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PI:
ID: PRO00007939
Status: Protocol Approved
Expiration Date: 10/31/2020

Combinatorial Therapeutics for Regenerative Endodontics - PRO00007939

Protocol ID: PRO00007939
Protocol Name: Combinatorial Therapeutics for Regenerative Endodontics
Protocol PI:
Approval Date: 10/31/2017
Expiration Date: 10/31/2020
Renewed From:

PI

Name	Appointment
	391700, DENT

Protocol Role:	Contact Information	First	Middle	Last			
Role Name							
Principal Investigator	**Campus Address**						
Lab Contact/Email Recipient							
Authorized Signer	**City**	Ann Arbor	**State**	MI	**Zip**	48109	
Animal Handler							
Animal Health Contact	**Phone**		**eRAM System Email**				
Application/Protocol Editor	**Alt Phone**		**eRAM System Override Email**				

List degrees and briefly describe animal handling experience and qualifications relevant to the species selected for this particular study. Note: This text field contains information relevant to all approved protocols this individual is listed on. Do not delete information relevant to species and procedures on other animal use protocols.

is a dentist-scientist with significant expertise in various animal models of tissue regeneration in rats, rabbits, and dogs.

UM Employed:

General Information

Protocol Title:
Combinatorial Therapeutics for Regenerative Endodontics

Indicate the types of animal use proposed in the application.
Basic / Applied Research

Briefly explain the overall scope of the study and why the study is important to human or animal health, the advancement of knowledge, or the good of society. This section should be clearly written in language that a layperson can easily understand.
Dental pulp (i.e. nerve) injury caused by cavities (caries) or physical trauma, leads to inflammation, which if left untreated, results in pulp tissue necrosis (death).
Loss of an immature (young children) permanent tooth can be devastating, mostly because dental implants (artificial tooth) placement are contraindicated in these young patients.
Globally, 21% of children aged 6 to 11 have caries in their permanent teeth and 18% of school children in the United States experience dental trauma.
Traditional treatment of a young tooth that has its pulp tissue dead is a root canal treatment. Although there is a good success rate for teeth that received a root canal treatment, the materials used to fill the canals are non-biological (synthetic-based plastic material), and do not support the continuous development of the root or the regeneration of the lost (dead) dental pulp tissue. This increases the risk of tooth loss following re-infection or secondary trauma.
Meanwhile, existing regenerative-based therapy has shown limited success, with only one study describing the regeneration of the dental pulp following a clinical procedure termed evoked bleeding (EB). This lack of progress is due to patient-to-patient variability in regenerative response and to the extreme cell toxicity of antibiotic pastes used for disinfection in the EB method. We have identified a biocompatible disinfection strategy, which uses triple antibiotic eluting nanofibers as 3D drug delivery constructs (3D-C) capable of ablating periapical infection and eliminating dentin biofilm with minimal dental pulp stem cell (DPSC) toxicity. Disappointingly, no clinical therapy combines biocompatible disinfection with predictable pulp-dentin regeneration. With this in mind, we are proposing to establish an innovative therapeutics to treat young permanent teeth with necrotic pulps.
The overall goal of this proposal is to establish an innovative combinatorial 2-step therapeutics to treat immature (open apex) permanent teeth with necrotic pulps. In Step-1, we will ablate periapical infection using our biodegradable 3D-C. In Step-2, we will concentrically inject our tissue-specific collagen-fibril matrices to enable dentin and pulp regeneration at the appropriate locations within the root canal system. Based on our strong and exciting preliminary data, we hypothesize that collagen-fibril matrices of precise stiffness can induce dental pulp stem cells (DPSCs) differentiation into odontoblastic and endothelial lineages, leading to predictable dentin and pulp regeneration. We anticipate that the study findings will aid in the development of a novel biocompatible disinfection strategy by using triple antibiotic eluting nanofibers as 3D-C and highly tunable injectable oligomeric collagen-fibril matrices for dentin-pulp regeneration.
Taken together, the proposed research is relevant to public health because its successful execution offers the basis for novel therapeutics for pulp regeneration and thus enhanced tooth vitality (sensations to cold/hot and pain) for millions of juvenile patients. Our findings are anticipated to lead to novel therapeutics to treat necrotic immature (open apex) permanent teeth and will be further validated in future human clinical trials.

Explain why animals must be used to accomplish your proposed work. For each species selected in this protocol, describe why it was chosen for this project. Explain the physical and/or physiological characteristics of the species that makes it ideal for this research, and explain why a lesser sentient species cannot be used. Further explain why non-animal models (cell or tissue culture, isolated organ preparations, computer simulations, etc.) cannot be used.
The experiments described here intend to investigate the possibility of engineering a metabolically active dental pulp tissue capable to form dentin (mineralized tissue) aiming to minimize the consequences of dental caries and trauma. Although it is possible to evaluate certain aspects of tissue regeneration in vitro, no single or group of in vitro assays permit a thorough analysis of the unique regeneration process of the dentin-pulp complex.

We are investigating pulp tissue healing/regeneration, particularly in permanent (2nd dentition) but immature teeth, i.e., teeth that may be affected by trauma and/or caries while in their developmental stages (that is the reason the requested animal are at young age). Thus, we will need to use vertebrate animals to determine whether the experimental biomaterials do, in fact, promote root canal disinfection (triple antibiotic eluting nanofibers as 3D drug delivery constructs/3D-C) and tissue regeneration (collagen-fibril matrices/scaffolds). There are no non-animal alternatives or alternative procedures available to achieve the proposed goals, as described in the protocol.

The scientific dental community, more specifically the one related to prevention, maintenance, and treatment of endodontic- and periodontal-related infections (i.e., periapical and periodontal diseases) has established clinically relevant models in the dog, in particular beagles, because of the pathophysiology as well as the size and anatomy of the canine tooth and more importantly the metabolism of periodontal structures (e.g., bone remodeling) are comparable to humans.

As humans cannot be used for extensive experimentation, the establishment of suitable animal models which will closely parallel human reactions is a major concern [J Endod. 1979 Nov;5(11):322-7. Pulpal response to minimal exposure in presence of bacteria and Dycal.]. In this way, McWalter et al. [1973; Pulp capping in monkeys with a calcium-hydroxide compound, an antibiotic, and a polycarboxylate cement. Oral Surg 36(1):90-100, 1973] demonstrated that the pulp of monkeys appears to be much less sensitive to injury than human pulp tissue based on the significant healing potential. Weiss and Bjorvatn [Weiss, M.B., and Bjorvam, K. Pulp capping in deciduous and newly erupted permanent teeth of monkeys. Oral Surg 29: 769-775, 1970] reported

that that even when death of the pulp occurs in the monkey, periapical lesions may not necessarily develop. Meanwhile, Citrome et al. [A comparative study of tooth apexification in the dog. J Endod 5(10):290-297, 1979] showed that the pulpal tissue of dogs is as sensitive as that of humans. In detail, that apexification study, four of the five "blunderbuss" (immature permanent teeth, exact what we are proposing, so that one can determine the impact of the biomaterial/regenerative strategy on the tooth development) canals left unfilled, but cleaned and sealed, developed periapical lesions within 11 weeks with significant resorption of cementum and dentin. Collectively, these findings suggest that the pulp of dogs is equally or perhaps more sensitive than that of humans and is, therefore, an excellent model for endodontic research.

Based on the aforementioned, wa respectfully submit and propose that we continue (PI: had the proposed protocol previously approved at Indiana University IACUC Committee, available upon request) to use of the well-established dog model of periapical disease (Wang X et al. Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis, J Endod. 2010;36(1):56-63) to determine the regenerative potential of the biomaterials and strategy proposed in this project. In fact, the PI: is about to submit the preliminary data obtained in that study for publication.
Moreover, due to the size adequacy (similar size teeth with compatible overall dimension of the pulp chamber in which the biomaterials will be placed either for disinfection or to promote tissue healing/regeneration), root canal can be accessed with clinical instruments used in humans, which also represents an important step toward clinical translation of the biomaterials and overall regenerative strategy proposed in this project.

Most of in vivo studies using animal models have performed the aforementioned protocol to evaluate different regenerative therapies. Taken together, endodontic treatment in canine model is the smallest animal model that will allow placement of the proposed scaffolds in the immature root canals where the materials are intended to be clinically used and allow us to draw conclusions overall the potential benefits to humans. This might become an ideal animal model system for these type of research because it will allow for the investigation of dental pulp tissue regeneration.

Funding

Will this project be fully or partially funded through one or more external sources?
Yes

Will this project be fully or partially funded through one or more internal sources?
No

External Sponsor / Funding Sources
NOTE: column names starting with "PAF" display information directly from Proposal Management.

PAF Sponsor Name	PAF Sponsor Description	PAF Official Sponsor Desc	PAF Sponsor Role	PAF ID	PAF Title	PAF PI	PAF State	PAF Has Vertebrate Animals?	External Funding Documents	Attached Documents?	Award
NIH	NIH	Health and Human Services, Department of-National Institutes of Health	Direct	18-PAF00030	Novel Therapeutics to Treat Necrotic Teeth	(UM Principal Investigator)	Awarded	yes		no	AWD0063

Personnel

Will ULAM Technical Services (IVAC, ASOR, ULAM Breeding Colony Management) be utilized?

Yes

Name	Role(s)
Name:	Application/Protocol Editor

Protocol Roles:

Role Name	Contact Information					
Application/Protocol Editor	Campus Address					
	City	Ann Arbor	State	MI	Zip	48109
	Phone		eRAM System Email			
	Alt Phone		eRAM System Override Email			

Species

Dog

Species Detail - Dog

Will any of the animals used, generated, or acquired for this protocol be transgenic or genetically modified?
No

Animal Acquisition

ULAM Acquired? No

Non-ULAM sources? Yes

If animals are acquired via non-ULAM sources (e.g., any field research, purchases outside of ULAM) the "Report Non-Traditional Animal Acquisition" use form is required to regularly report animal use. Refer to the tutorial on creating animal use forms in eRAM for further instruction. For animals acquired through breeding refer to the Policy on Counting Animals Bred for reporting needs.

Animal Transportation

Will personal vehicles be used to transport animals? No

Quarantine and Conditioning

Will any animals be singly housed for experimental reasons? No

Will you be withholding enrichment from any animals for scientific reasons? Yes

Provide scientific justification for withholding enrichment.
Enrichment may be provided as long as the dogs cannot chew on it (i.e., nyla bones/rawhide).

Location - Dog

Location:

Intended Purpose(s) for this location.

Housing?
No

Use?
Yes

Will recovery surgery be conducted in this location?
Yes

Will non-recovery surgery be conducted in this location?
No

Will euthanasia be conducted in this location?
Yes

Will CO2 overdose be conducted in this location?
No

Location - Dog

Location:

Intended Purpose(s) for this location.

Housing?
Yes

Use?
No

Will the laboratory provide daily husbandry care instead of ULAM?
No

Procedure Summary - Dog

Will non-pharmaceutical grade (reagent-grade) substances be administered to the animals?
Yes

List each reagent-grade drug and provide justification for its use. Justification must be based on unavailability of an acceptable veterinary or human pharmaceutical-grade compound or on scientific necessity. (Note: USDA and OLAW do not consider expense an appropriate justification for use of reagent-grade substances.)
The triple antibiotic containing 3D polymer constructs are processed in our laboratory via electrospinning (i.e., a nanotechnology processing method that transforms a polymer solution in a 3D construct composed of nano/microfibers). The polymer used in the fabrication of the 3D construct is polydioxanone, an FDA-approved resorbable suture material.
The antibiotics used will be pharmaceutical grade.

GeniPhys is a Purdue University based start-up business that specializes in the commercialization of the first standardized, tunable collagen polymers (Collymers) and collagen-fibril materials for research and medical applications. The collagen-fibril materials are currently manufacture research-grade.

The growth factors, namely bone morphogenetic protein-2 (BMP2) and vascular endothelial growth factor (VEGF) are not available in pharmaceutical grade.

Describe the steps that will be taken to ensure stability, potency, and sterility when preparing each non-pharmaceutical grade substance:

1. **How will you ensure sterility (e.g., use of sterile container, sterile diluents, 0.2 um filter)?**
2. **What is the shelf life/use by date for each substance, and how was it determined?**
3. **How will stock and working solutions be stored (e.g., in freezer or refrigerator, at room temperature), include the temperature and duration of storage.**

The triple antibiotic 3D polymer constructs are processed in a chemical hood using using sterile instruments, pipettes and tips. The constructs will be submitted to ethylene oxide sterilization prior to animal implantation. The collagen-fibril biomaterials (scaffolds) are received from the company (GeniPhys) as a sterile compound, it is aliquoted into sterile tubes using sterile pipette tips. All tooth scaffolds, root canal tips and scaffolds are prepared in a bio-safety cabinet using sterile instruments, pipettes and tips. The growth factors (i.e., BMP2 and VEGF) will be prepared using sterile tubes tips and reagents.
The shelf life for the collagen-fibril biomaterials (GeniPhys) is 6 months (as provided by the manufacturer) when stored
at 4°C
The shelf life for the growth factors follows the recommended protocol of the manufacturers': the Lyophilized growth
factors (BMP2 or VEGF) although stable at room temperature for 3 weeks, should be stored desiccated at -20°C. Upon
reconstitution the growth factors should be stored at 4°C between 2-7 days and for future use below -20°C.

Procedure Summary

1. **Provide an outline for each proposed experiment, including pertinent timepoints and endpoints.**
2. **Provide a complete description of each procedure performed on live animals, including euthanasia. Do not describe procedures done post-euthanasia or in vitro work.**

Note: The selected housing room and surgery suite are next to each other and the dogs do not need to travel through any hallways to go between the two. We will transport dogs in covered transport carts or just carry them by hand and expect the trip to take less than one minute total. We expect minimal anxiety during this brief process

Surgery in dogs will be performed in an operating room used only for surgery. The dogs will be given carprofen 4 mg/kg SQ 1-2 hours before surgery, and pre-medicated with buprenorphine (0.01-0.02 mg/kg IM) 10-15 minutes before an indwelling intravenous cathether is placed, followed by induction with propofol (2-8 mg/kg intravenous). Animals will be intubated and maintained on isoflurane. Cefazolin 20 mg/kg IV will be administered as an antibiotic. Atropine may be utilized as needed for the treatment of bredycardia or hypersalivation. Nerve block anesthesia (Bupivacaine 1%/Lidocaine 1% 1:1 ratio, local anesthesia for inferior alveolar nerve) will be given before the clinical procedures.

Signs of pain will include the observation of overall attitude (scared/submissive appearance, unwilling to eat/interact with people, inability to lay down), body movement (trembling), facial expression (tense facial muscles with furrowed brows, dilated pupils, ears flattened against head), guarding (growling when approached), posture (hunched up), respiratory pattern (short, shallow breathing pattern), and vocalization (crying, whining...). Pain monitoring will be done approximately every 8-12 hours for the first 48 hours post-op. Then, will be done twice a day for the following 24-48 hours by observation and pain scoring, and for the appropriate decision to administer pain-relieving medications. Animals will be given buprenorphine 0.01-0.02 mg/kg SQ or IM every 8-12 hours for a minimum duration of 2 days post-op, then for rescue analgesia based on pain scoring. In addition, carprofen 4 mg/kg SID will be given for a minimum of 3 days.

The dogs post-surgery will be checked daily by the PI/lab staff until the surgical wound heals. PI/lab staff will request further veterinary support if any complications are observed. Pain scores will be done approximately every 8-12 hours for a minimum of 72 hours post-op. The animals will be administered; a) Buprenorphine 0.01-0.02 mg subcutaneous or intramuscular 8-12 hours (2 doses minimum) post-op and b) Carprofen 4 mg/kg subcutaneous or oral once daily for 3 days minimum post-op. The animals will be able to chew/eat normally after the surgeries. The animals will be given water and canned dog food everyday as long as the animals are fully recovered.

All Dates listed are approximate (~)
X-rays will be conducted under sedation with Ketamine 5-10mg/kg + midazolem 0.1-0.2mg/kg IV or IM.

STUDY 1:
Week 0: Arrival of animals
Week 1: Mandibular pre-molars (P2, P3, and P4 – bilaterally) and maxillary pre-molars (P2 and P3 – bilaterally) will be submitted to periapical lesion induction (Bacterial Sampling, Baseline Sampling/S1)
Week 5: X-rays to confirm periapical lesions (Bacterial Sampling/S2), endodontic treatment and disinfection strategy either with the triple antibiotic containing 3D polymer construct (3D-C) or triple antibiotic paste (TAP).
Week 9: X-rays, (Bacterial sampling/S3), euthanasia of animals, and preparation for microCT and Histological analysis

Immature permanent double-rooted premolar teeth will be selected in ~4-5-months-old dogs. Radiographs wil

be taken to confirm incomplete root development. Within each dog, the teeth will be randomly divided: 3D drug delivery construct (3D-C, n=4), TAP (1g/mL) (TAP, n=4), and negative control (NC, n=2, normal healthy pulp). Three interventions will be conducted: 1. Periapical Lesion Induction - An intravenous injection of propofol in addition to local anesthetic will be given to all animals prior to pulp exposure and disruption in all experimental teeth. Sterile sponges will be soaked in a supragingival plaque suspension (dogs' teeth) and placed into the pulp chamber. The teeth will be temporarily sealed with a restorative cement and radiographically monitored until periapical lesion is confirmed (~28 days). 2. Baseline Bacterial Sampling - Under anesthesia, the teeth will be isolated (rubber dam) and re-entered. Pulp chamber will be dried (cotton pellets) prior to injection of dental transport fluid (DTF) into the mesial canal of each tooth. Samples will be collected after DTF agitation and then soaked up and transferred with sterile paper points to the DTF bottle (S1). Next, each canal will be irrigated with 1.25% NaOCL, flushed with sodium thiosulfate, irrigated with saline, and dried with paper points. DTF will be injected into the mesial canals, adsorbed with paper points and transferred to the DTF bottle (S2). 3. Disinfection - Under rubber dam isolation, each canal will be irrigated with sterile saline and dried prior to 3D-C insertion or TAP injection followed by placement of a restoration. After 4 weeks, the coronal seal will be removed, chamber irrigated with sterile saline, and the canals dried with peper points. Again, DTF will be injected into the mesial canals and paperpoints will be used to absorb the DTF prior to transferring to the DTF bottle (S3). Premolars (n=8, 2/dog) allocated to the negative control (i.e., hasithy pulp) will be accessed and sampled identically to the test groups. These will serve as controls to verify the effectiveness and asepsis of the sampling technique. Dogs will be euthanized 30 days post-disinfection
Radiographic Evaluation Radiographs will be taken before and after periapical lesion induction and compared with follow-up radiographs, which will be taken after disinfection.
X-rays will be taken with the animals under general anesthesia and/or sedated (if not major procedure is being performed, i.e., one of the three scheduled surgical interventions).

STUDY 2:
Week 0: Arrival of animals
Week 1-4: Mandibular pre-molars (P2, P3, and P4 – bilaterally) and maxillary pre-molars (P2 and P3 – bilaterally) will be submitted to periapical lesion induction
Week 5-8: X-rays to confirm periapical lesions. Then once disease is confirmed (apical radiolucency), under anesthesia, the teeth will be re-entered to initiate the disinfection protocol. Under rubber dam isolation, the restorative sealing material and sponge will be removed and root canals will be irrigated with NaOCl and dried with sterile paper points prior to insertion/injection of the disinfecting materials/strategies, namely 3D-C or TAP injection. Teeth will be sealed and the medications allowed to act for 4 weeks. Then, the coronal seal will be removed, chamber irrigated with sterile saline, and the canals dried with paper points. Abundant saline irrigation will be performed to ensure complete removal of any remaining pieces of the degradable 3D-C.
Week 9-20 Regenerative Strategy - Transduced autologous DPSCs will be encapsulated within the distinct collagen-fibril matrices. Teeth treated for 4 weeks either with 3D-C (Group 3, proposed strategy) or TAP (Group 4) as well as non-infected teeth (Group 5) will be subjected to the concentric injection of the collagen-fibril matrices. An endodontic fiber post of suitable size will be stabilized inside the tooth and the first collagen solution (outer) will be injected. Upon setting (3-5 min), the post will be removed and the second solution will be injected (inner). Non-operated premolars (P1 maxilla and mandible) will be left to develop naturally for comparison. All operated teeth will be closed with white MTA and composite. All teeth will be radiographically assessed every month, until the animals' euthanasia.

Immature permanent double-rooted premolar teeth will be selected in ~4-6-months-old dogs. Within each dog, the teeth will be randomly divided into 5 groups that will undergo different disinfection and regenerative strategies Since evoked bleeding [EB] is an established procedure, after disinfection, for Groups 1-2 a sterile #30 stainless steel pre-curved K-file will be introduced 2 mm past the apical foramen to allow blood invasion to the CEJ level. A resorbable collagen-based matrix will be placed over the blood clot. Then, these teeth will be closed with white mineral trioxide aggregate (MTA) and resin composite. Intervention 1 = Periapical Lesion Induction - Pulp tissue will be exposed in the premolar teeth allocated to Groups 1-4. Sterile sponges will be soaked in a supragingival plaque suspension and positioned into the pulp chamber prior to placement of a temporary (IRM) restoration. Intervention 2 (Procedure 1) Disinfection - once the development of periapical disease is confirmed (apical radiolucency), under general anesthesia, the teeth will be re-entered. Under rubber dam isolation, IRM and sponge will be removed and root canals will be irrigated with NaOCl and dried with sterile paper points prior to insertion of 3D-C [novel proposed drug delivery strategy] or [TAP] injection. Teeth will be sealed and the medications allowed to act for 4 weeks. Intervention 2 (Procedure 2) Pulp tissue collection from different set of teeth (canines) - On the day of disinfection, dental pulp will be extirpated from the upper canines. These teeth will only serve as the source of DPSCs and will be restored with MTA and resin composite. DPSCs will be isolated from the pulp tissue by enzymatic digestion. Intervention 3 (Regenerative Strategy) The treated premolar teeth will have their coronal seal removed, chamber irrigated with sterile saline, and the canals dried with paper points. Abundant saline irrigation will be performed to ensure complete removal of any remaining pieces of the degradable 3D construct [3D-C]. Transduced autologous DPSCs will be encapsulated within the distinct collagen-fibril matrices. Teeth treated for 4 weeks either with 3D-C (Group 3, proposed strategy) or TAP (Group 4) as well as non-infected teeth (Group 5) will be subjected to the concentric injection of the collagen-fibril matrices. An endodontic fiber post of suitable size will be stabilized inside the tooth and the first collagen solution (outer) will be injected. Upon setting (3-5 min), the post will be removed and the second solution will be injected (inner). Non-operated premolars (P1 maxilla and mandible) will be left to develop naturally for comparison. All operated teeth will be closed with white MTA and composite. All teeth will be radiographically assessed every month, until the animals' euthanasia.
X-rays will be taken with the animals under general anesthesia and/or sedated (if not major procedure is being performed, i.e., one of the three scheduled surgical interventions).

All items are prepared in a biosafety cabinet using sterile vials, pipette tips and tubes. Sterile needles will be used for administration to the dogs.
Compounds to be used in this protocol include:
collagen scaffold (800 Pa stiffness)
collagen scaffold (235 Pa stiffness)
Bone morphogenetic protein 2 (BMP2). It will be incorporated into the collagen scaffold (800 Pa stiffness)
Vascular endothelial growth factor (VEGF). It will be incorporated into the collagen scaffold (235 Pa stiffness)
All reagents will be of pharmaceutical grade and prepared using sterile vials and tips with sterile solutions in a bio-safety
cabinet. For administration to the dogs, sterile needles and syringes will be used

Procedures

Anesthetic, Analgesic, Tranquilizing or Neuromuscular Blocking Agents
Yes

Anesthetic/Analgesic Details

Will the Guidelines for anesthesia, analgesics, and post anesthetic care be followed for this species?
Yes

Agent	Type	Dose	Unit
Atropine	Drug - Other	0.01 - 0.04	mg/kg

Does the Dose Range differ from the recommendation?
No
Routes of Administration
Route
SC
Does the Route of Administration differ from the recommendations?
No
Is there any additional information related to this agent?
Yes
Detail the additional agent information.
Atropine 0.04 mg/kg will be given SC approximately 15-20 minutes before induction with propofol.

Agent	Type	Dose	Unit
Bupivacaine	Analgesic	1 - 1	%

Does the Dose Range differ from the recommendation?
No
Routes of Administration
Route
SC
Does the Route of Administration differ from the recommendations?
No

Description of Analgesic Administration	Duration of Analgesic Effectiveness	Unit
nerve block Lidocaine 1%/Bupivacaine 1% 1:1 ratio, local anesthesia for alveolar nerve block	4-8	Hour(s)

Does this differ from UCUCA Policy?
No
Is there any additional information related to this agent?
No

Agent	Type	Dose	Unit
Buprenorphine	Analgesic	0.01 - 0.02	mg/kg

Does the Dose Range differ from the recommendation?
No

Routes of Administration

Route

IM

SC

Does the Route of Administration differ from the recommendations?
No

Description of Analgesic Administration	Duration of Analgesic Effectiveness	Unit	Does this differ from UCUCA Policy?
will be given preemptively	6-8	Hour(s)	No

Is there any additional information related to this agent?
Yes

Detail the additional agent information.
Pain will be evaluated at correct intervals by observation and pain scoring, and for the appropriate decision to administer pain-relieving medications. Animals will be given buprenorphine 0.01-0.02 mg/kg SQ or IM every 8-12 hours for a minimum duration of 2 days post-op, then for rescue analgesia based on pain scoring

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Agent	Type	Dose	Unit
Carprofen	Analgesic	1 - 4	mg/kg

Does the Dose Range differ from the recommendation?
No

Routes of Administration

Route

SC

Does the Route of Administration differ from the recommendations?
No

Description of Analgesic Administration	Duration of Analgesic Effectiveness	Unit	Does this differ from UCUCA Policy?
dogs will be given carprofen 4 mg/kg SQ 1-2 hours before surgery	18-24	Hour(s)	No

Is there any additional information related to this agent?
Yes

Detail the additional agent information.
In addition, carprofen 4 mg/kg SID will be given for a minimum of 3 days, and Baytril 5-10 mg/kg BID for 5 days will also be administered.

Agent	Type	Dose	Unit
Isoflurane - Vaporizer	Anesthetic	1 - 3	%

Does the Dose Range differ from the recommendation?
No

Routes of Administration

Route

Inhalation

Does the Route of Administration differ from the recommendations?
No

Duration of Anesthesia	Unit	Max Supplemental Dose (See Guidelines)	Unit	Select method(s) for scavenging waste gases.
4-8	Hour(s)	5	%	Active scavenging with a vacuum system

Will there be variations from standard anesthetic administration? No

Is there any additional information related to this agent?
No

Agent	Type	Dose	Unit
Ketamine	Anesthetic	5 - 10	mg/kg

Does the Dose Range differ from the recommendation?
No

Routes of Administration

Route

IV

Does the Route of Administration differ from the recommendations?
No

Duration of Anesthesia	Unit	Max Supplemental Dose (See Guidelines)	Unit
10-20	Minute(s)	another dose (5 mg/kg)	mg/kg

Will there be variations from standard anesthetic administration? No

Is there any additional information related to this agent?
Yes

Detail the additional agent information.
Isofluorane inhaled from precision vaporizer using endotracheal tube with waste gas scavenging.

Agent	Type	Dose	Unit
Lidocaine	Analgesic	1 - 1	%

Does the Dose Range differ from the recommendation?
No

Routes of Administration

Route

SC

Does the Route of Administration differ from the recommendations?
No

Description of Analgesic Administration	Duration of Analgesic Effectiveness	Unit
nerve block Lidocaine 1%/Bupivacaine 1% 1:1 ratio, local anesthesia for alveolar nerve block	2-4	Hour(s)

Does this differ from UCUCA Policy?
No

Is there any additional information related to this agent?
No

Agent	Type	Dose	Unit
Midazolam	Tranquilizer	0.2 - 0.3	mg/kg

Does the Dose Range differ from the recommendation?
No

Routes of Administration

Route

IV

Does the Route of Administration differ from the recommendations?
No

Is there any additional information related to this agent?
Yes

Detail the additional agent information.
Ketamine/Midazolam for induction followed by isofluorane

Agent	Type	Dose	Unit
Propofol	Anesthetic	2 - 8	mg/kg

Does the Dose Range differ from the recommendation?
No

Routes of Administration

Route

IV

Does the Route of Administration differ from the recommendations?
No

Duration of Anesthesia	Unit	Max Supplemental Dose (See Guidelines)	Unit
60-90	Second(s)	1.6	mg/kg

Will there be variations from standard anesthetic administration? No

Is there any additional information related to this agent?
Yes

Detail the additional agent information.
induction with propofol 2-8 mg/kg intravenous (IV). Animals will be intubated and maintained on isoflurane

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Euthanasia **Yes**

Dog

Methods used to ensure that the animals will not revive.
Other

Other Description:
Beuthanasia®-D (Sodium pentobarbital 390mg + sodium phenytoin 50 mg/ml)), followed by observation of heartbeat cessation greater than 1 minute to ensure death, as recommended by the Panel on Euthanasia of the American Veterinary Medical Association

Will animals be perfused with paraformaldehyde or another fixative?
No

Euthanasia Method: Barbiturate overdose

Justify why an acceptable method cannot be used.

Is the source of the carbon dioxide compressed gas calibrated to a flow rate of 10-30%?
(note: All ULAM euthanasia rooms comply with this requirement)

Agent	Trade Name	Controlled Substance	Dose	Route of Administration
Sodium Pentobarbital-Euthanasia		yes	100-130	IV

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Imaging **Yes**

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Surgery **Yes**

Have animals undergone major surgical procedures prior to use in this protocol?
No

Will animals undergo more than one surgical procedure from which they are allowed to recover while under this protocol?
Yes

List surgeries in order of which they are performed.
For Study 1: Three interventions will be conducted (lesion induction, bacterial sampling, and disinfection). For Study 2: Three interventions will be conducted (lesion induction, disinfection, injection of the collagen-fibril matrices)

Duration between recovery surgeries:
Lesion induction (4 weeks recovery), Disinfection (4 weeks recovery), and injection of collagen-fibril matrices (12 weeks recovery)

Type of Surgery
Oral

Is this a recovery surgery? Yes

Is this a non-recovery surgery? No

Will the recommendations within surgical guidelines for rodents or mammals be followed regarding:

Preparation of the surgical area and supplies?
Yes

Preparation of the animal and surgeon?
Yes

Suture material and closure?
Yes

Post-operative monitoring and care?
Yes

All other recommendations within the guidelines?
Yes

Adverse Consequences - Dog

Describe how you have refined experimental procedures to minimize pain and distress and enhance animal well-being. Examples of refinement include, but are not limited to, use of anesthetics and analgesics, supportive care, acclimation to procedures, techniques that reduce stress, using less-invasive routes of administration or blood collection, modifying surgical procedures, replacing a surgical model with a non-surgical one.
We will take all actions to minimize discomfort, distress, pain and injury in animals as possible while still achieving the goals of the study. In order to comply with federal regulations (Animal Welfare Act and the Public Health Service Policy), all survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments and aseptic techniques, in a sterile environment. Surgery in dogs will be performed in an operating room used only for surgery. Pain will be evaluated at correct intervals by observation and pain scoring, and for the appropriate decision to administer pain-relieving medications. Animals will be given analgesics pre-emptively and post-operatively as described elsewhere, than as needed based on pain scoring.
The PI has previous experience with the proposed model and we do not anticipate any major/severe adverse physical and/physiological consequances or adverse effects as a result of the procedures.

Describe any expected or possible severe adverse physical and/physiological consequences or adverse effects on well-being that the animals may experience as a result of the procedures. Include adverse phenotype expression in genetically-modified animals and death as a result of euthanasia.
Pain is anticipated mainly due to the surgical interventions. Postoperative administration with analgesic (Buprenorphine) is expected to resolve thosa potential complications.

Periapical lesion induction for treatment with Regenerative endodontics procedure. The periapical lesion will be monitored with radiographic exams in order to see regression of radiolucency.

PI will consult ULAM veterinary staff immediately should any other complication arises. If deemed appropriate by the veterinarian, the animal would be euthanized."

Describe how the consequences or events stated above will be monitored and alleviated. Include criteria for premature euthanasia (euthanasia due to reaching a humane endpoint prior to a planned experimental endpoint).
Premature euthanasia will be conducted under the following circumstances
- Animal appears to be in excessive pain
- Infection can't be cleared through repeated antibiotic treatment
- Animal shows excessive weight loss of more than 20%
- Animal exhibits prolonged anorexia
- Animals will monitored for drooling and excessive scratching at muzzle

Use Justification - Dog

Use Category	Procedure Description	Examples	Number of Animals
E	Category E (original categories 7-9) Procedures that cause pain or distress which is NOT alleviated by use of anesthetics, analgesics, or tranquilizers. Category E procedures require strong scientific justification as to why appropriate anesthetics, analgesics, or tranquilizers cannot be applied, including evidence that all possible alternatives have been evaluated and determined to be unfeasible.	Contact RCA to assist with Use Category selection as needed.	0
D	Category D (original categories 3-6) Procedures that cause pain or distress which is alleviated by use of anesthetics, analgesics, or tranquilizers.	Contact RCA to assist with Use Category selection as needed.	10
C	Category C (original categories 1-2) Procedures that cause no pain or distress, or only slight or momentary pain or distress and do not require use of analgesics.	Contact RCA to assist with Use Category selection as needed.	0
Total			**10**

Justify the number of animals needed to achieve the scientific aim(s) of the project. Numbers justification should include the following:

1. **For experimental animals: Calculations illustrating how you arrived at the total needed per experiment. Calculations should include the number of animals per experimental group, number of groups (experimental and control), number of repeats, and any extras needed to cover experimental failure, attrition, etc.**
2. **For breeding animals: Calculations that break down your breeding scheme, including number of adult breeders and replacements, number of breeding cages, average number of pups expected per litter, number of matings, and progeny both wanted and unwanted.**
3. **For field studies: Calculations that include number of collection sites, number of collections, and average number of animals expected to be collected each time.**
4. **For teaching courses: Calculations should include number of courses taught, average number of students per course, and number of animals per student.**
5. **For all study types: Explanation for how you determined the number of animals per group/collection/student is necessary. This should be based on statistical or power analysis whenever possible. If based on previous studies or literature, please provide references. If based on statistical or power analysis, please describe the parameters used in your analysis.**

As highlighted in previous sections of this application, the PI has performed a pilot study (under IACUC approval at Indiana University). Our findings indeed support further investigation and also from animal model perspective corroborates with the literature as reiterating that the proposed animal model in the dog (beagle) is the most clinically translatable model prior to clinical trials in humans.

STUDY #1
We will use a total of 10 teeth (bi-rooted premolars) per dog. Six maxillary premolars (P2, P3, and P4 per each quadrant) and 4 mandibular premolars (P2 and P3 per each quadrant) will be used. Four dogs, with 4 teeth per dog for each experimental group, 2 teeth per dog for the negative control, and two roots for each tooth and assuming within dog correlations of 0.5, will detect 10-fold differences in CFU counts (CV=2.1) and 50% differences in improved periapical radiolucency.

STUDY #2
We will use a total of 10 teeth (bi-rooted premolars) per dog. Six maxillary premolars and 4 mandibular premolars will be used. With a sample size of 6 dogs, with 2 teeth per dog for each treated group and 2 extra teeth (non-operated, premolars P1) as negative control, the study will have approximately 80% power to detect a difference of 50% between the groups for the presence of radicular wall thickening, apical closure, and improved periapical radiolucency, assuming within dog correlations of 0.5.

Alternatives

The following questions should be used to answer how scientific advances are monitored that would enable the use of:

1. **Less painful or distressful procedures,**
2. **Anesthetics, analgesics, or tranquilizers that would reduce pain or distress, or**
3. **Methods that do not utilize animals.**

Database Search

Specify the databases that were searched for alternatives.
BDR, NIH RePORT, Agricola, Altweb

Indicate the keywords or search strategy used. Keywords should indicate you've searched for alternatives to painful and distressful procedures in this project, as well as for alternatives to using animals.
animal use alternatives, endodontics, revascularization, antibiotic pastes, pulp tissue regeneration, biomaterials, animal model, 3D bioactive scaffolds

Last search conducted:
7/4/2017

Indicate how many years were covered by this search.
36

Other Methods

Were scientific journals reviewed?
No

Were subject matter experts consulted?
No

Were scientific meetings attended?
No

PI Assurance

Acknowledgment

I acknowledge that as Principal Investigator I am responsible for this project and all work performed by personnel listed on this protocol.

I have read the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training and certify that this project will be conducted in compliance with those principles.

I confirm that I have read all IACUC policies and guidelines pertaining to this project.

I assure that I will not initiate new procedures or modifications to existing procedures without IACUC approval.

I confirm that this project does not unnecessarily duplicate previous research or instructional projects.

I assure that I have made every effort to refine this project to reduce animal pain and distress and to look for alternatives to painful or distressful procedures.

I verify that all personnel working with animals on this project are qualified or will be fully trained to conduct the project in a humane and scientific manner. I will maintain training records for personnel who are trained on procedures by myself or other personnel working on this project.

I understand that this project is approved for up to three years and that renewal of the project after the expiration date will require re-submission for a complete de novo review by the IACUC.

I understand that failure to comply with IACUC policies and guidelines will jeopardize the University of Michigan's Animal Welfare Assurance agreement with OLAW, USDA registration, and AAALAC International accreditation status. I understand that serious and continuing noncompliance may lead to suspension of this project or revocation of my privileges to conduct animal research at the University of Michigan.

In the event that unexpected outcomes or adverse events occur, I will report these events to the IACUC, ACUO, and/or veterinarian.