Al Nasser University
FACULTY OF MEDICINE
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (FOM IACUC)

Application for Approval of Animal Use Protocol (AUP)

1. In order for the Faculty of Medicine Institutional Animal Care and Use Committee (FOM IACUC) to perform its obligations in accordance with the Al Nasser University Faculty of Medicine IACUC Policy, it needs a complete description of the proposed animal use and care. Incomplete application will be returned to the applicant resulting in delay in the granting of approval.

2. Hand-written documents will not be accepted for review.

3. Submission is to be made on the prescribed form electronically or by email, and accompanied by a signed hard copy to:

   The Commission facilitator, Faculty of Medicine Institutional Animal Care and Use Committee, c/o Deputy Dean (Research),
   Faculty of Medicine,
   Al Nasser University.
   Phone: +967-775253300
   Email: wafalmadhaji2020@gmail.com

   All enquiries should be directed to Commission facilitator at the above mentioned address and/or contact number.

4. Any changes to the approved Animal Use Protocol (AUP) (procedure, species, personnel, etc.) must be documented through submission of an Amendment Form and approved by the FOM IACUC before implementation.

   a) Any **SIGNIFICANT** changes to an approved project in progress (e.g. the inclusion of new procedures involving potential pain, surgery or anaesthetization, or a change in Principal Investigator or course director) require IACUC review (and approval) prior to initiation.

   b) Any **NON-SIGNIFICANT** changes to an approved project in progress (e.g. increase in number of animals used within 10%, changes in location of experiment or changes in personnel) require administrative review (and approval) prior to initiation.

5. Approval of AUP will be for a **maximum of 3 years**. Following this, a new application is required.

Office Use Only
Date received:
Application No.:
Approval No.:
Expiry date:

Al Nasser University

FACULTY OF MEDICINE

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (FOM IACUC)

Animal Use Protocol (AUP) Application

This completed Animal Use Protocol (AUP) Application needs to be submitted to and approved by the FOM IACUC prior to commencement of the animal study.

SECTION 1a: PRINCIPAL INVESTIGATOR/COURSE INSTRUCTOR

Principal Investigator here refers to the main person responsible for the care and use of animals in this protocol (i.e. not necessarily be the grant holder)

Full Name:			**Tel :**	
Academic Title: Dr.			**Fax:**	
Department:			**Mobile:**	
Email:				
Experience or trained working with animals?	/	**Yes (provide evidence) ***		**No #**

*Evidence includes certificate of training, publication involving animal studies

#Provide tentative date for training ( )

SECTION 1b: DESIGNATED EMERGENCY CONTACT(S)

Full Name	Mobile phone	Email

SECTION 1c: CO-INVESTIGATOR(S)/RESEARCH ASSISTANTS

List the names of all other individuals (besides the PI) authorised to conduct procedures involving animals under this protocol:

Full Name:			**Tel :**	
Academic Title:			**Fax:**	
Department:			**Mobile:**	
Email:				
Experience or trained working with animals?		**Yes (provide evidence) ***	/	**No #**

*Evidence includes certificate of training, publication involving animal studies

#Provide tentative date for training (1/2015)

Al Nasser University

FACULTY OF MEDICINE

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (FOM IACUC)

Full Name: Najihah Binti Mohd Hashim		**Tel :**		
Academic Title:		**Fax:**		
Department:		**Mobile:**		
Email:				
Experience or trained working with animals?	/	**Yes (provide evidence) ***		**No #**

*Evidence includes certificate of training, publication involving animal studies

#Provide tentative date for training ( )

Full Name:		**Tel :**		
Academic Title:		**Fax:**		
Department:		**Mobile:**		
Email:				
Experience or trained working with animals?	/	**Yes (provide evidence) ***		**No #**

*Evidence includes certificate of training, publication involving animal studies

#Provide tentative date for training ( )

SECTION 2: PROJECT TITLE/PROJECT TYPE/PROJECT COMMENCEMENT

Project Title (In lay terminology, please give a descriptive title of your research project or course taught):

Please specify the type of AUP application (☒all that apply):

Research ☒ Others ☐

Pilot study ☐ Please specify: _____

Breeding protocol ☐

Teaching ☐

Type of application:

☒ First submission

☐ Modification (Registration no.: _____

Proposed length of study using animals:

Start date: _____ End date: _____

SECTION 3: FUNDING

Grant type:

Others: _____

Funding status: Awarded * Date awarded: _____

** Please delete where appropriate*

SECTION 4: LAY DESCRIPTION

Provide a typed abstract of 250 words or less in simple language. Outline the objectives of the project, the experimental approach, and the significance of the expected results to human and/or animal health.

Please cite three (3) recent references related to the proposed study.

SECTION 5 (a-d): JUSTIFICATION OF ANIMAL USE AND THE THREE R's

The FOM IACUC requires "that animals should be used only if the researcher's best efforts to find an alternative have failed". The three R's (Replacement, Reduction and Refinement) are the cornerstone of ethical animal research, and FOM IACUC requires investigators to implement the 3R's when they are preparing to use animals for scientific or teaching purposes.

Please cite up to three (3) relevant references for Sections 5.a and 5.c.

5.a Are there alternative non-animal methods used by other investigators for the type of work proposed in this AUP (e.g. tissue cultures, in vitro monoclonal antibody, computer model, etc.)? If yes, describe why these alternatives are not appropriate for this project.

5.b Why must animals be used in these experiments (☒all that apply)?

- ☐This is a study of animal behaviour
- ☐This phenomenon under study cannot be reproduced in vitro
- ☒This is a pre-clinical study of the in vivo effectiveness of a treatment or procedure
- ☐This is for teaching
- ☐Others (elaborate in the space below)

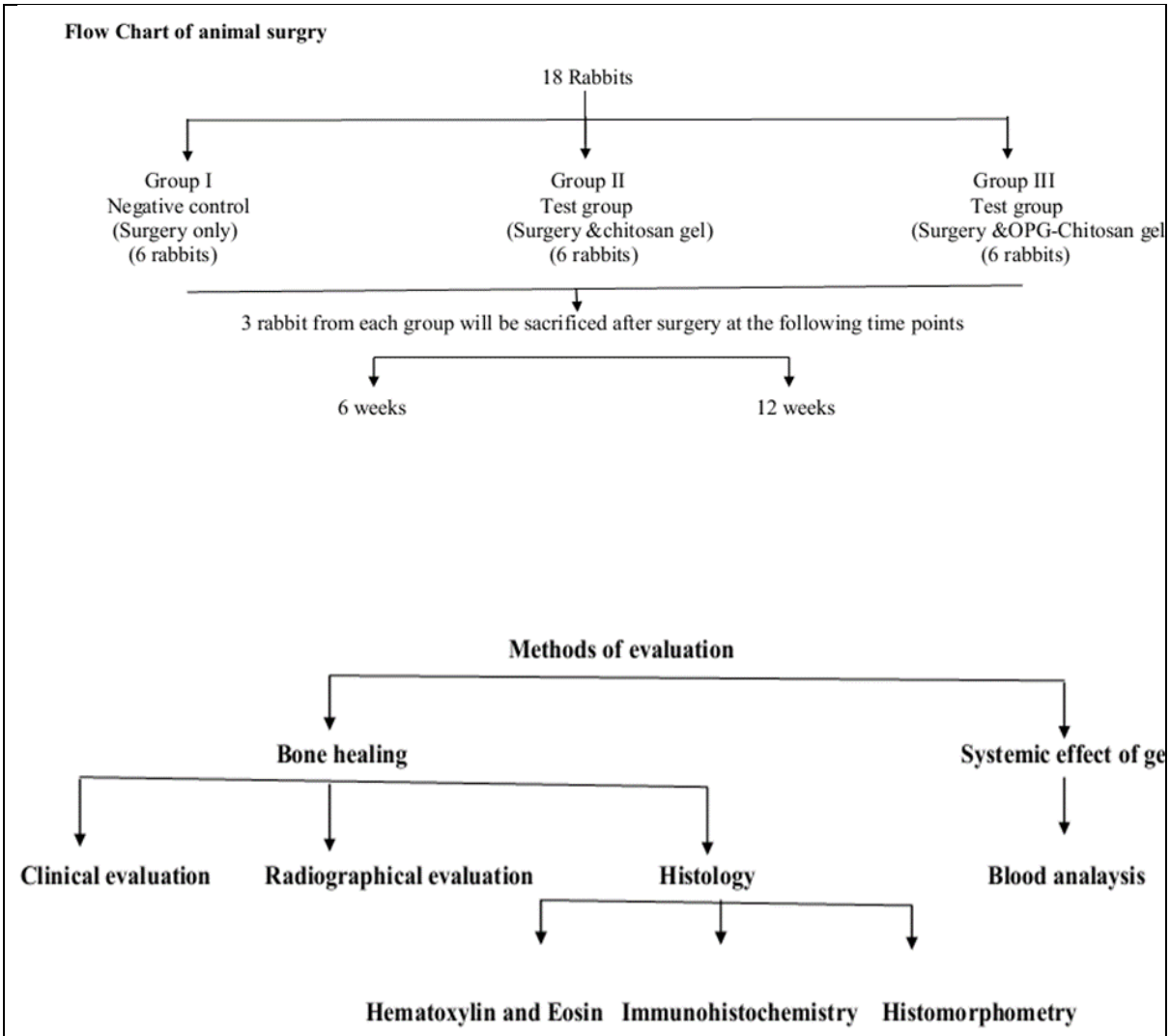
5.c What characteristics of this/these species make them appropriate for the proposed study? These might include structural, behavioural, physiological, biochemical, or other features or considerations (such as availability of species-specific reagents, or the use of well-established model) which make the model compatible with the research objectives. **Cost is not a primary consideration**.

5.d If animals are housed for more than 24 hours in the animal facility, please specify the environmental enrichment provisions and any housing restrictions required, i.e. social housing, specific materials, space, objects, etc.

SECTION 6: EXPERIMENTAL OBJECTIVES AND DESIGN

6.a Describe the objectives of the experiments.

6.b Describe the experimental design; what will be done to the animals in a step-by-step description when applicable and the statistical techniques to be employed. Where possible, use charts and diagrams (may be added as appendices) to show relationships between different activities and demonstrate the distribution of animal numbers in different procedures. Please cite up to three (3) relevant references related to the proposed study**. (example)**



Material and methods

i. Rabbit model

Eighteen New Zealand White male rabbits (6 months old, 3.5- 4 kg) will be used. The animals will be housed in the Animal Experimental Block (AEB) under standard laboratory conditions. They will be allowed for free access to food supply throughout the experimental period. Number coding will be used to identify each rabbit.

ii. Type of defect

For each rabbit, a cranial critical size defects (15 mm) will be created on occipital bone. The oval shape defect will be created with a bone trephine bur. The defect will be filled with either

1- OPG-chitosan gel

2- Chitosan gel

3- Surgery only with out fill.
Three rabbit for each time point. Therefore, 18 experimental sites will be obtained in all rabbits.

iii. Surgical procedure

All groups will be yielded to the same operation.

1- **Animal anesthetization**

General anesthesia will be induced by an intramuscular injection of 30 mg/kg of ketamine 100 mg/ml (Troy laboratories PTY. Limited, Smithfield, Australia) and 3 mg/kg of xylazine 20 mg/ml which is analgesic, sedative and relaxant injection (Troy laboratories PTY. Limited, Smithfield, Australia) (Flecknell, 1996). Local anesthetic solution (2% lidocaine with adrenaline) will be used for local hemostasis {Dentak (1) injection lidocaine HCL anhydrous 20.0 mg (2% W/V)}. The drape will be placed over the animal leaving only the desired surgical site will be exposed.

2- **Surgical operation**

The surgical sites of the Rabbits' skull will be shaved and disinfected using Povidone Iodine. Skin-periosteal flap will be raised to expose the parietal bone. One oval bone defects will be created with bone trephine bur on the occipital bone. Then the defects will be filled with the gel in test group or not be filled in negative control group. The periosteal is closed with 4.0 vicryl on a half round needle. The flap over the defect will be sutured with 3.0 vicryl on a half round needle.

3- **Post operative**

Tincture iodine and dressing will be applied over the suture incision. Each animal will be post-medicated according to their weight with meloxicam between 0.3 - 1.5 mg/kg (Turner et al., 2006) and an intramuscular injection Kombitrim® 1ml/10 kg (sulfamethoxazole and trimethoprim) (Kela Labratoria n.v, Hoogstraten, Belgium) for three days to protect against the Post-Operation infection. The rabbits will be placed in cages and will be allowed for water and food supply.

a. Blood Samples

Blood samples were collected before the surgery and before killing. All blood samples were centrifuged and collected sera was stored until assay. Kidney and liver functions test will be performed.

iv. Euthanasia

The rabbits will be euthanized by using Barbiturates (Dolethal, Pentobarbitone sodium 200 mg/ml solution, 0.7ml /kg IV) after 3 month. A skin incision was made to expose the cranium bone. The previously grafted sites will be excised with a bone cutting fissure bur. The size of bloc will be around 20 mm x 20 mm so bone containing surgical site with a margin of preserved 2 mm normal bone around the surgical site will be harvested in formaline filled container for histological investigations.

Methods of evaluation

Clinical Evaluation

The bone healing is evaluated clinically in the rabbit bone defect without treatment, with chitosan gel only and bone defect filled with OPG-chitosan gel.

Radiographic evaluation

Periapical radiograph is used to immediately after getting bloc.

Tissue preparation

The ratio of tissue from implant site to fixative and decalcification solutions is 1:50. The bloc are fixed in buffered formalin. Ethylenediaminetetracetic acid (EDTA). The specimen are decalcified with 100 ml 10% ethylned EDTA. The decalcification solutions are changed twice a week.

Chemical testing for complete decalcification is carried out using 3% ammonium oxalate. Five ml decalcified solution is taken and concentrated hydrochlorid acid is added until pH 3.2-3.6 is obtained and following pH correction, 5 ml of 3% di- ammonium oxalate monohydrate extra pure is added and the resulting solution monitored for 30 minutes resulting for precipitate formation.
Once decalcification is completed, specimens are washed in PBS for 30 minutes. The decalcified specimens are trimmed using trimmed. The bone formation is evaluated by H and E stain immunohistochemistry and histomorphometery.

Gantt Chart (example)

Year	2014	2015							
Month	Dec	Jan	Feb	Mar	April	May	June	July	Aug
Material synthesis									
Ethical application									
Purchase of materials									
Clinical work (surgery)									
Laboratory work									
Data collection and statistical analysis									

5- Statistical techniques to be employed :
Using SPSS system

SECTION 7: SURGICAL/NON-SURGICAL INTERVENTION AND PAIN MANAGEMENT

7.a Give details of the **surgical procedure** and pain management during, and/or after surgical intervention in live animal studies. Please specify the anaesthetic, analgesic, antibiotic and other drugs used in pain management.

7.b Give details of the **non-surgical procedure** and pain management during, and/or after the procedure in live animal studies. Please specify the anaesthetic, analgesic, antibiotic and other drugs used in pain management.

7.c List *all* procedures, manipulations, and/or measurements that will be performed on the animals. Include post-operative care, specify analgesics & anaesthetics with dosages and routes of administration, and special procedures used.

	PROCEDURES Including physical or chemical restraint, blood sampling, injection of compounds, e.g. antibiotics, chemicals, etc.	**Frequency** (no. of times each animal is subjected to the same procedure)	**No. of animals involved**	**Pain/distress classification (C, D, E)**	**Anaesthetics/ analgesics Antibiotics** Drug, dosage, route
1	Surgery	Twice	18	D	Anethesia - Ketamin (30 mg/kg/IM) - xylazine (3 mg/kg/IM) - 2 % Lidocain HCL with Adrenaline (20 mg (2 %)
3	Postoperarive	Once for 3 days	18	D	Anti-inflammatory (Meloxicam (0.3 - 1.5 mg/kg/ Im)) Antibiotics (Kombitrim (1ml/10 kg/ IM))

SECTION 8: CLASSIFICATION OF PAIN/DISTRESS

Please check one ☒. Information and examples on the classification can be obtained from these websites:

http://tulane.edu/asvpr/iacuc/hsc/upload/3-USDA_Classification.pdf
http://www.esf.edu/animalcare/documents/USDApainLevels.pdf

☐ C	**Classification C:** Animals upon which teaching, research, experiments, or tests will be conducted involving no pain, distress, or use of pain-relieving drugs.
☒ D	**Classification D:** Animals upon which experiments, teaching, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which appropriate anaesthetic, analgesic, or tranquilizing drugs will be used.

☐ E	**Classification E*:** Animals upon which teaching, experiments, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which the use of appropriate anaesthetic, analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.

*An explanation of the procedures producing pain or distress in these animals and the justification for not using appropriate anaesthetic, analgesic or tranquilising drugs must be provided below.

SECTION 9: ANIMAL USE

9.a List ALL ANIMALS involved in the study.

Species/Strain	Quantity	Weight/ Age	Gender	Accommodation (Building & Room)	Experimental Area (Building & Room - surgery and/or procedure rooms)
New-Zealand white rabbits	18	3.5- 4 kilo , 6 month	Male	Animal experimental unit facility	Surgery and procedure room of Animal experimental unit facility

9.b Explain how the total number of animals to be used was determined:

e.g. 6 animals x 2 treatments x 2 replicates = 24 animals.

3 rabbits x 3 Surgery treatment x 2 = 18 rabbits

SECTION 10: SOURCE OF ANIMALS

Please specify details of the animals in table below and indicate if health certificate (or equivalent) is available for the animals.

Species	Source/Supplier	Address/Location	Phone number	Health Certificate	Mode of transportation
Rabbits	Animal experimental unit	FOM- Animal experimental unit	7967 7515	---------- -	Rabbits

SECTION 11: EXPERIMENTAL AND/OR HUMANE ENDPOINT

When experimental procedures produce animals that may become ill, it is necessary to define an endpoint to ensure that an experimental animal's discomfort, pain and/or distress is terminated, minimized or reduced.

11.a Indicate any clinical conditions or abnormalities expected or that could arise as a result of the proposed study or teaching exercise (e.g. behavioural changes such as increased grooming, vocalization or postural changes, or physical abnormalities such as anorexia, dehydration, diarrhoea, etc.)

non

11.b In terms of species-specific behavioural changes and physiological signs, list the criteria that will be used to trigger the decision to remove an animal from the teaching exercise or experiment, or to terminate the teaching exercise or experiment. If necessary, consult the Attending Veterinarian for further advice at (email???)

IF they get anorexia after surgery, subcutaneous (under the skin) fluid in combination with oral fluid will be given.

SECTION 12: ANIMAL DISPOSAL

Indicate how animals are to be disposed of after completion of the project/research.

Euthanasia [☒ select preferred technique(s)]:

☒ Anaesthetic overdose (specify agent: Dolethal, Pentobarbitone sodium)

☐ Cervical dislocation*

☐ Exsanguination (under anaesthesia)

☐ Decapitation *

☐ CO_2

☐ Others (Specify _____)

* Provide justification for using physical methods of euthanasia, and state the location that it is done:

Method of carcass disposal (Include method of disposing contaminated organs/tissues):

Will be done by licensed biodisposable agent

SECTION 13: HAZARDOUS AGENTS & MATERIALS

Specify each agent/material to be used and hazardous dosage:

NOTE: If a Biosafety and/or Radiation Safety risk assessment is required then a separate application must be submitted to the relevant bodies.

Potential Hazard to ***Animals***	☒ None
Biological	
Chemical	
Carcinogen	
Drug	
Ionizing Radiation	
Other (i.e. allergen)	

Potential Hazard to ***Humans***	☒ None
Biological	
Chemical	
Carcinogen	
Drug	
Ionizing Radiation	
Other (i.e. allergen)	

Describe potential health risks to animals or humans. Specify any special animal care required because of the hazard(s) involved. Specify precautions to be taken by personnel. Specify any special containment requirements (i.e. storage, waste/disposal requirements, etc)

- Sharp instruments such as needles and blades
- Mask, gloves, guan
- Drugs and anaesthesia

(All mentioned above will be handled in careful manner)

SECTION 14: SIGNATURES

Your signature indicates that (check each box where applicable before signing):

1	☒	Animals used in this research or teaching project will be cared for in accordance with the principles contained in **Guide for the Care and Use of Laboratory Animal (8th Edition), until the Malaysia Code of Practice for the Care and Use of Animals for Scientific Purposes is made available.** http://oacu.od.nih.gov/regs/guide/guide.pdf
2	☒	You have considered alternative procedures that do not involve the use of living animals.
3	☒	You will use the minimum number of animals consistent with objectives of described research/teaching program.
4	☒	You have carefully selected the species that you propose to use.
5	☒	You will use techniques and facilities that are in accordance with the **Guide for the Care and Use of Laboratory Animal (8th Edition)** http://oacu.od.nih.gov/regs/guide/guide.pdf
6	☒	You will notify the FOM IACUC of any revisions to this AUP.
7	☒	You will keep copies of all approved AUPs, revisions and amendments in an accessible file.
8	☒	This project has been reviewed for scientific merit.
9	☒	The consultant Attending Veterinarian has been contacted for consultation prior to AUP submission.
10	☒	Experimental animals are housed in **Animal Experimental Unit**. Animals from external sources need to be quarantined or housed according to the Standard of Procedure for Quarantine of Laboratory Rodent and Rabbit.
11	☐	Experimental animals are housed in other **Satellite Animal Facilities** in the Faculty of Medicine.

		Animals from external sources need to be quarantined or housed according to the Standard of Procedure for Quarantine of Laboratory Rodent and Rabbit.

Approval from the FOM IACUC is valid for a period of **three (3) years**. If required, AUP must be renewed after the expiry date even if no revisions are made. At the end of the animal experiment**, a closure report** of the animal use is to be submitted to http://www.resfom.com for FOM IACUC review.

AUP form completed by:

Email address :

Principal Investigator's signature

Date signed

Supervisor's signature and stamp

Date signed

Head of Department' signature and stamp

Date signed

Al Nasser University

FACULTY OF MEDICINE

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (FOM IACUC)

FOR FOM IACUC OFFICE USE ONLY

Decision of Faculty of Medicine Institutional Animal Care and Use Committee (FOM IACUC)

- ☐ Approved
- ☐ Approved Pending Minor Modification
- ☐ Withhold Approval Pending Justification and Clarification

This AUP form has been reviewed by the FOM IACUC, and is approved based on the information provided

_____

Signature of FOM IACUC Chair Date

Date of AUP Approval: ☐