University of Delaware
Institutional Animal Care and Use Committee
Application to Use Animals in Research and Teaching

Title of Protocol: Non-viral gene delivery to excisionally-wounded mice

AUP Number: 1229-2015-0 ← **(4 digits only — if new, leave blank)**

Principal Investigator: Millicent Sullivan

Common Name: Mouse

Genus Species: Mus musculus

Pain Category: ***(please mark one)***

USDA PAIN CATEGORY: ***(Note change of categories from previous form)***	
Category	**Description**
☐**B**	Breeding or holding where NO research is conducted
☐**C**	Procedure involving momentary or no pain or distress
☐**D**	Procedure where pain or distress is alleviated by appropriate means (analgesics, tranquilizers, euthanasia etc.)
X E	Procedure where pain or distress cannot be alleviated, as this would adversely affect the procedures, results or interpretation

Official Use Only

IACUC Approval Signature: Glenn Tolbert, DVM

Date of Approval: 3/9/2015

Principal Investigator Assurance

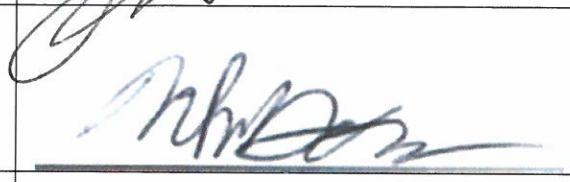
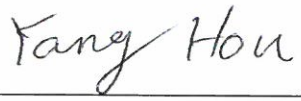
1. I agree to abide by all applicable federal, state, and local laws and regulations, and UD policies and procedures.
2. I understand that deviations from an approved protocol or violations of applicable policies, guidelines, or laws could result in immediate suspension of the protocol and may be reportable to the Office of Laboratory Animal Welfare (OLAW).
3. I understand that the Attending Veterinarian or his/her designee must be consulted in the planning of any research or procedural changes that may cause more than momentary or slight pain or distress to the animals.
4. I declare that all experiments involving live animals will be performed under my supervision or that of another qualified scientist. All listed personnel will be trained and certified in the proper humane methods of animal care and use prior to conducting experimentation.
5. I understand that emergency veterinary care will be administered to animals showing evidence of discomfort, ailment, or illness.
6. I declare that the information provided in this application is accurate to the best of my knowledge. If this project is funded by an extramural source, I certify that this application accurately reflects all currently planned procedures involving animals described in the proposal to the funding agency.
7. I assure that any modifications to the protocol will be submitted to by the UD-IACUC and I understand that they must be approved by the IACUC prior to initiation of such changes.
8. I understand that the approval of this project is for a maximum of one year from the date of UD-IACUC approval and that I must re-apply to continue the project beyond that period.
9. I understand that any unanticipated adverse events, morbidity, or mortality must be reported to the UD-IACUC immediately.
10. I assure that the experimental design has been developed with consideration of the three Rs: reduction, refinement, and replacement, to reduce animal pain and/or distress and the number of animals used in the laboratory.
11. I assure that the proposed research does not unnecessarily duplicate previous experiments. ***(Teaching Protocols Exempt)***
12. I understand that by signing, I agree to these assurances.

Millicent Sullivan — January 12, 2015

Signature of Principal Investigator — Date

NAMES OF ALL PERSONS WORKING ON THIS PROTOCOL

I certify that I have read this protocol, accept my responsibility and will perform only those procedures that have been approved by the IACUC.

Name	Signature
1. Millicent Sullivan	
2. Frank Warren	
3. Nikki Ross	
4. Theresa Freeman	
5. Yang Hou	
6. Morgan Urello	
7. Click here to enter text.	
8. Click here to enter text.	
9. Click here to enter text.	
10. Click here to enter text.	
11. Click here to enter text.	
12. Click here to enter text.	
13. Click here to enter text.	
14. Click here to enter text.	

The Animal Use Protocol form has been developed to facilitate review of requests for specific research, teaching, or biological testing projects. The review process has been designed to communicate methods and materials for using animals through administrative officials and attending veterinarians to the Institutional Animal Care and Use Committee (IACUC). This process will help assure that provisions are made for compliance with the Animal Welfare Act, the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals.

Please read this form carefully and fill out all sections. Failure to do so may delay the review of this application. Sections that do not apply to your research must be marked "NA" for "Not Applicable."

This application form must be used for all NEW or THREE-YEAR RENEWAL protocols.

All answers are to be completed using Arial 12 size font.

All questions must be answered in their respective boxes and NOT as attachments at the end of this form.

Please complete any relevant addenda:

- Hybridoma/Monoclonal Antibodies ("B")
- Polyclonal Antibodies ("C")
- Survival Surgery ("D")
- Non-Survival Surgery ("E")
- Wildlife Research ("F")

If help is needed with these forms, contact the IACUC Coordinator at extension 2616, the Facility Manager at extension 2400 or the Attending Veterinarian at extension 2980.

1. Principal Investigator Information:	
a. Name:	Millicent Sullivan
b. University/Company:	University of Delaware
c. Department:	Chemical & Biomolecular Engineering
d. Building/Room:	213 Colburn Laboratory
e. Office Phone:	302-831-8072
f. Lab Phone(s):	N/A
g. Home Phone:	302-652-1762
h. Mobile Phone:	412-725-5657
i. E-Mail Address:	msullivan@udel.edu

2. Protocol Status:

a. ☐New Protocol ***OR*** x Re-submission due to three (3) completed years.
If re-submission, enter Protocol Number: 1229

b. **X** Research ***OR*** ☐ Teaching

c. **X** Laboratory Animals ***OR*** ☐ Wildlife
If "Wildlife" please complete Addendum "F"

d. Proposed Start Date: March 1, 2015

e. Proposed Completion Date: February 28, 2018

f. Funding Source: National Science Foundation and National Institutes of Health

g. Award Number: NSF CBET 1159466; NIH 1 R01 EB017766 01

3. Personnel involved in Protocol *(Include Principal Investigator):*

***Status*:** Indicate Prof, Post-Doc, Grad Student, Lab Manager, Research Assistant, Technician, etc.

***Qualifications*:** Include **procedures this person is proficient in performing** on proposed species and the time they have been doing the procedure.
Be specific (e.g. sub-mandibular bleeding on mice-2yrs, performing castrations on mice and rats-1yr, tail-vein injections on mice-2yrs, etc.) **(If no experience, list who will train.)**

***Responsibilities*:** Include **all responsibilities** this person will have with live animals on this protocol, including euthanizing animals.

Name	E-mail	Office Phone Number	Home/Cell Phone Number	Received Animal Facility Training	
				Yes	No
a. Millicent Sullivan	msullivan@udel.edu	302-831-8072	412-725-5657	x	Click here to enter text.

Status: PI, Associate Professor

Qualifications: I have 6 years of mouse model experience, including experience with wound repair studies. Specifically, my postdoctoral research (3 years) investigated the foreign body response (FBR) and the wound repair response (WRR) in wild-type and hevin-null animals (hevin is an matricellular protein whose absence alters the organization of dermal extracellular matrix, and subsequently, the FBR and WRR), as well as the inflammatory response to intraperitoneally-injected lipopolysaccharide (LPS) in the same two murine strains. Additionally, for the past 3 years I have supervised research on the current protocol, which has involved the subdermal injection of collagen-based gels containing model genes and the analysis of gene expression and wound repair in mice. I have used the following animal procedures: administration of anesthesia (routine), biomaterial implantation (with assistance), incisional and excisional wounding (with assistance), intraperitoneal injection of LPS (occasional, with assistance), peritoneal macrophage collection (occasional, with assistance), tail bleeding (with assistance), organ collection and euthanasia (routine).

Responsibilities: Supervision of all animal work and overall project direction.

Name	E-mail	Office Phone Number	Home/Cell Phone Number	Received Animal Facility Training	
				Yes	No
b. Frank Warren	fwarren@udel.edu	302-831-2400	302-738-2286	x	Click here to enter text.

Status: Laboratory Manager

Qualifications: 37 years experience in laboratory animals involving research (injections, bleeding, surgeries, treatment), disease prevention, diagnostics, euthanasia, and laboratory management.

Responsibilities: Surgeries, euthanasia, training of laboratory personnel in general animal handling procedures.

Name	E-mail	Office Phone Number	Home/Cell Phone Number	Received Animal Facility Training	
				Yes	No
c. Nikki Ross	nross@udel.edu	302-831-6851	717-715-6973	x	Click here to enter text.

Status: Ph.D. Student

Qualifications: Three years of experience with animal studies on the current protocol, including all general animal handling procedures, anesthesia, subdermal injection, bioimaging analysis, euthanasia, and organ collection.

Responsibilities: Nikki is an experienced graduate student who is co-leading the execution of the experiments proposed in this protocol (along with Morgan Urello), including subdermal injections, bioimaging analyses, euthanasia, and organ collection. Prior to her graduation (TBD), Nikki will assist in the training of a new graduate student or postdoctoral fellow in these procedures.

Name	E-mail	Office Phone Number	Home/Cell Phone Number	Received Animal Facility Training	
				Yes	No
d. Theresa Freeman	theresa.freeman@jefferson.edu	Click here to enter text.	215-955-1068	x	Click here to enter text.

Status: Co-I, Associate Professor

Qualifications: I have had >8 years of mouse and rat model experience, including experience with fracture repair studies. Specifically, my previous research investigated the effect of ultrasound on fracture healing, a rat osteomyelitis model, mouse breeding and the subdermal endochondral ossification model (this is the model on which the proposed procedure is based, and involves the same technical processes of subdermal injection of a hydrogel solution). I have used the following animal procedures: administration of anesthesia, biomaterial implantation (a little experience, with assistance), incisional and excisional wounding (with assistance) and organ collection (routine, no assistance).

Responsibilities: Dr. Freeman is our primary scientific collaborator at TJU, and she is an expert in the biology of tissue repair within biomaterials (in contrast, Dr. Hou is the technical expert with the proposed procedures). She will accompany Dr. Hou (see below) to UD to train UD personnel in all proposed procedures. Dr. Hou will take the lead in technical training of UD personnel in project-specific procedures, and Dr. Freeman will take the lead in scientific planning discussions, and answer scientific questions that may arise during technical training in the animal facility.

Name	E-mail	Office Phone Number	Home/Cell Phone Number	Received Animal Facility Training	
				Yes	No
e. Yang Hou	Click here to enter text.	215-955-1068	Click here to enter text.	x	Click here to enter text.

Status: Visiting Scientist

Qualifications: Dr. Hou has multiple years' expertise with animal handling procedures, including all proposed procedures (e.g., surgeries, anesthesia, biomaterial injection, euthanasia, and organ collection). Specifically, Dr. Hou is very experienced with implementing the subdermal biomaterial mouse model that we have proposed to use, including surgical prep and surgery; anesthesia; ear notching procedures; subdermal injection of biomaterials; ip injection; euthanization; and tissue collection.

Responsibilities: Dr. Hou will lead in the technical training of all UD personnel on all proposed procedures.

Name	E-mail	Office Phone Number	Home/Cell Phone Number	Received Animal Facility Training	
				Yes	No

f. Morgan Urello	urellom@udel.edu	302-831-6851	609-254-7260	x	Click here to enter text.

Status: Ph.D. Student

Qualifications: Two and a half years of experience with animal studies on the current protocol, including all general animal handling procedures, anesthesia, subdermal injection, bioimaging analysis, euthanasia, and organ collection.

Responsibilities: Morgan is an experienced graduate student who is co-leading the execution of the experiments proposed in this protocol (along with Nikki Ross), including subdermal injections, bioimaging analyses, euthanasia, and organ collection. Morgan will assist in the training of a new graduate student or postdoctoral fellow in these procedures.

Name	E-mail	Office Phone Number	Home/Cell Phone Number	Received Animal Facility Training	
				Yes	No
g. Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

Status: Click here to enter text.

Qualifications: Click here to enter text.

Responsibilities: Click here to enter text.

Name	E-mail	Office Phone Number	Home/Cell Phone Number	Received Animal Facility Training	
				Yes	No
h. Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

Status: Click here to enter text.

Qualifications: Click here to enter text.

Responsibilities: Click here to enter text.

4. Non-Scientific Summary: In language understandable to a ***high-school senior, very briefly describe*** the goals and significance of this study.

a. Specific Scientific Goals: The long-term goal of our research is to develop new gene delivery biomaterials that will stimulate tissue repair in chronic wounds (such as diabetic wounds) by promoting the local production of growth factors. In the proposed project, we will test DNA-modified collagens for their ability to trigger cells to produce model proteins [such as green fluorescent protein (GFP) and/or luciferase (Luc)] in subdermal injection sites in mice that model the wound environment. Mice will be used because they represent an excellent and widely used model of human type II diabetes mellitus (DM), and because they are considered an appropriate model for humans in studies of diabetic wound repair. They have already been used extensively to test the effects of delivered growth factors and genes in wounds.

b. Significance of this Research (including the possible benefits to human and/or animal health, the advancement of scientific knowledge, or the betterment of society): Patients with DM have a 15% risk of developing an ulcer, and 69% of diabetic foot ulcers have not healed within 20 weeks. Ulcers are the leading cause of hospitalization for diabetics, and require amputation in 14 – 24% of cases, indicating that there is a significant need for improved wound healing therapies. Gene delivery approaches can overcome current issues (e.g., low stability and rapid degradation) associated with protein-based therapies, and build on previously demonstrated promise for gene delivery approaches to improved wound repair.

5. Experimental Design: Explain the experimental design. This description should allow the IACUC to understand fully the experimental course of an animal or group of animals from its entry into the experiment to the endpoint of the study.

The inclusion of flow charts, diagrams, and/or tables are greatly encouraged to explain experimental design or sequential events.

Be sure to include all animal events and related details, i.e.,

- **All Procedures**-bleedings, injections, identification methods, genotyping methods, surgical procedures, euthanasia, etc.
- **Procedural details**–number of animals involved in procedure, approximate animal weight, if relevant (for injections, bleeding, etc.), route, frequency, volume, etc.

- **Pharmaceutical-grade and non-pharmaceutical grade compounds** – Identify any drugs, biologics, or reagents that will be administered to animals. If these agents are not human or veterinary pharmaceutical-grade substances, provide a scientific justification for their use and describe methods that will be used to ensure appropriate preparation.
- **Names of surgical procedures** (but reserve the surgical details for the proper Surgical Addenda)
- **Monitoring**–observations, measurements (animal weight, tumor size, etc.)
- **Monitoring details**–criteria, frequency, names of personnel monitoring, conditions for removing an animal from the study, etc.
- **Endpoints**–include endpoints for the animals/study and how will they be determined.

(Describe): Click here to enter text.

Proposed use of animals. 8 week-old sex-matched CD-1 mice (*Mus musculus*) will be obtained from Charles River Laboratories and used in this study. The mice will be used to collect dermal fibroblast cells for *in vitro* studies of gene expression, and will also be used for *in vivo* studies of gene expression by using a subdermal injection model of the wound environment. Prior to all procedures, mice will be quarantined and acclimated for 7 d.

For *in vitro* cell collection:
Animals will be euthanized by CO_2 asphyxiation and cervical dislocation (double kill). The dorsal region of the animals will be shaved and disinfected, and skin sections will be removed and washed; subsequently, the collagen matrix will be digested and cells collected by incubation of the sections at 37°C in collagenase solutions, agitation, and centrifugation. Subsequent to isolation, cells will be maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal bovine serum (FBS), 1% penicillin G/streptomycin sulfate, and 1 μg/mL fungizone. Cells will be passaged 2 – 3 times prior to use, and maintained until ~passage 7, based on our previous experience with dermal fibroblast collection and maintenance.

For *in vivo* studies of gene expression: The mice will be also used to test whether, and to what extent, DNA-modified collagen gels stimulate model gene transfer *in vivo*. Biomaterials (collagen gels containing DNA and polymer) will be subdermally injected into the dorsa of mice. The subdermal model is used as a model of wound tissue.

Mice will be prepared for subdermal injection procedures by anesthesia (inhalation of isoflurane [3-5% during induction, 1-3% during maintenance] in 0.2-0.5 L/min oxygen). Ears will be notched with one-millimeter diameter ear punches (cleaned in chlorhexidene, and rinsed in sterile water or saline) to differentiate treatment groups. Subsequent to anesthesia, two specified locations on the abdomen of each animal will be shaved and surgically scrubbed (chlorhexiderm or povidone-iodine followed by rinsing with sterile saline). Biomaterial solutions (~300 μL/injection, 1 injection in each of 4 abdominal site; thus, 4 injections per animal) will be injected subdermally **using a non-thermal plasma probe needle (2 mm o.d.)**. Injection sites will be cleaned with sterile saline and mice will be returned to their cages to awaken. Blood loss is usually minimal and the injection does not cause a significant injury; however, mice will be observed until normal activity resumes and bleeding ceases. After recovery from anesthesia, mice will be given full access to food and water.

Gene delivery within the subdermal injection sites will be analyzed at 4, 7, and 10 d post-subdermal injection (5 mice per group per time point), when the difference between normal and impaired wound healing peaks (and thus, when therapeutic gene expression in future studies will be desirable). The *in vivo* analysis of luciferase activity will be performed according to well-established procedures that have been successfully implemented with mice. Initially, mice will be administered luciferin *via* ip injection (0.2 mL of a 15 mg/mL solution); 8 minutes later, the animals will be anesthetized (inhalation of isoflurane [3-5% during induction, 1-3% during maintenance] in 0.2-0.5 L/min oxygen) on the stage of a Caliper IVIS Lumina Imaging System equipped with premium animal handling features including a heated stage and a 5-position manifold that allows anesthesia to be maintained during imaging. This method of anesthesia has also previously been reported for CD-1 mice, and will enable anesthesia to be appropriately maintained during imaging procedures. Immediately following imaging, the animals will be euthanized (isoflurane overdose followed by cervical dislocation) and subdermal injection site tissues will be removed for histochemical analyses of transfection efficiency, tissue morphology, and cell viability. Luciferase activity, transfection efficiency (GFP expression), and cell viability can all be assessed with the same animals, thereby minimizing the number of animals required for the planned end-point analyses.

Summary of biomaterial samples to be tested: Control treatments: Collagen gels or Matrigel solutions (BD Biosciences); Matrigel is a basement membrane protein solution containing collagen IV, laminin, fibronectin, and will serve as an inert scaffold used in place of collagen in some experiments. Matrigel will be supplemented with commercially-available recombinant human bone morphogenetic protein-2 (rhBMP-2) [1.25 μg rhBMP-2 per 250 μL growth factor-reduced, phenol red-free Matrigel]; inclusion of rhBMP-2 is expected to stimulate cellular infiltration of subdermal injection sites and enhanced gene delivery when used with DNA-containing complexes. Experimental samples: Collagen gels or Matrigel/rhBMP gels containing GFP-Luc DNA/polymer complexes of various formulations. All samples will be prepared in an aseptic environment to ensure sterility, and the starting solutions (Matrigel or collagen solutions) are shipped as sterile solutions from the manufacturer. Polymer and DNA solutions are sterile-filtered (0.2 μm) prior to assembly of the DNA/polymer complexes, which are also prepared using aseptic procedures.

Veterinary care. The animals will be housed under specific pathogen free conditions in the University of Delaware's Animal Facility that is overseen by the UD Office of Laboratory Animal Medicine. This facility includes appropriate quarantine, breeding, and isolation facilities. Routine veterinary care and surveillance will be overseen by the OLAM veterinarian and other OLAM staff. Mice will be monitored daily, and weighed daily by OLAM staff, and signs of poor health, including weight loss, lethargy, ruffled coat, anorexia, labored breathing, prostration, squinting eyes, and hunched posture will be used to determine whether an animal should be euthanized.

Animal welfare. All procedures outlined in this proposal conform to the NIH Guide for the Care and Use of Laboratory Animals and the Federal Animal Welfare Act, and will be overseen by the University of Delaware Institutional Animal Care and Use Committee. All surgeries are preceded by the administration of anesthetic, at doses that have been widely reported for CD-1 mice. Furthermore, the materials that will be utilized in the proposed studies have been tested previously *in vitro* and *in vivo* and shown to be non-toxic. The

components of the materials (collagen, peptides, plasmid DNA, polymers) have previously been utilized in other *in vivo* studies at a range of compositions, and all components are non-toxic. The animals will have free access to food and water following the procedure and will be administered analgesics during recovery as needed. Heating pads (~38°C) will be used during anesthesia. Tissue collections will be performed after animals are euthanized.

Euthanasia. Animals for *in vitro* cell collection will be euthanized by CO_2 asphyxiation followed by cervical dislocation (double kill). This method is well-accepted and humane, and is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the Guide for the Care and Use of Laboratory Animals. In addition, animals will be euthanized (CO_2 asphyxiation followed by cervical dislocation) should they be found to exhibit weight loss greater than 20% and/or if they exhibit significant signs characteristic of poor health (see criteria in "Veterinary Care"). Animals already under anesthesia for imaging experiments will be euthanized immediately following imaging by isoflurane overdose followed by cervical dislocation.

REFINEMENT, REDUCTION & REPLACEMENT

When using animals for research, it is important to consider the three Rs: reduction, refinement, and replacement to reduce both animal distress and the number of animals used in the laboratory.

Reduction: Minimizing the number of animals used
Refinement: Using techniques and procedures to reduce pain and distress
Replacement: Using non-animal methods or lower phylogenetic organisms

6. Justification for the Use of Animals (instead of *in vitro* methods)
(Check all that apply and explain):

a. X The complexity of the processes being studied cannot be duplicated or modeled in simpler systems: ***(Explain)***: *In vitro* culture models cannot duplicate the complexities of the repair response and the *in vivo* cellular microenvironment, necessitating the use of animal models. The repair response includes multiple different cell types infiltrating the repair site from the surrounding tissues, and also involves various aspects of the immune response. These responses cannot be modeled in culture.

b. ☐ There is not enough information known about the processes being studied to design non-living models: ***(Explain)***: Click here to enter text.

c. ☐ Other: ***(Explain)***: Click here to enter text.

7. Justification for Species Appropriateness:

(Check all that apply and explain):

a. X A large database exists, allowing comparisons with previous data: ***(Explain)***: CD-1 mice have been routinely used to implement the subdermal injection model for the testing of biomaterials, and hence there is a large quantity of data available, using this model, for comparison with the results of our proposed studies.

b. ☐ The anatomy or physiology is uniquely suited to the study proposed: ***(Explain)***: Click here to enter text.

c. X This is the lowest species on the phylogenic scale suitable to the proposed study: ***(Explain)***: Mammalian models are necessary to mimic the structure and basic physiology of repair environment in skin and underlying tissues, due to the need for similar tissue structure, conservation in various proteins involved in the healing response, and similarities in basic aspects of the immune response. Murine models, in particular, have been extensively studied for the purpose of analyzing tissue and wound repair, and mice are the lowest species that is considered acceptable for this purpose.

d. X Other: ***(Explain)***: A greater variety of well-characterized reagents are available for mice as compared with other non-human animals, and thus, we feel that these animals are the best choice for our short- and long-term studies.

8. Justification for Number of Animals Requested: (Note: numbers should include animals used for breeding and all animals born)

a. ☐ Pilot study or preliminary project where group variances are unknown at the present time. Describe the information used to estimate how many animals will be needed: (Only a limited number of animals will be permitted.)
(Explain): Click here to enter text.

b. X Group sizes are determined statistically. Describe the statistical analysis used to estimate the number (N) of animals needed: N may be estimated from a power analysis for the most important measurement in the study, usually based on the expected size of the treatment effect, the standard error associated with the measurement, and the desired statistical power (e.g. $P < 0.05$). Data analysis methods should not be submitted unless directly applicable to the estimate of N.

An online calculator may be found at: http://www.math.uiowa.edu/~rlenth/Power/
or a stand-alone calculator that can be downloaded from
http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3

(Explain)*: Number of animals.** We will minimize the number of animals to be used in these studies by completing preliminary transfection screens *in vitro*, with primary (or low passage) murine dermal fibroblasts. Cellular experiments will be miniaturized to the extent possible to reduce the number of animals required for cellular collection. However, *in vitro* culture models cannot duplicate the complexities of the repair response and the *in vivo* cellular microenvironment, necessitating the use of animal models. The number of animals requested for the *in vivo* study is based on previous reports documenting the expected effect size for similar gene transfection analyses. A power analysis (power = 0.8, $\alpha = 0.05$) suggests that 5 animals per group are required to detect a 20% difference in transfection. Thus, ***assuming ~15% mortality based on prior reports of surgical death rates, 52 mice will be needed to obtain statistically significant results for *in vivo* studies:

Test treatment	Group size	Time points	Concentrations	Total
DNA-modified collagen gels	5	3	3	45
DNA-collagen mixed gels (polymer 1)	5	3	3	45
DNA-collagen mixed gels (polymer 2)	5	3	3	45
				135 / 3 samples per animal x 1.15 = 52

c. X Group sizes are based on the quantity of harvested cells or the amount of tissue required for *in vitro* st animals needed.) ***(Explain)*:** Please see "Number of Animals" section above.

In addition, based on previous experience with fibroblast collection from mouse dermis, we will need approximately 40 animals for cell collection. Specifically, the dermis from ~4 animals generates ~5 x 10^6 – 1 x 10^7 fibroblast cells, which can then be passed ~6 – 7 times. 10 collections of this quantity of cells will be sufficient for the proposed experimental time period. Thus, **92 (52 + 40) animals in total** are needed for the proposed studies.

d. ☐ Teaching protocol. Specify the number of students in the class, the student to animal ratio and how that ratio was determined: Animal numbers should be minimized to the fullest extent possible without compromising the quality of the hands-on teaching experience for students or the health and welfare of the animals. ***(Explain)***: Click here to enter text.

e. ☐ Study involving feral or wild animals. Animals will be captured and released in an attempt to maximize the sample size within logistical constraints. Describe the process by which you estimate these numbers and estimate the precision needed: ***(Explain)***: Click here to enter text.

f. ☐Observational, non-manipulative study. Animals will not be captured, their behavior will not be interfered with, and exact animal numbers cannot be predicted: ***(Explain)***: Click here to enter text.

g. ☐ Product testing. The number of animals needed is based on FDA guidelines. Provide the citation from the regulations, the IND tracking number, or relevant FDA correspondence: ***(Explain)***: Click here to enter text.

h. ☐ Other. Elaborate, indicating the method used to determine the group size. ***(Explain)***: Click here to enter text.

9. Animals Requested:

Common Name	Genus and Species	Total Number of Animals for Three Years
1. Mouse	*Mus musculus* / CD-1 *In vivo* studies Tissue collection	 52 40
2. Click here to enter text.	Click here to enter text.	Click here to enter text.
3. Click here to enter text.	Click here to enter text.	Click here to enter text.
4. Click here to enter text.	Click here to enter text.	Click here to enter text.
5. Click here to enter text.	Click here to enter text.	Click here to enter text.

10. Where will animals be housed (or captured for wildlife)? University of Delaware Laboratory for Animal Medicine

11. Where will the experiments take place?

If animals must be removed from the vivarium, please describe how they will be transported (such as hand carried in covered microisolator cages). All subdermal injection experiments will take place in the LSRF. Animals will be euthanized immediately following imaging experiments, and the carcasses will be used immediately for tissue collection. Carcass disposal will occur in the LSRF.

12. Will any animals be humanely killed, without treatment or manipulations, to be used to obtain tissue, cells, etc.? ☒ Yes ☐ No

If **Yes,** list types of tissue, etc: Dermal tissues for the collection of dermal fibroblast cells.

13. Physiological Measurements ☐ Yes ☒ No

If **Yes,** list and explain: Click here to enter text.

14. Dietary Manipulations ☐ Yes ☒ No

If **Yes,** list and explain (Note: if food or fluid will be restricted, describe method for assessing the health and wellbeing of the animals. Body weights must be recorded at least weekly. Amount earned (if animals work for food or fluid) during testing and amount freely given must be recorded. A scientific justification must be provided for departures from the recommendations of the Guide.) Click here to enter text.

15. Environmental Stress (e.g. cold, restraint, forced exercise) ☐ Yes ☒ No

If **Yes,** list and explain: Click here to enter text.

16. Trauma or Burn Injury ☒ Yes ☐ No

If **Yes,** list and explain: Subdermal injections of the collagen biomaterial solutions will be made on the abdomen of anesthetized animals. To minimize the number of animals required for the study, each animal will receive a total of 4 injections, two each on opposing sides of their abdomen (with 3 control treatments and 1 test treatment per animal).

17. Production of Hybridoma/Monoclonal Antibodies ☐ Yes ☒ No

If **Yes,** please complete Addendum "**B**".

18. Production of Polyclonal Antibodies ☐ Yes ☒ No

If **Yes,** please complete Addendum "**C**".

19. Administration of Hazardous Chemicals, Drugs, Toxins, or Nanoparticles

☐ Yes CAS#_____ ☒ No

If **Yes,** describe hazards posed to personnel: Click here to enter text.

Methods to control exposure: Click here to enter text.

Methods of Disposal of Animals and Bedding: Click here to enter text.

20. Administration of radioactive materials ☐ Yes ☒ No

a. Type to be used. Include radioisotope(s) and chemical form(s): Click here to enter text.

b. Describe the practices and procedures to be followed for minimization of radiation exposure to workers and for the handling and disposal of contaminated materials associated with this study:

(Include the methods for management of radioactive wastes and monitoring facility for radioactive contamination, if applicable.) Click here to enter text.

c. Who will be responsible for the daily care of animals containing radioactive materials?

Click here to enter text.

d. Approval received from UD- Environmental Health and Safety? ☐Yes ☐No ☐Pending

Click here to enter text.

Please attach a copy of any approvals or provide the approval number.

Click here to enter text.

21. Study of Irradiation *in vivo*? ☐Yes (gamma irradiator? ☐ or x-ray irradiator? ☐)

☒ No

a. Make, model, and location of irradiator to be used:

Click here to enter text.

b. Approval received from UD- Environmental Health and Safety? ☐Yes ☐No ☐Pending

Please attach a copy of any approvals or provide the approval number.Click here to enter text.

22. Administration of Biological Agents (eg microorganisms, recombinant DNA, **HUMAN** serum, tissue, cell lines, etc.) X Yes ☐No

Animal Biosafety Level X 1 ☐2 ☐3 ☐4

Describe hazards posed to personnel:Plasmid DNA encoding GFP and/or luciferase reporter genes will be administered within a collagen/polymer gel.

Methods to control exposure: Experiments involving plasmid DNA will be handled according to UD procedures for the use of recombinant DNA. The plasmid that will be utilized contains a GFP-Luc fusion reporter gene, and no other genes. Therefore, both the plasmid and the genes it expresses are BSL1. An rDNA registration form for the proposed work has been approved. Collection of murine tissues will be conducted and contained in a biological safety cabinet, or as appropriate, will be conducted on the bench. Personnel will employ standard and approved techniques for handling biological materials, including the use of personal protective equipment (gloves, goggles, lab coat), proper sharps and biological waste disposal (e.g., autoclaving), and restricted access to the laboratories where work is being performed.

Methods of Disposal of Animals and Bedding: All animal carcasses and bedding will be disposed of in the OLAM in an EHS-approved manner.

Approval received from UD- Institutional Biosafety Committee? X Yes ☐No ☐Pending

Please attach a copy of any approvals or provide the approval number. Click here to enter text.

23. Will tumor cells, tissue, sera, viral vectors or other biologics of RODENT origin – other than those isolated from rodents already housed in the facility – be administered to animals?

☐ Yes X No

If Yes, this material must be tested for rodent pathogens and test results must be attached (Please contact the Attending Veterinarian for details). Click here to enter text.

24. Use of Genetically Engineered Animals

☐ Yes X No

If Yes, please describe any anticipated phenotypes that may cause pain or distress and any special care or monitoring that the animals will require.

Click here to enter text.

Does the proposed work involve creating new genetically modified animals, or involve crossing two genetically modified animals to produce offspring with a new genotype.

☐ Yes X No

Approval received from UD- Institutional Biosafety Committee?

☐Yes ☐No ☐Pending ☐Exempt (breeding of two lines of genetically-modified rodents is exempt if 1) both parents can be housed under BL1 containment and 2) neither parent strain incorporates more than one half of the genome of an exogenous eukaryotic virus or incorporates a transgene under the control of a gammaretroviral long terminal repeat and 3)the rodent that results from the breeding is not expected to contain more than one half of an exogenous viral genome)

Please attach a copy of any approvals or provide the approval number.

Click here to enter text.

25. Special Study Requirements: Please describe any special study requirements such as a requirement for single housing of the animals, exemption from environmental enrichment, or special caging:Click here to enter text.

26. Any other procedures? X Yes ☐ No

If **Yes,** explain: The *in vivo* analysis of luciferase activity will be performed according to well-established procedures. Initially, mice will be administered luciferin *via* ip injection (0.2 mL of a 15 mg/mL solution); 8 minutes later, the animals will be anesthetized (inhalation of isoflurane [3-5% during induction, 1-3% during maintenance] in 0.5-1.0 L/min oxygen) on the stage of a Caliper IVIS Lumina Imaging System equipped with premium animal handling features including a heated stage and a 5-position manifold that allows anesthesia to be maintained during imaging. This method of anesthesia will enable anesthesia to be appropriately maintained during imaging procedures. Immediately following imaging, the animals will be sacrificed (see description of procedures for euthanasia) and subdermal injection tissues removed for histochemical analyses of transfection efficiency, tissue morphology, and cell viability. Luciferase activity, transfection efficiency (GFP expression), and cell viability can all be assessed with the same animals, thereby minimizing the number of animals required for the planned end-point analyses.

27.Will this study involve surgery? ☐ Yes x No

If **Yes,** and it is "Survival Surgery," please complete Addendum "**D**".

If **Yes,** and it is "Terminal Surgery," please complete Addendum "**E**".

28.Will any animal undergo anesthesia for any reason other than surgery? x Yes ☐ No

If **Yes,**

a. List Procedures and Reason(s) for using anesthesia: Please see description in item #5.

b. Check the type of anesthesia to be used.

X Isoflurane

☐Injectable (***For injectable, complete the following):***

Drug:	Click here to enter text.
Dose:	Click here to enter text.
Route:	Click here to enter text.

29. Animal Use and Pain Distress. If you have indicated that **animals in your study will experience pain or distress**, even if it will be fully alleviated, please mark the appropriate check boxes below and fill in the requested information for each item marked.

You must conduct at least two (2) searches.

I have considered alternatives to the use of animals in my study. Alternatives refer to methods or approaches which result in refinement of procedures which lessen pain and/or distress; reduction in numbers of animals required; or replacement of animals with non-whole-animal systems or replacement of one animal species with another, particularly if the substituted species is non-mammalian or invertebrate. I have used the following methods and sources to search for alternatives:

Note: You may need to do more than one search per database to look for alternatives if there are multiple procedures that may cause pain and/or distress.

Database Used:

X **Medline**	☐ **Agricola**
☐ **Toxline**	☐ **CAB Abstracts**
☐ **Biosis**	☐ **Other *(Specify)***: Click here to enter text.

Date of Search: January 12, 2015

Years Covered: All

Keywords Used (must include the word *alternative*): alternative, mouse, wound, gene therapy, biomaterial

Number of Papers Found: 4

Discussion of the Relevancy of the Papers Found: The four articles found describe murine wound repair studies involving gene therapy. One article studies the biological effects on wound repair of delivering the growth factor VEGF from a hydrogel scaffold; two articles study/report on the improvements in repair mediated by wound glues (one is a review article); and one article describes the healing effects of injecting stem cells. None of the articles report alternatives to the murine models for repair.

Database Used:

☐ **Medline**	☐ **Agricola**

X **Toxline**	☐ **CAB Abstracts**
☐ **Biosis**	☐ **Other *(Specify)***: Click here to enter text.
Date of Search: January 12, 2015	
Years Covered: All	
Keywords Used (must include the word *alternative*): alternative, mouse, wound, gene therapy, biomaterial	
Number of Papers Found: 1	
Discussion of the Relevancy of the Papers Found: The article describes an alternative therapy for wounds using a murine model, but does not identify alternative models to reduce pain.	

30. Unnecessary Duplication of Work. Activities involving animals must not unnecessarily duplicate previous experiments performed by you or others. Provide a written narrative that assures that the activities of this project comply with this requirement and support this assurance by performing a literature search.

The search should return, at minimum, the related previous work from your laboratory.

You must conduct at least two (2) searches.

(NOT REQUIRED FOR TEACHING PROTOCOLS)

Note: You may need to do more than one search per database to look for duplication of work, especially if you are doing more than one experiment.

Database Used:	
X **Medline**	☐ **Agricola**
☐ **Toxline**	☐ **CAB Abstracts**
☐ **Biosis**	☐ **Other *(Specify)***: Click here to enter text.
Date of Search: January 12, 2015	
Years Covered: All	
Keywords Used: Gene delivery and collagen mimetic peptide	
Number of Papers Found: 1	
Discussion of the Relevancy of the Papers Found: The identified paper used a substantially different biomaterials approaches than the proposed approach to improve tissue repair. The focus was also on bone repair as opposed to cutaneous repair. Publications from my laboratory are still in press and therefore were not identified. No duplicative work identified.	

Database Used:

☐ **Medline**	☐ **Agricola**
☐ **Toxline**	☐ **CAB Abstracts**
☐ **Biosis**	X **Other *(Specify)***: Web of Science

Date of Search: January 12, 2015

Years Covered: All

Keywords Used: Gene delivery and collagen mimetic peptide

Number of Papers Found: 6

Discussion of the Relevancy of the Papers Found: The papers identified describe various biomaterials containing peptides and collagen, and their use for gene delivery during tissue repair. None of the approaches uses collagen mimetic peptides to direct gene delivery and hence there is no unnecessary duplication.

31. What is the expected disposition of animals at the end of the experiments?
(Check all that apply):

X Euthanized

☐Maintained

☐Released ***(Wildlife Only)***

☐Other ***(Specify)***: Click here to enter text.

32. Euthanasia*

Select methods that will be used in case of emergency and/or at the end of the procedure/experiment.

***NOTE:**

- Methods must be approved by the AVMA or must be scientifically justified.
- A "Primary" and "Secondary" method must be selected (UD Double Kill Policy).
- **If different methods will be used for different groups** of animals, indicate the group after the procedure (e.g., write "Neonates" after Decapitation, "Adults" after CO_2, "Terminal Surgery Animals" after Isoflurane Anesthesia Overdose, etc.).

☐Animals will NOT be under anesthesia when euthanasia is performed.

X Animals will be under anesthesia when euthanasia is performed. ***(Check drug used below)***:

X Isoflurane

☐ Injectable ***(Complete the following)***:

Drug: Click here to enter text.
Dose: Click here to enter text.
Route: Click here to enter text.

PRIMARY method(s) of euthanasia

X CO_2 by compressed gas cylinder ***(Not for animals already under anesthesia or neonates)***

☐Barbiturate Euthanasia Solution - Injectable ≥150mg/kg ***(Check route below)***:

☐IV ☐IP ☐IC

X Isoflurane Anesthesia Overdose - Inhalant

☐Cervical Dislocation ***(only under anesthesia)***

☐Decapitation ***(only under anesthesia or neonates)***

☐Exsanguination or Perfusion ***(only under anesthesia)***

☐Incision of Chest Cavity – Bilateral Pneumothorax ***(only under anesthesia)***

☐Pithing – ***(only under anesthesia) (amphibians, reptiles only)***

☐Removal of Vital Organ(s) ***(only under anesthesia) (Check all that apply):***

☐Brain ☐Kidneys

☐Heart ☐GI Tract

☐Liver ☐Lungs

☐Other Vital Organ(s) – ***(Specify):*** Click here to enter text.

☐Other Method of Euthanasia: ***(Describe and Scientifically Justify)***:

SECONDARY method(s) of euthanasia that will be used to ensure that the animal does not survive:

X Cervical Dislocation

☐Decapitation

☐Exsanguination or Perfusion

☐Incision of Chest Cavity – Bilateral Pneumothorax

☐Barbiturate Euthanasia Solution - Injectable ≥150mg/kg ***(Check route below)***:

☐IV ☐IP ☐IC

☐Pithing – Double pithing required ***(fish, amphibians, reptiles only)***

☐Removal of Vital Organ(s): ***(Check all that apply)***:

☐Brain ☐Kidneys

☐Heart ☐GI Tract

☐Liver ☐Lungs

☐Other Vital Organ(s) – ***(Specify):*** Click here to enter text.

☐ Other Method of Euthanasia: ***(Describe and Scientifically Justify)***: Click here to enter text.