately, using the same protocol number.

The protocol number, protocol title, name of investigator and name of institution will be kept confidential in all cases. The remaining information will be used in the preparation of annual statistics on the use of animals in Canadian science.

1 CI Category of Invasiveness
2 Protocol Description Please give a descriptive protocol title that indicates, in lay terms, the nature of the procedures used (preferably in 40 words or less)
3 AA No. of Animals Approved
“partial-thickness burn wounds” in rats. again, for a “proprietary gel”

not peer-reviewed

“The veterinary technician will also be responsible for performing all burn procedures after adequate training from [blanked out]

manufacturer of DRDC dressing is blanked out

“This scald model has been used in previous DRDC experiments”

130 x 2 rats [half bred in-house]

weirdly, they list the

Animal Housing Room: 1412
Experimental Site Room: 1404

method of “Euthanasia” is “Cervical Dislocation under anaesthesia”

“Animals that will lose more than 15% WB, show signs of withdrawal, abnormal breathing rate, undue pain or distress will be sacrificed. The animals will be humanely euthanized within 24 h following burn injury if the nature of the signs of illness (hunched position, reduced muscle tone, lack of planar reflex), their rate of onset and a marked hypothermia (<33C) strongly suggest impending death.”
DRDC TORONTO

ANIMAL USE PROTOCOL

PROTOCOL NO. 1/04

FOR OFFICE USE ONLY

Expiry Date

<table>
<thead>
<tr>
<th>Surname</th>
<th>First Name</th>
<th>Initial</th>
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<th>Section</th>
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<td>OM</td>
<td>TG</td>
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</table>

<table>
<thead>
<tr>
<th>DRDC Telephone</th>
<th>Residence Telephone</th>
</tr>
</thead>
</table>

NAME OF DESIGNATED ALTERNATE(S) FOR EMERGENCIES

<table>
<thead>
<tr>
<th>Name</th>
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<th>Residence Telephone</th>
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<td></td>
<td>Residence Telephone</td>
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</tbody>
</table>

Title

Evaluation of the wound-healing properties of a proprietary gel in a rat model of uncontaminated, partial-thickness burn wounds

PROPOSED START DATE OF RESEARCH

Day | Month | Year
--- | ----- | ----
01  | 10    | 04

EXPECTED DATE OF COMPLETION

Day | Month | Year
--- | ----- | ----
31  | 12    | 05

CATEGORY OF INVASIVENESS (Refer to Canadian Council on Animal Care Categories A, B, C, D, E).

- [ ] Burn injury
- [ ] s.c. injection analgiesic
- [ ]

LIST EACH experimental condition or manipulation that could cause pain or discomfort in the conscious animal and for each indicate the expected degree of pain/discomfort using the CCAC Codes.

FOR EACH, indicate (by checking the box) the conditions that will be alleviated and ensure that the drugs to be used are stated in Section 13 and procedures described in Section 6.

TYPE OF EXPERIMENT

- [ ] Research
- [x] Testing

SURGICAL

- [ ] Acute
- [x] Survival

NON-SURGICAL

- [x] Acute
- [ ] Chronic

FUNDING SOURCE NUMBER: 300005 (i.e., Financial Coding, WBE)

PEER REVIEWED

- [ ] YES
- [x] NO

IF YES, ATTACH REVIEW DOCUMENTATION.

NOTE 1. AN INDEPENDENT PEER REVIEW OF SCIENTIFIC MERIT IS REQUIRED FOR ALL NEW RESEARCH PROJECTS.

This protocol has previously been submitted

NOTE 2. IF A PROTOCOL IS SUBMITTED BEFORE SCHEDULED ACC MEETINGS, IT MAY BE GIVEN 'PROVISIONAL' APPROVAL BY THE CHAIR, SECTION HEAD, VETERINARIAN, AND COMMUNITY MEMBER COLLECTIVELY. A COPY, HOWEVER, MUST BE SUBMITTED TO ALL MEMBERS OF THE COMMITTEE AT THAT TIME FOR FORMAL APPROVAL AT THE NEXT MEETING.
DESCRIPTION OF PROJECT AND PROCEDURES. Describe in DETAIL all procedures, techniques to be used, emphasizing those performed on animals. Include all justifications required from Sections 7-16. Append additional page(s) as necessary.

See document enclosed for details of experimental procedures.

and the will be responsible for performing all pre-operative procedures, incl. anesthesia induction; analgesic administration. The veterinary technician will also be responsible for performing all burn procedures after adequate training from

Co-op students will initially be responsible for preparation of gel formulations, care of animals during acclimation period, and post-operative observation of the animals. After an appropriate period of observation (i.e., at least 16 animals having undergone the burn protocol), they will take over duties (i.e., pre-operative procedures described above only).

An on-call staff from familiar with the l.p. injection of capsaicin in newborn rats, will be responsible for performing this technique.
IDENTIFY THE PRIMARY OBJECTIVE(S) OF THE PROJECT. In layman's terms, please summarize the primary objective(s) and benefit(s) expected from the study.

The objective of the present study is to determine whether a 6-hour application of the 2nd generation of DRDC dressing, now manufactured by ,
exerts any changes in whole body or skin temperature in a non-lethal rat model of non-contaminated burn wounds. This scalable model has been used in previous DRDC experiments (ACC 1/97 amendment #2).

<table>
<thead>
<tr>
<th>Name</th>
<th>Prof</th>
<th>Tech</th>
<th>PD</th>
<th>Grad</th>
<th>UnderGrad</th>
<th>Term</th>
<th>Training</th>
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<tbody>
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<tr>
<td>Co-op students</td>
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<td>(TBD)</td>
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</tr>
</tbody>
</table>

Note: Universally one-day course, at minimum, is mandatory for all personnel, including students, post-docs, visiting fellows, RAS.
Check if the individual has completed this or equivalent training.
Note: The appended experimental methods should indicate the responsibilities of each individual listed above.

<table>
<thead>
<tr>
<th>Animal Species (Common Name)</th>
<th>Total Number of Animals</th>
<th>Source of Animals</th>
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</thead>
<tbody>
<tr>
<td>Rat</td>
<td>130</td>
<td>bred in house</td>
</tr>
</tbody>
</table>

JUSTIFICATION FOR: A. Species (see Note 1) and B. Number of Animals Used (See Note 2)

A. The rat species has been used extensively in studies of burn injury, especially for testing wound-care products.

B. The number of animals per experimental group should yield sufficient data for completing statistical analysis.

Note 1: Is the choice of animal the most scientifically appropriate one, and of the lowest level of sentience consistent with the Objectives? Justified in Section 6.06.06.06.01

Note 2: Is the number of animals based on a plan incorporating statistical considerations that ensure sufficient, but not excessive numbers for drawing reliable conclusions? Justified in Section 5.06.06.06.01

ALTERNATIVES: Are non-animal alternatives available for this project? ☐ YES ☒ NO
SPECIAL ANIMAL CARE REQUIREMENTS. Specify, if any, special housing, nutritional, temperature, lighting, post-operative care or other facilities or conditions that are or may be required.

N/A

SPECIFY ANIMAL HOUSING ROOM 1412

SPECIFY EXPERIMENTAL SITE ROOM 1404

<table>
<thead>
<tr>
<th>DRUGS FOR ANAESTHESIA/ANALGESIA</th>
<th>DRUG</th>
<th>DOSAGE</th>
<th>ROUTE OF ADMINISTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Pre-Anaesthesia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Anaesthesia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Anaesthesia</td>
<td>Halothane</td>
<td>to effect</td>
<td>inhalation</td>
</tr>
<tr>
<td>Anaesthesia</td>
<td>nitrous oxide</td>
<td>to effect</td>
<td>inhalation</td>
</tr>
<tr>
<td>C. Pre-Analgicic</td>
<td>buprenorphine</td>
<td>50 µg/kg</td>
<td>s.c.</td>
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<tr>
<td>Post-Analgicic</td>
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<tr>
<td>D. Other</td>
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</tr>
</tbody>
</table>

POST-OPERATIVE CARE needs defined in Section 12 are fully detailed in annex A, p. 2 l. 12.13.

If anaesthesia or analgesia NOT TO BE USED in invasive protocol, justification in Section 6 para

☐ Anaesthetic Overdose (specify agent)

☒ Cervical Dislocation under anaesthesia

☐ Exsanguination (under Anaesthesia)

☐ Stunning

☐ Carbon Dioxide

NOTE: Physical euthanasia can be used only with pre-anaesthesia. If this is not possible, justification must be included:

☐ Decapitation

☐ Pithing

☐ Other

IF NOT EUTHANIZED, please indicate how and where disposed of.

Specify each Agent and Potential Hazard (include amount, route, and Frequency of admin, precautions OR Indicate if included in Section 6).

☐ Biological

☐ Chemical

☐ Carcinogen

☐ Radiolotope/Radiation (Include R/A Permit No. and Expiration Date)

NOTE: If special precautions are required, ensure these are detailed in Section 12.

16

Specify Endpoints and Criteria (in detail):

All animals will be closely examined and weighed daily. Animals that will lose more than 15% WB, show signs of withdrawal, abnormal breathing rate, undue pain or distress will be sacrificed. The animals will be humanely euthanized within 24 h following burn injury if the nature of the signs of illness (hunched position, reduced muscle tone, lack of plantar reflex), their rate of onset and a marked hypothermia (<33°C) strongly suggest an impending death.

Defining humane endpoints is imperative if the project will lead to pain or discomfort or any physical or physiological abnormality that would affect the animal's well-being. The criteria that will be employed to remove the animal from the study and euthanized prior to the onset of unrelievable pain or distress, moribundity or death MUST be stated here and in the protocol.
All animals in this research/testing proposal will be maintained and used in accordance with the recommendations of the Canadian Council on Animal Care, the requirements under the Animals for Research Act, R.S.O. 1990, and the DCIEM Animal Care Policies And Guidelines, and other applicable DCIEM policies and procedures.

Principal Investigator __________________________ Date __________________________

Not Approved

Section or Group Head __________________________ Date __________________________

ACC Approval

Chair/Animal Care Committee __________________________ Date __________________________

DCIEM Consultant Veterinarian __________________________ Date __________________________

OBSERVATIONS, RESTRICTIONS OR CONDITIONS (as applicable). INCLUDE THE NEED FOR (and details of) EXTERNAL PEER REVIEW.

THIS PROTOCOL IS APPROVED FOR THE PERIOD SPECIFIED IN SECTION 3. IT WILL BE REVIEWED SEMI-ANNUALLY AT SCHEDULED DCIEM ACC MEETINGS. IF AN EXTENSION IS REQUIRED, A FORMAL AMENDMENT TO THE PROTOCOL MUST BE SUBMITTED FOR APPROVAL BY THE SECTION HEAD, CHAIR AND VETERINARIAN.
form D [2006]

box check: “liver injury”

blacked-out but readable: “The objectives of this protocol are to 1) perform a pilot study to assess the feasibility of establishing a reliable rabbit model of liver hemorrhage; and 2) determine the hemostatic efficacy of various hemostatic agents”

on 215 [maximum] New Zealand rabbits

Source of Animals: blanked

justified in part because “there are currently no in vitro model [sic] simulating bleeding time.”

euthanasia: intracardiac [injection into heart]

looks like there’s no relevant post-operative care - the liver laceration is under surgical anaesthesia, and the animal is killed by intracardial injection on the operating table. from what i can tell.
**DRDC Toronto**

**ANIMAL USE PROTOCOL**

<table>
<thead>
<tr>
<th>Surname</th>
<th>First Name</th>
<th>Initial</th>
</tr>
</thead>
</table>

<table>
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<tr>
<th>Rank/Position</th>
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<th>Group</th>
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<td>OMS</td>
<td>Trauma</td>
</tr>
<tr>
<td>DCIEM Telephone</td>
<td>Residence Telephone</td>
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</tr>
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</table>

**NAME OF DESIGNATED ALTERNATE(S) FOR EMERGENCIES**

<table>
<thead>
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<tbody>
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<table>
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<tr>
<th>Title</th>
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**PROPOSED START DATE OF RESEARCH**

<table>
<thead>
<tr>
<th>Day</th>
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<tr>
<td>1</td>
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**EXPECTED DATE OF COMPLETION**

<table>
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<tr>
<td>31</td>
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<td>2006</td>
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</table>

**CATEGORY OF INVASIVENESS (Refer to Canadian Council on Animal Care Categories A, B, C, D, E)**

- ☑ B. Shaving (under anesthesia)
- ☑ B. Liver injury (under anesthesia)
- ☑ B. Wound (under anesthesia)

**LIST EACH experimental condition or manipulation that could cause pain or discomfort in the conscious animal and for each indicate the expected degree of pain/discomfort using the CCAC Codes. FOR EACH, Indicate (by checking the box) the conditions that will be alleviated and ensure that the drugs to be used are stated in Section 13 and procedures described in Section 6.**

**TYPE OF EXPERIMENT**

<table>
<thead>
<tr>
<th>SURGICAL</th>
<th>Research</th>
<th>NON-SURGICAL</th>
<th>Acute</th>
<th>Chronic</th>
</tr>
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</table>

| FUNDING SOURCE NUMBER: | 16ci01; 30d05 (i.e., Financial Coding, WBE) |

<table>
<thead>
<tr>
<th>FEER REVIEWED</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
</table>

**IF PROTOCOL IS SUBMITTED BETWEEN SCHEDULED ACC MEETINGS, IT MAY BE GIVEN 'PROVISIONAL' APPROVAL BY THE CHAIR, SECTION HEAD, VETERINARIAN, AND COMMUNITY MEMBER COLLECTIVELY. A COPY, HOWEVER, MUST BE SUBMITTED TO ALL MEMBERS OF THE COMMITTEE AT THAT TIME FOR FORMAL APPROVAL AT THE NEXT MEETING.**
DESCRIPTION OF PROJECT AND PROCEDURES. Describe in DETAIL all procedures, techniques to be used; emphasizing those performed on animals. Append additional page(s) as necessary.
IDENTIFY THE PRIMARY OBJECTIVE(S) OF THE PROJECT. In layman's terms, please summarize the primary objective(s) and benefit(s) expected from the study.

RESEARCH STAFF

<table>
<thead>
<tr>
<th>Name</th>
<th>Prof</th>
<th>Tech</th>
<th>PD</th>
<th>Grad</th>
<th>UnderGrad</th>
<th>Term</th>
<th>Training</th>
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Note: University one-day course, at minimum, is mandatory for all personnel, including students, post-docs, visiting fellows, RAS. Check if the individual has completed this or equivalent training.

Note: The appended experimental methods should indicate the responsibilities of each individual listed above.

ANIMALS

<table>
<thead>
<tr>
<th>Animal Species (Common Name)</th>
<th>Total Number of Animals</th>
<th>Source of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mus musculus (Lab)</td>
<td></td>
<td>Masco</td>
</tr>
</tbody>
</table>

JUSTIFICATION FOR: A. Species (see Note 1) and B. Number of Animals Used (See Note 2)

A. ☒ The choice of animal is the most scientifically appropriate one, and of the lowest level of sentient consistent with the Objectives? Justified in Section 6.

B. ☒ The number of animals is used in the study is necessary and minimal. Justified in Section 6.

Note 1: ☒ Is the choice of animal the most scientifically appropriate one, and of the lowest level of sentient consistent with the Objectives? Justified in Section 6.

Note 2: ☒ Is the number of animals based on a plan incorporating statistical considerations that ensure sufficient, but not excessive numbers for drawing reliable conclusions?

ALTERNATIVES: Are non-animal alternatives available for this project? [ ] YES [ ] NO

There are currently no in vitro model simulating bleeding time.
12 SPECIAL ANIMAL CARE REQUIREMENTS. Specify, if any, special housing, nutritional, temperature, lighting, post-operative care or other facilities or conditions that are or may be required.
N/A

SPECIFY ANIMAL HOUSING ROOM [X]

SPECIFY EXPERIMENTAL SITE ROOM [X]

13 DRUGS FOR ANAESTHESIA/ANAGESIA
Is Anaesthesia to be used? ☑ YES ☐ NO Is Analgesia to be used? ☑ YES ☐ NO
If yes, complete Section 13 below. If no to either, justification is in Section 6 para

A. Pre-Apneaesthesia
Pre-Anaesthesia
B. Anaesthesia
Anaesthesia
C. Pre-Analgesic
Post-Analgesic
D. Other

POST-OPERATIVE CARE needs defined in Section 12 are fully detailed in Section 6 para N/A.

14 EUTHANASIA

☑ Anaesthetic Overdose (specify agent)
☐ Cervical Dislocation
☐ Exsanguination (under Anaesthesia)
☐ Stunning
☐ Carbon Dioxide

NOTE: Physical euthanasia can be used only with pre-anaesthesia. If this is not possible, justification must be included:

☐ Decapitation ☐ Pithing ☑ Other [X]

IF NOT EUTHANIZED, please indicate how and where disposed of:

HAZARDOUS AGENTS AND PRECAUTIONS

Specify each Agent and Potential Hazard (include amount, route, and Frequency of admin, precautions OR indicate if included in Section 5).

☐ Biological
☐ Chemical
☐ Carcinogen
☐ Radiosotope/Radiation (include R/A Permit No. and Expiration Date)

NOTE: If special precautions are required, ensure these are detailed in Section 12.

15 ENDPOINTS
Specify Endpoints and Criteria (in detail):
N/A

Defining humane endpoints is imperative if the project will lead to pain or discomfort or any physical or physiological abnormality that would affect the animal's well-being. The criteria that will be employed to remove the animal from the study and euthanized prior to the onset of inreleasable pain or distress, mortality or death MUST be stated here and in the protocol.
DECLARATION
All animals in this research/testing proposal will be maintained and used in accordance with the recommendations of the Canadian Council on Animal Care, the requirements under the Animals for Research Act, R.S.O. 1980, and the DCIEM Animal Care Policies And Guidelines, and other applicable DCIEM policies and procedures.

Principal Investigator __________________________ Date __________________________

MANAGEMENT APPROVAL

Section or Group Head __________________________ Date __________________________

ANIMAL CARE COMMITTEE APPROVAL

Chair/Animal Care Committee __________________________ Date __________________________

DCIEM Consultant Veterinarian __________________________ Date __________________________

OBSERVATIONS, RESTRICTIONS OR CONDITIONS (as applicable). INCLUDE THE NEED FOR (and details of) EXTERNAL PEER REVIEW.

THIS PROTOCOL IS APPROVED FOR THE PERIOD SPECIFIED IN SECTION 3. IT WILL BE REVIEWED SEMI-ANNUALLY AT SCHEDULED DCIEM ACC MEETINGS. IF AN EXTENSION IS REQUIRED, A FORMAL AMENDMENT TO THE PROTOCOL MUST BE SUBMITTED FOR APPROVAL BY THE SECTION HEAD, CHAIR AND VETERINARIAN.
more detail on rabbit hemorrhage study - likely the study of 2006 in Form D

looks like they tried with rats but had trouble because “this species possesses an enormous ability to control even massive bleeding (e.g., 30% total blood volume shed) without the need for further adjuncts, a finding recently confirmed by another scientist”

after “liver-resection”, bleeding is monitored for 30 mins, “after which period they will be humanely euthanized by intracardiac injection of T-61.”
ATLAS OF SURGICAL OPERATIONS

ZOLLINGER / ZOLLINGER
Evaluation of hemostatic agents using an acute rabbit model of moderate liver hemorrhage

Introduction

Uncontrolled bleeding remains a major cause of death in combat, up to 25% of the evacuated casualties dying of hemorrhagic complications at the first echelon hospital. Up to 90% of all hemorrhagic deaths in Vietnam were from truncal injury. First responders still have limited means at their disposal to stop truncal hemorrhage. While hemostatic dressings such as the and the bandages have proven useful in situations where it is possible to mechanically suppress the bleeding, they are expensive. The cheap, hemostatic powder was initially touted as the magic bullet in that it required only a sprinkling over the severely bleeding tissue or organ. However, concerns related to the high temperatures generated during the chemical process leading to hemostasis have limited its use to life-threatening situations only, and it is not recommended for use on soft internal tissues such as liver and spleen. Not surprisingly, medics in the recent Operations Enduring Freedom and Iraqi Freedom have therefore recommended that further R&D be performed to develop better solutions for the treatment of non-compressible hemorrhage.

We have recently attempted to develop a non-heparinized rat model of non-compressible liver hemorrhage (ACC 5_04). However, this species possesses an enormous ability to control even massive bleeding (e.g., 30% total blood volume shed) without the need for further adjuncts, a finding recently confirmed by another scientist (personal communication). The experiments described in the present protocol will be carried out under Business Line 3 Gel; 30dd05) as well as the MOM Thrust 6c (specifically in support of the Work Unit on Hemorrhage Control; 16ci01).
Objectives

The objectives of the present protocol are to establish a rabbit model of liver bleeding, and to determine the efficacy of two hemostatic agents in this model of non-compressible hemorrhage.

Methods

Up to 225 male, New Zealand white rabbits, weighing 2.0 – 2.5 kg, will be obtained from... We will use up to 10 rabbits to establish the liver injury model. If the pilot study is successful, up to 15 rabbits per experimental group tested (1 control group for each of the 3 studies listed in Table 1; 10 experimental groups; incl. 5 spare animals). The animals will be housed individually and allowed to adapt to the environmental conditions (22°C, 12 h light/dark cycle) for 2-3 days before undergoing surgery. Animals have free access to food and water until pre-anesthesia.

Table 1. Hemostatic agents or dressings to be tested

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Sample size*</th>
<th>Name</th>
<th>Manufacturer</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1-5</td>
<td>75</td>
<td>Hemostatic peptide (up to 5 doses)</td>
<td>DRDC Toronto</td>
<td>Gelatin-based material</td>
</tr>
<tr>
<td>A2</td>
<td>6-8</td>
<td>45</td>
<td>Recombinant activated Factor VIIa (up to 3 doses; 75, 100 and 125 μg/kg body weight)</td>
<td>powder</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>15</td>
<td></td>
<td></td>
<td>gel</td>
</tr>
<tr>
<td>C</td>
<td>10-11</td>
<td>30</td>
<td>TIF material with peptide (up to 2 formulations; effective dose in study A)</td>
<td>powder</td>
<td></td>
</tr>
</tbody>
</table>

* Add 15 animals per study (n=45)
Pilot study: development of the liver-resection model in the heparinized rabbit

We intend to develop a simple, reproducible, heparinized liver injury model of uncontrolled, moderate hemorrhage recently described by [1]. Briefly, rabbits will be wrapped in a blanket and gently held to induce anesthesia (2.0% halothane: O₂). Their abdomen will be clipped, depilated, and cleansed using standard procedures. Heparin (5 IU/mL in normal saline; will be administered through a 22 G i.v. catheter by continuous infusion syringe infusion pump model 55-4153;
in the rabbit marginal ear vein at a rate of 1 mL/min for 20 min. Following a 40-min waiting period to allow the coagulation parameters (i.e., activated partial thromboplastin time, APTT) to return to normal [1], a mid-line laparotomy will be performed. The left lateral lobe of the liver will then be gently positioned into a pre-weighed funnel leading into a 100-mL beaker. Care will be taken to keep the other lobes (i.e., right lateral, caudate, and median) outside the funnel during the entire study period by gently holding them back with a string of tubular elastic net bandage.

We have previously determined that this method was very effective to isolate the left lateral lobe (unpublished data; ACC 5_04). An area (40 mm x 4 mm) of the left lateral lobe will be marked with a ruler, then resected using curved scissors while holding the lobe between dry gauzes. A strip of pre-weighed, sterile, unmedicated 2- ply gauze will immediately be applied against the cut surface, and the bleeding will be monitored for the ensuing 30 min. This time period was selected based on [1], who have shown that the hemostasis is achieved within 14 ± 3 min, with an average blood loss of 49 ± 7 mL (i.e., approximately 30% of the total blood volume).
In an attempt to reduce the inter-individual variability in liver anatomy (and thus vascularity), blood loss (BL; in mL) will be standardized for the amount of liver tissue removed:

\[ BL = \frac{1}{Lobe_{resected}} \times \left[ (F_{30\, \text{min}} - F_{0\, \text{min}}) + (T_{30\, \text{min}} - T_{0\, \text{min}}) + (G_{30\, \text{min}} - G_{0\, \text{min}}) - V_A \right] \]

where \( F, T \) and \( G \) correspond to the weights of the funnel, beaker and gauze, respectively; \( V_A \) is the volume of hemostatic agent solution used; and, the rabbit's blood density is 1.0431 g/mL [2]. The animals will be monitored for 30 min, after which period they will be humanely euthanized by intracardiac injection of T-61. The remaining portion of the left lateral lobe will then be dissected out and weighed. No mortality occurring within the 30-min bleeding period [1].

These experimental procedures will be performed initially in three rabbits. This methodology is comparable to that described in [1], with the exception that these authors initiated their experimental treatment immediately following the cessation of the infusion period (while the APTT value was elevated to approximately three times the normal range). Their study also showed that no significant changes in APTT were observed over the 20-min infusion period in a very lightly heparinized animal (i.e., using 0.2 IU/mL), the total blood loss and bleeding times averaging 18 ± 9 mL (i.e., 10% total blood loss) and 6 ± 2 min, respectively. As the half-life of heparin is relatively short in the rabbit [3], it is possible that we measure comparable values to those stated in the lightly heparinized rabbit model above, despite initially using a higher heparin concentration, as the APTT will have returned to close to a normal value during the 40-min waiting period. If this is the case, we will then opt to initiate the treatment immediately after completion of the infusion procedures in the remaining 7 animals, while the APTT values remain presumably high. One may then question the relevance of this model to the battlefield scenario (i.e., non-
heparinized casualty). However, to our knowledge, all current animal models of liver [1] or kidney injury [4] have involved heparinized animals, likely due to the difficulty in otherwise ensuring that large volumes of blood (comparable to those expected in battlefield casualties) will be lost. Nevertheless, this animal model would remain highly relevant to the clinical setting as it will mimic the conditions following a full heparinization prior to hepatic surgery. The pilot will be judged successful and testing will proceed if a reproducible blood loss can be measured (i.e., coefficient of approx 10%; 25-30% blood volume shed).

**Experimental protocol for testing an hemostatic agent or wound dressing using the liver injury wound model**

Liver injury will be induced in the anesthetized rabbits, as described above. No blood will be removed from the damaged liver surface before application of the dressing or hemostatic agent under study. The liver should be actively bleeding when the experimental treatment is applied. A pre-weighed amount of will be applied on a sterile gauze. Alternately, the gauze will be pre-moistened with a solution containing various concentrations of the peptide or Factor VIIa. Additional volumes of these agents or saline will be applied to the blood-drenched gauze (using a syringe) every 30 s for the first 5 min due to expected loss of agent through turbulence. Animals will be monitored for 30 min after application of the gauze to the wound, as described previously.

Pre-screening of the hemostatic properties of treatments #1-8 will be performed in 80 rabbits (n=10 per treatment). Recombinant activated Factor VIIa has been shown repeatedly to be hemostatic when injected systemically at doses ranging from 75-125 μg/kg body weight [5-6]. To our knowledge, this agent has never been applied topically. However, it is known that application of anti-Factor VIIa will induce thrombolysis [7]. Recent *in vitro* data has shown that the
contractor-designed peptide is hemostatic (DRDC Toronto, personal communication). If no significant hemostatic effect is apparent in Study 1A compared to non-treated animals, no further testing of treatments #1-5 will be performed, and study C will not be performed. Alternately, sample size will be increased to 15 in the event that these treatments exert a nearly significant hemostatic effect when applied to the injured liver. Furthermore, if the result is already significant (p<0.05) at the smaller sample size (i.e., n=10), no further animals will be tested. If deemed hemostatic, the peptide will be incorporated into the DRDC dressing material at its maximum effective dose. Their ability to diffuse through the material and exert their hemostatic effect will be tested using the liver injury model (n=8). Sample size will be increased to fifteen if the hemostatic effect measured is significant, to achieve statistical significance. Furthermore, if the result is already significant at the smaller sample size, no further animals will be tested.

Statistical analysis

Statistical analyses will be completed using Statistica (Version 6.1, Statsoft, Inc.). In each study, a one-way analysis of variance will be used to determine statistical significance among groups for respective differences in hemostatic efficacy. When statistical significance will be determined, a Neumann-Keuls post-hoc analysis will be performed to locate significant differences. Significance will be deemed to exist when p<0.05.

REFERENCES
“to establish a non-lethal model of contaminated open wounds in pigs.”: up to 21 Yorkshire pigs

justification for species: “The pig is used extensively in animal models of wound healing since pig skin is very similar to that of humans.”

involves hazardous biological agents Ps. aerughosa, Staph.epidermis, and Fusobacterium necrotum

“wound contamination characteristics over a 21-d study period.”

“Two samples will be taken (using a 4-mm biopsy punch) from pre-selected wounds . . . on days 0, 1, 3, 7, 10, 14, 17 and 21, with the animal under general anesthesia”

animals to be euthanized at end of 21 days using T61 (i.v.) following sedation
**DCIEM**

**ANIMAL USE PROTOCOL**

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**NAME OF DESIGNATED ALTERNATE(S) FOR EMERGENCIES**

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**Title**

Establishment of a non-lethal model of full thickness, contaminated wounds in pigs

**PROPOSED START DATE OF RESEARCH**

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**EXPECTED DATE OF COMPLETION**

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**CATEGORY OF INVASIVENESS**

(Refer to Canadian Council on Animal Care Categories A, B, C, D, E).

- X B. Shaving (under anaesthesia)
- X B. Wound injury (under anaesthesia)
- X B. Pre-med, mild discomfort, I.M. injection
- X D. Recovery from wound injury

**LIST EACH experimental condition or manipulation that could cause pain or discomfort in the conscious animal and for each indicate the expected degree of pain/discomfort using the CCAC Codes.**

**FOR EACH, indicate (by checking the box) the conditions that will be alleviated and ensure that the drugs to be used are stated in Section 13 and procedures described in Section 6.**

**TYPE OF EXPERIMENT**

- X Research
- X Testing

**SURGICAL**

- Acute
- Survival
- Non-Surgical

**FUNDING SOURCE NUMBER:** 16aw1993400 (l.e., Financial Coding, WBE)

**PEER REVIEWED**

- X YES
- No

IF YES, ATTACH REVIEW DOCUMENTATION.

This protocol has been used extensively in the laboratories of

IF PROTOCOL IS SUBMITTED BETWEEN SCHEDULED ACC MEETINGS, IT MAY BE GIVEN 'PROVISIONAL' APPROVAL BY THE CHAIR, SECTION HEAD, VETERINARIAN, AND COMMUNITY MEMBER COLLECTIVELY. A COPY, HOWEVER, MUST BE SUBMITTED TO ALL MEMBERS OF THE COMMITTEE AT THAT TIME FOR FORMAL APPROVAL AT THE NEXT MEETING.

A0369287_1-A-2012-00705-0136
DESCRIPTION OF PROJECT AND PROCEDURES. Describe in DETAIL all procedures, techniques to be used, emphasizing those performed on animals. Append additional page(s) as necessary.

See Annex A.

and/or veterinarian technician will be responsible for performing all pre-operative procedures, incl. anesthesia induction; analgesic administration. will also be responsible for performing all wound injury procedures.

Co-op students will be responsible for preparation of gel formulations, dressing materials, etc., care of animals during acclimation period, and post-operative observation of the animals.
IDENTIFY THE PRIMARY OBJECTIVE(S) OF THE PROJECT. In layman’s terms, please summarize the primary objective(s) and benefit(s) expected from the study.

To establish a non-lethal model of contaminated open wounds in pigs. This model will be useful in future studies to evaluate the efficacy of wound care agents, including dressings and antiseptics.

RESEARCH STAFF

<table>
<thead>
<tr>
<th>Name</th>
<th>Prof</th>
<th>Tech</th>
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Note: University one-day course, at minimum, is mandatory for all personnel, including students, post-docs, visiting fellows, RAG. Check if the individual has completed this or equivalent training.

Note: The appended experimental methods should indicate the responsibilities of each individual listed above.

ANIMALS

<table>
<thead>
<tr>
<th>Animal Species (Common Name)</th>
<th>Total Number of Animals</th>
<th>Source of Animals</th>
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</thead>
<tbody>
<tr>
<td>Yorkshire Pig</td>
<td>Up to 21</td>
<td></td>
</tr>
</tbody>
</table>

JUSTIFICATION FOR: A. Species (see Note 1) and B. Number of Animals Used (See Note 2)

A. The pig is used extensively in animal models of wound healing since pig skin is very similar to that of humans.

B. The number of animals should yield sufficient data for completing statistical analysis.

Note 1: Is the choice of animal the most scientifically appropriate one, and of the lowest level of sentience consistent with the Objectives? Justified in Section 6. Alternatives?

Note 2: Is the number of animals based on a plan incorporating statistical considerations that ensure sufficient, but not Excessive numbers for drawing reliable conclusions? Justified in Section 6 para. 5.1.3.6.

ALTERNATIVES: Are non-animal alternatives available for this project? ☐ YES ☒ NO
SPECIAL ANIMAL CARE REQUIREMENTS. Specify if any, special housing, nutritional, temperature, lighting, post-operative care or other facilities or conditions that are or may be required.

See Annex A. Daily injection of long-acting analgesics for 21 d or as required.

SPECIFY ANIMAL HOUSING ROOM 0631

SPECIFY EXPERIMENTAL SITE ROOM 0324

DRUGS FOR ANAESTHESIA/ANAGESIA

Is Anaesthesia to be used? ☑ YES ☐ NO

Is Analgesia to be used? ☑ YES ☐ NO

If yes, complete Section 13 below. If no to either, justification is in Section 6 para

A. Pre-Anaesthesia

DRUG:

Induction:

DOSAGE:

ROUTE OF ADMINISTRATION:

B. Anaesthesia

To effect:

Inhalation

C. Post-Anaesthesia

D. Other

POST-OPERATIVE CARE needs defined in Section 12 are fully detailed in Section 6 para Annex A p 4, 13-15.

EUTHANASIA

☐ Anaesthetic overdose/overdose with ketamine and/or xylazine
☐ Cervical Dislocation
☐ Exsanguination (under Anaesthesia)
☐ Stun Gun
☐ Carbon Dioxide

NOTE: Physical euthanasia can be used only with pre-anaesthesia. If this is not possible, justification must be included:

☐ Decapitation ☐ Pithing ☐ Other

IF NOT EUTHANIZED, please indicate how and where disposed of:

HAZARDOUS AGENTS AND PRECAUTIONS

Specify each Agent and Potential Hazard (include amount, route, and frequency of admin, precautions OR indicate if included in Section 6).

☐ Biological: Ps. aeruginosa, Staph. epidermidis

☐ Chemical

☐ Carcinogen

☐ Radiotrace/Radiation (include FDA Permit No. and Expiration Date)

NOTE: If special precautions are required, ensure these are detailed in Section 12.

ENDPOINTS

Specify Endpoints and Criteria (in detail):

All animals will be closely examined and weighed daily. Animals that will lose more than 15% of their body weight, show signs of withdrawal, abnormal breathing rate, fever or undue pain or distress will be euthanized.

Defining humane endpoints is imperative if the project will lead to pain or discomfort or any physical or physiological abnormality that would affect the animal's well-being. The criteria that will be employed to remove the animal from the study and euthanized prior to the onset of unrelievable pain or distress, moribundity or death MUST be stated here and in the protocol.
DECLARATION
All animals in this research/proposal will be maintained and used in accordance with the recommendations of the Canadian Council on Animal Care, the requirements under the Animals for Research Act, R.S.O. 1990, and the DCEM Animal Care Policies and Guidelines, and other applicable DCEM policies and procedures.

Principal Investigator Date

MANAGEMENT APPROVAL

Section or Group Head Date

ANIMAL CARE COMMITTEE APPROVAL

Chair/Animal Care Committee Date

DCEM Consultant Veterinarian Date

OBSERVATIONS, RESTRICTIONS OR CONDITIONS (as applicable). INCLUDE THE NEED FOR (and details of) EXTERNAL PEER REVIEW.

APPROVED WITH THE CHANGE OF ANALGESIC FROM BUPRENORPHINE I.M. TO PHENTANYL PATCH.

THIS PROTOCOL IS APPROVED FOR THE PERIOD SPECIFIED IN SECTION 3. IT WILL BE REVIEWED SEMI-ANNUALLY AT SCHEDULED DCEM ACC MEETINGS. IF AN EXTENSION IS REQUIRED, A FORMAL AMENDMENT TO THE PROTOCOL MUST BE SUBMITTED FOR APPROVAL BY THE SECTION HEAD, CHAIR AND VETERINARIAN.
Establishment of a Non Lethal Model of Full-thickness, Contaminated Wounds in Pigs

Introduction

Open wounds are injuries frequently encountered in military combat operations. The use of antibiotics and antiseptic agents as well as marked improvements in operative wound management have virtually eliminated the incidence of mortality associated with these injuries. However, inevitable delays in providing adequate therapy in a battlefield scenario can still result in high wound infection rates and devastating infectious complications. The experiments described in the following protocol will be carried out under the MOM Thrust 6c, and are specifically in support of the Work Units on Wound Care Management (16ca19) and Business Line 3 Activity “Gel” (30dd05).

Background and Objective

Several animal models of infected wounds have been described in the literature, with variations in experimental parameters such as wound size and depth, number of wounds, location and shape of the wounds, as well as species used (1-4). However, it is generally acknowledged that pigs may be the most appropriate experimental animal before undertaking clinical trials, since pig skin possesses many anatomical features that makes it very comparable to human skin (5, 6). The objective of the present study is to establish at DRDC Toronto a non-lethal model of contaminated full-thickness wounds in pigs that will be used in subsequent studies to investigate the bactericidal or wound healing properties of wound care agents.

The porcine model will be based on that developed in the laboratories of (7). Both the PI as well as a senior OMS research technician have experience using this model through the performance of contract W7711-7783. In
that model, twenty wounds are contaminated with ATCC strains of *Pseudomonas aeruginosa*, *Staphylococcus epidermis*, and *Fusobacterium* sp. Our intent is to develop this model using *Ps. aeruginosa*, *Staph. epidermis*, and *Fusobacterium* sp. strains previously isolated from pig wounds. It has been shown that bacteria passed previously in a host have enhanced virulence characteristics, likely due to an activation of the bacteria by repeated exposure to the immune cells of the host (personal communication). This trauma model will be used in future studies to evaluate the efficacy of wound-care agents.

10 **Methods**

Up to 21 male Yorkshire pig (15-20 kg) will be obtained from experiments will be conducted using up to three pigs at a time, to ascertain the adequacy of the various technical procedures involved (e.g., wound covering, number of biopsies per wound, bacterial challenge, etc.) as well as wound contamination characteristics over a 21-d study period. After the first pair of pigs has undergone the wound sampling protocol (see below), data will be analyzed to determine:

- whether the inoculum concentration is sufficient to ensure that the wound bacterial counts are at least $10^6$ CFU/g tissue (i.e., clinical infection threshold);
- how many samples of a given wound are required to offer a representative assessment of wound bacterial count (intra-wound contamination variability should be < 10%);
- how many wounds must be sampled at a given time point to provide a representative assessment of overall wound contamination (inter-wound contamination variability should be < 10%);
- whether a 21-d study period is sufficient to allow wound closure or is too long.
It is also noteworthy that the *Ps. aeruginosa* and *Staph. epidermidis* bacteria isolated from the pig wounds will be stored using standard microbiological procedures and will be used for the establishment of the porcine model. Experimental parameters will be re-adjusted as required and the revised protocol will be applied to another pair of pigs. Ideally, the final wound sampling parameters selected should not only optimize the assessment of wound bacterial contamination, but also aim to minimize disturbance of the wound healing process. It is expected that the final protocol be tested on 6 pigs, to ensure that the data are reproducible.

The animals will be allowed to adapt to the environmental conditions (20-25°C, 12 h light/dark cycle) for at least 7 days before undergoing surgery. Animals will be housed individually, and have free access to pig chow and water at all times during the study period. The study will be conducted in accordance with the guidelines from the Canadian Council on Animal Care (CCAC).

**Bacterial challenge**

Isolates of *Ps. aeruginosa* (i.e., a Gram-negative bacteria), *Staph. epidermis* (i.e., a Gram-positive bacteria), and *Fusobacterium* sp. (an anaerobe) will be used to infect the wounds. The bacterial strains will be grown at 37°C in nutrient broth for 18 h in a shaking water bath to obtain a log-phase growth culture. The suspensions will be washed three times in sterile phosphate-buffered saline (PBS), re-suspended in sterile PBS, and diluted to approximately 10^7 colony forming units (CFU) per mL. Serial dilutions will be plated on Pseudomonas Isolation agar (PIA; for *Ps. aeruginosa*), Staphylococcus Medium 110 (SM110; for *Staph. epidermidis*) or Tryptic Soy agar (TSA; for *Fusobacterium*) to assess bacterial concentrations in the inoculum. On the experimental day, the three bacterial cultures will be mixed together in a ratio approximating 1:1:0.5 (*Pseudomonas: Staphylococcus: Fusobacterium; 10^7 CFU in 50 mL*).
Surgical Procedures

On the experimental day, each pig will be pre-anesthetized with ketamine (15 mg/kg body weight, i.m.) and acepromazine (0.5 mg/kg body weight, i.m.) followed by gas inhalation (oxygen: 1-2% isoflurane). The dorsal and lateral thorax will be clipped, and the skin prepared for wounding by washing with an antibiotic-free soap. Columns of wounds on the dorsum will be labeled (using an indelible marker) as A through D, and rows marked as 1 through 4. Sixteen full-thickness (down to the deep fascia) wounds will be created using a 3-cm diameter tissue trephine. Wounds will be made 4 cm apart, with columns B & C set 2 cm on each side of the pig’s spine. Sterile gauze compresses will be applied on the wounds, soaked with a saline/epinephrine solution (1:100 v/v), and allowed to remain in situ until complete haemostasis has occurred. Each wound will then be measured in two directions using digital calipers, to ascertain the initial wound area, and digitally photographed.

The wounds will be loosely packed with 2.5 cm x 2.5 cm sterile gauzes and inoculated with 3 mL the bacterial suspension. The wounds will then be covered for 20 min with an occlusive film to prevent drying. At the end of the infection period, the gauzes will be removed, and the wounds will be covered with a piece of sterile absorbent gauze. Layers of adhesive PVC tape will then be applied to the back of the pig to hold down firmly the gauze dressings. The entire trunk of the pig will finally be wrapped with a layer of elastic adhesive bandage (self-adhesive bandage,

A dose of narcotic (i.m. buprenorphine, 0.1-0.3 mg/kg body weight) will be administered before returning the animal to its pen, and twice daily thereafter for 21 d, or as required. Pigs will be followed closely for 3 h to ensure that no deaths be attributed to anesthesia or open wound injury per se. However, no
mortality is expected after completing these procedures (personal communication). Nevertheless, CCAC guidelines for judging morbidity and moribundity will be used to assess the animal’s health status, if necessary. Thus, the animals will be humanely euthanized before the end of the 21-d study period if the nature of the signs of illness, their rate of onset and persistence strongly suggest an impending death. Inclusion of subjective (e.g. withdrawal, breathing rate, mobility) and objective measurements (e.g. food and water intake) will facilitate the monitoring of the animal’s health status.

Experimental Protocol

Two samples will be taken (using a 4-mm biopsy punch) from pre-selected wounds (see sampling schedule below) on days 0, 1, 3, 7, 10, 14, 17 and 21, with the animal under general anesthesia. All but three wounds will be sampled 4 times over the 21-d study period, with at least 7 days between 2 consecutive wound samplings. Wounds A3, B2 and C4 will not be sampled, to provide an estimation of the normal time required for healing to be completed (in the absence of biopsies). Prior to sampling, the size of the wound will be measured in two directions, and photographs will be taken. Wounds will then be covered as previously described. Tissues will be placed into pre-weighed tubes, homogenized in cold PBS, and plated serially on SM110 and PIA to determine the microbiological counts. The sampling schedule will be as follows:
Wound sampling procedure

Day 0  Wounds A1 A1 A1 D3 D3 D3
       B1 A2 B3 C1 D1 C2

Day 1  Wounds B1 A2 C2 D2 D4 A4

Day 3  Wounds B3 C1 D1 D3 C3 A4

Day 7  Wounds B1 A2 C2 D2 D4 B4

Day 10 Wounds B3 C1 D1 C3 A4 B4

Day 14 Wounds C2 D2 D4 C3 A4 B4

Day 17 Wounds B1 A2 D1 C3 B4

Day 21 Wounds A1 B3 C1 D2 D4

Animals will be humanely euthanized at the end of the 21-d study period using T61 (i.v.) following sedation (ketamine, 15 mg/kg body weight; acepromazine, 0.5 mg/kg body weight, i.m.).

REFERENCES
Page 147

is withheld pursuant to section
est retenue en vertu de l'article

17

of the Access to Information Act
de la Loi sur l'accès à l'information
form G [2004]

amendment to porcine protocols? indicates the DRDC has authorized a Contractor for “testing in porcine models of injury the in vivo bactericidal efficacy (full-thickness infected wounds) and wound healing properties”

these are tests for “four different DRDC materials developed under a Technology Investment Fund”
Amendment #1 to protocol ACC 2/04

Background information

We have recently issued a contract (W7711-05-7958) for testing in porcine models of injury the in vivo bactericidal efficacy (full-thickness infected wounds) and wound healing properties (partial-thickness wounds as per ACC 4/04) of the four different DRDC materials developed under a Technology Investment Fund (16ci04).

However, the preparation of the different types of DRDC materials remains very time-consuming despite our efforts to scale-up the process, and we must be careful not to "waste" any material. Furthermore, we intend to use similar technical procedures to secure the materials during the different studies that will be performed by the Contractor. We will thus combine the two different models on a same animal, keeping the total area to be injured in a given animal (i.e. full- and partial-thickness wounds) at least 4 times below those previously accepted under ACC 2/04 (i.e., 113 cm²; 16 wounds of full-thickness wounds of 3-cm diameter) and ACC 4/04 (i.e., 80 cm²; 80 partial-thickness wounds of 1 cm²).

This pilot study (1 pig) will allow us to determine the type of commercial tape that should cover the materials to ensure the full the recovery of the material after 1 day. It will also indicate whether the size of the material applied to the partial-thickness wounds is appropriate, as we do not know whether the material will shrink or expand during the 3-d period. Lastly, we will determine whether puffs and meshes undergo any significant degradation over a 24-h period, as suggested by our in vitro testing [1]. No harvesting of the partial-thickness wounds will be performed throughout the experiment.

Amendments to Protocol 2/04

1. Create 14 partial-thickness wounds on the left side of the animal (1 cm²; total area 14 cm²).
2. Apply 1 strip of the following dressings over 5 wounds:
   a. Wet Aam IPN films (1 strip; 5 wounds)
   b. Freeze-dried benchmark IPN films (1 strip; 4 wounds)
   c. Wet benchmark IPN films (1 strip; 5 wounds)
3. Cover the wounds with a sterile sheet of dressing then adhesive PVC tape (This set-up will allow easy removal of the material after 24 h).
4. Create 8 full-thickness wounds on the right side of the animal (10 mm diameter; total area 6 cm²).
5. Infect 4 full-thickness wounds using ATCC strains of *Pseudomonas aeruginosa* and *Staphylococcus epidermis* similar to those used by the Contractor. Note that *Fusobacterium sp.* will not be used as the Contractor’s model does not include this bacterial strain.

6. Pack loosely the 4 wounds with 0.5 cm x 0.5 cm sterile gauzes, and inoculate them with 0.5 mL of the bacterial suspension for 20 min.

7. Cover the wounds for 20 min with an occlusive film (to prevent drying. At the end of the infection period, remove the gauzes.

8. Place either a re-moistened DRDC messy puff or a DRDC IPN mesh (n=4 per material) directly in the wounds.

9. Repeat step 3 as described above.

10. Wrap the entire trunk of the pig with a layer of elastic adhesive bandage self-adhesive bandage,

11. After a 24-h period, re-anesthetize the animal:
   a. compare the status of the puffs and meshes in the non-inoculated and inoculated wounds (e.g., degradation, presence of exudates, etc.)
   b. observe the status of the two types of films (e.g., shrinkage, swelling, etc.)

12. Cover the full-thickness wounds with sterile gauze then. No further observations will be made.

13. Re-apply the same dressings on the partial-thickness wounds and repeat step 3 above.

14. Re-anesthetize the animals at 48 and 72 h post-injury to observe the wounds and status of the dressings covering the partial-thickness wounds, replacing the restraining system (i.e., tape, and

15. Humanely euthanize the animal.

REFERENCES

1.
Amendment #2 to protocol ACC 2/04

Background information

We have recently tested the different DRDC polymeric films in a pig model of partial-thickness wounds (amendment #1 to protocol ACC 4/04) and determined that the re-moistened DRDC material should be applied to the partial-thickness wounds to prevent their complete degradation. Furthermore, the data also suggested that the use of a semi-permeable membrane (such as to cover the re-moistened DRDC material might be preferable to using an wound cover as the material's texture was somewhat altered after 24 h.

This study is designed to:

- indicate whether the size of the material applied to the partial-thickness wounds (i.e., strips) is appropriate, as we do not know whether the material will shrink or expand;
- determine the best method for freeze-drying the puffs and meshes (complete coverage, ease of insertion and handling, etc.)
- determine the 24-h bactericidal efficacy of mafenide acetate-loaded puffs and meshes.

No harvesting of the partial-thickness wounds will be performed throughout the 2-d experiment, as the Contractor will not assess wound healing until day 4 post-injury.

Amendments to Protocol 2/04

1. Create 10 partial-thickness wounds on the left side of one pig (1 cm²; total area 10 cm²).
2. Apply 1 strip of the following dressings over 5 wounds:
   a. Re-moistened Aam IPN film (1 strip; 5 wounds)
   b. Re-moistened benchmark IPN film (1 strip; 5 wounds)
3. Cover the wounds with: a sterile sheet of dressing; sterile gauze; and, adhesive cotton tape. This set-up will allow easy removal of the material after 24 h.
4. Create 18 full-thickness wounds on the right side of the animal (8 mm diameter; total area 9 cm²).
5. Infect 4 full-thickness wounds using ATCC strains of Pseudomonas aeruginosa and Staphylococcus epidermis similar to those used by the Contractor.
Table 1. Types of Meshes and Puffs tested

**Puffs:**

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<th>Type</th>
<th>Freeze-Drying Procedure</th>
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<tr>
<td>P1</td>
<td>Loaded FD</td>
<td>15mL test tube with 5mL on top</td>
</tr>
<tr>
<td>P2</td>
<td>loaded FD</td>
<td>15mL test tube with 5mL on top</td>
</tr>
<tr>
<td>P3</td>
<td>loaded REM</td>
<td>15mL test tube with 5mL on top</td>
</tr>
<tr>
<td>P4</td>
<td>loaded REM</td>
<td>15mL test tube with 5mL on top</td>
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<td>P5</td>
<td>loaded FD</td>
<td>50mL falcon tube with 15mL test tube on top (vertical)</td>
</tr>
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<td>unloaded REM</td>
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<td>P8</td>
<td>unloaded REM</td>
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**Meshes:**

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<td>M2</td>
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<td>U2B</td>
<td>unloaded REM</td>
<td>50mL falcon tube with 15mL test tube on top (vertical)</td>
</tr>
</tbody>
</table>

REM - remoistened  FD - freeze-dried.
2. Apply one 15-mm disc of the following dressings on each wound:
   a. Re-moistened Aam IPN film (n=9)
   b. Re-moistened benchmark IPN film (n=9)
3. Cover the wounds with: a sterile sheet of 
   sterile gauze; and, adhesive cotton tape
   This set-up will allow easy removal of the material after 24 h.
4. Create 8 full-thickness wounds on the right side of the animal (6 mm
   diameter; total area 2 cm²).
5. Place one of the dressings described in Table 1 in each of the wounds.
6. Repeat step 3 as described above.
7. Wrap the entire trunk of the pig with a layer of elastic adhesive
   bandage (self-adhesive bandage).
8. After a 24-h period, re-anesthetize the animal:
   a. Remove the films covering the full-thickness wounds, and make
      observations (adherence to wounds, appearance of 3D
      structures, ease of removal, excessive presence of exudate, etc.)
   b. Cover the new full-thickness wounds with sterile gauze then
      PVC
      No further observations will be made.
   c. Remove the films covering the partial-thickness wounds, and
      make observations (adherence to wounds, expansion/
      shrinkage of discs, etc.)
   d. Re-apply the same films on the partial-thickness wounds (as the
      Contractor’s study will call for leaving the dressings unaffected
      for 4 days post-injury) and repeat step 3 above.
9. Re-anesthetize the animals at 72 h post-injury and repeat steps 8 (c)
   and 8 (d), paying special attention to the integrity of the material after
   being left unattended for 48 h.
10. Humanely euthanize the animal.
### Table 1 Types of meshes and puffs tested

<table>
<thead>
<tr>
<th>Cut</th>
<th>Code</th>
<th>Freeze-drying procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>half puff</td>
<td>P1A</td>
<td>15mL test tube</td>
</tr>
<tr>
<td></td>
<td>P1B</td>
<td>50mL Falcon tube</td>
</tr>
<tr>
<td>half mesh</td>
<td>M1A</td>
<td>15mL test tube</td>
</tr>
<tr>
<td>1/3 mesh</td>
<td>M2A</td>
<td>5mL test tube</td>
</tr>
<tr>
<td></td>
<td>M2B</td>
<td>15mL test tube</td>
</tr>
<tr>
<td>1/3 mesh</td>
<td>M3A</td>
<td>Eppendorf tube (top)</td>
</tr>
<tr>
<td></td>
<td>M3B</td>
<td>HPLC vial top</td>
</tr>
<tr>
<td>1/3 mesh</td>
<td>M4A</td>
<td>Eppendorf tube (middle)</td>
</tr>
<tr>
<td></td>
<td>M4B</td>
<td>Eppendorf tube (bottom)</td>
</tr>
</tbody>
</table>

### REFERENCES

1.