

IACUC Protocol Comments Form

Part A: General Project Information

1. Project title:
To study differentiation, cell proliferation and signaling events in muscle injury and regeneration.
2. Principal Investigator(s), department(s), and contact telephone number(s):
Dr. Zhenguo Wu, Department of Biochemistry, HKUST.
Contact Phone no. 2358-8704
3. Names of staff/students involved in experiments & types of licences/endorsements held:

R Asso	ZHU Han	(16-140)	AH14010
PG	WANG Gang	(16-144)	AH12014
PG	SITU Chenghao	(16-145)	AH12013
PG	YU Youqian	(16-143)	AH13030
PG	WEI Xiuqing	(16-142)	AH14066
PG	ZHOU Shaopu	(16-139)	AH15053
PG	HAN Lifang	(16-146)	AH16028
RA	CHEN Xianwei	(16-141)	AH16026
PG	Heng Youshan	(17-109)	AH17035
PG	Wang Ruiyong	(17-108)	AH17034

4. with licenses under Section 7 of the Animals (Control of Experiments) Ordinance, chp. 340
4. Funding agency:
HKUST
5. Protocol Form Received on (date): 17/11/2017
6. Protocol Form Received by (staff): Crystal

William KW Chau	Signature: <i>Chau</i>
Approved / not Approved / <input checked="" type="radio"/> Modify (circle as appropriate)	Date: 20. 11. 2017
Comments: Part C.1. 4abc please complete properly.	

Siva WH Tsang	Signature:
Approved / not Approved / Modify (circle as appropriate)	Date:
Comments:	

Anthony E James	Signature:
Approved / not Approved / Modify (circle as appropriate)	Date:
Comments:	

If additional space is required for writing comments, please add additional sheets



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Chairman of AEC:	Signature:
Approved / not Approved / Modify (circle as appropriate)	Date:
Comments:	

The Hong Kong University of Science & Technology**Committee on Research Practices Review Form**2017 104
APR 2018**INSTRUCTIONS :**

- (i) **Parts A & E** must be completed. Complete Parts B, C & D, if appropriate.
(ii) Please use additional pages where deemed necessary.

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4. Funding agency:
HKUST

YES NO

Part B: Animal Research

Note: If the animal work will involve hazardous material, please also complete the appropriate sections of Part C.

1. Explanation of how the project advances scientific knowledge and confirmation that alternatives to experiments on live animals have been considered but are not appropriate:
It will advance our understanding of the mechanism of muscle regeneration.
2. Species/strain and number of animals to be used: **CCR2 RFP, Dusp27-floxed, HSA Cre, IRES-CreER, Pax7-Cre, Pax 7-CreERT , Pax7BP1, Pax7-GFP, Mdx, MST3, mTOR, Myf-5 Cre, MyH6 CreER, PI3K-floxed, Rosa YFP, Smad4-floxed mice, Stat3-floxed, TSC1-floxed, IGF1R, CC2-YFP, E2F4, Zmpste24 and B6 (Total ~1000).**
3. Proposed location where animals are to be kept: Animal and Plant Care Facility of HKUST
4. Place in which experiments will be carried out: Animal and Plant Care Facility of HKUST
5. The likely duration of the experiments: About 2 years.
6. Outline of procedures with special reference to procedure likely to cause suffering or injury:

Drug Treatment

To induce Cre-recombinase to knock down specific gene, **Tamoxifen** will be intraperitoneally injected to mice ranging from P5 to 2 months or above at different days in a month. The total dosage of 1.5mg/20g body weight of mice will be applied.

At mice adulthood ,under anesthesia (see below), CTX (**Cardiotoxin**) (30 μ L of 10 μ M) or Barium Chloride (will be injected into TA (Tibialis Anterior) muscles of transgenic mice.

Viral injection

Adult mice will be injected at TA muscle with 10^{7-9} titer of cloned cDNA Adenovirus or Lentivirus in 30 μ l PBS. The injected mice will be catered back to its IVC sentinel. Food and drink will be provided as usual. With one day rest, two consecutive days will be applied in total.

Note: For safety reasons, all manufacturers have generated viral particles replication incompetent for research or medical use.

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After different day, EdU (**5-ethynyl-2'-deoxyuridine**) (100ul of 10mM) will be injected to trace cells proliferation. This tracing injection will be applied three times a day and at different days, maximum 10 days in a month. Within a month, the mice will be sacrificed by means of cervical dislocation and perfusion fixation (see below) for the histological analysis.

Note: **Cardiotoxin** is a 60 AA peptide with 4 disulfide bridges, purified from Naja pallida snake venom.

Perfusion Fixation

Under full anesthesia, abdominal muscle extend through thorax to the chin will be incised and opened. Scissors will split the sternum to expose the heart. Aorta is identified and cut; while a perfusion needle 24G is ready to insert through into the Left Ventricle. About 30ml PBS will be injected. All the blood will then be drained off. The mice are died of exsanguination. After then, 30ml of 4% PFA (paraformaldehyde) will be applied through the 24G needle again to fix the whole body.

7. Measures to be taken to minimize animal suffering/injury, including details of anaesthetics, analyses and method of euthanasia, etc., to be used:
Ketamine (0.133mg/g of body weight) and Xylazine (0.013mg/g of body weight) by means of ip (intraperitoneal) injection will be applied to the mice by means of an ip (intraperitoneal) injection before the surgery.
8. Post-experiment care of animals: Carcass will be returned to Animal and Plant Care Facility for disposal.
9. Other information: No

Part C.1: Safety – Chemical Hazards Risk Assessment

YES NO

If YES, please complete ALL items below:

1. Location where experiments will be carried out:
Open bench in the lab or Animal Facility

2. Hazardous materials involved:

Hazard type <u>(pls. check)</u>	Name of compound <u>and maximum amount used in operation</u>	Amount in storage <u>and location</u>
<input type="checkbox"/> Asphyxiant		
<input type="checkbox"/> Carcinogenic	EdU	100ul of 10mM in APCF
<input type="checkbox"/> Corrosive	Tamoxifen	100ul of 15ug/ml in APCF
<input type="checkbox"/> Flammable		
<input type="checkbox"/> Irritant		
<input type="checkbox"/> Mutagenic		
<input type="checkbox"/> Reactive/Explosive		
<input type="checkbox"/> Sensitizer		
<input type="checkbox"/> Teratogenic		
<input type="checkbox"/> Toxic	Cardiotoxin	30ul of 10uM in APCF
<input type="checkbox"/> Others	Paraformaldehyde	30ml of 4% Paraformaldehyde

3. Description of work processes involving hazardous materials:

EdU and Taxmoxifen are applied by intraperitoneally injection at different days. CTX is injected on TA muscles only.

4. Proposed controls:

- a. Engineering control:

Local exhaust ventilation Fume cupboard Minimize quantity
 Substitution for less hazardous materials Others _____
 NA

b. Administrative control:

- Safety training Warning & label Access control
 Approved operating procedures Proper waste management practice
 Others _____
 N/A

c. Personal protective equipment:

- | | | |
|--|--------------------------------------|--|
| <input type="checkbox"/> Safety glasses | <input type="checkbox"/> Face shield | <input type="checkbox"/> Lab coat |
| <input type="checkbox"/> Mask | <input type="checkbox"/> Respirator | |
| <input checked="" type="checkbox"/> Appropriate gloves | | <input checked="" type="checkbox"/> Others Protective gown |
| Specify: _____ | | Specify: _____ |
| <input type="checkbox"/> N/A | | |

Part C.2: Safety – Biological Hazards Risk Assessment YES NO*If YES, please complete ALL items below:*

1. Location where experiments will be carried out:

Biosafety cabinet in APCF

2. Hazardous materials involved:

Hazard type <u>(pls. check)</u>	Name of agent and maximum amount used in operation	Amount in storage and location
<input type="checkbox"/> Biological toxins		
<input type="checkbox"/> Human bodily fluids (e.g blood or tissues)		
<input type="checkbox"/> Infectious microbes		
<input type="checkbox"/> Lab animals		
<input checked="" type="checkbox"/> Others: Virus	10 ⁷⁻⁹ titer of Adenovirus or Lentivirus in 30ul PBS	

3. Description of work processes involving hazardous materials:

4. Proposed controls:

a. Engineering control:

- Biosafety cabinet Facility containment Others _____ N/A

b. Administrative control:

- | | | |
|--|---|---|
| <input type="checkbox"/> Safety training | <input type="checkbox"/> Access Control | <input type="checkbox"/> Medical Surveillance |
| <input type="checkbox"/> Approved operating procedures | | <input type="checkbox"/> Proper waste management practice |
| <input type="checkbox"/> Others _____ | <input type="checkbox"/> NA | |

c. Personal protective equipment:

- | | | |
|--|--------------------------------------|--|
| <input type="checkbox"/> Safety glasses | <input type="checkbox"/> Face shield | <input checked="" type="checkbox"/> Lab coat |
| <input type="checkbox"/> Mask | <input type="checkbox"/> Respirator | |
| <input checked="" type="checkbox"/> Appropriate gloves | | <input type="checkbox"/> Others _____ |

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Specify: _____

Specify: _____

____ N/A

5. **FOR RECOMBINANT DNA WORK ONLY**

YES

NO

If YES, please complete ALL items below:

- a. The source(s) of DNA:
- b. The nature of the inserted DNA sequences:
- c. The hosts and vectors to be used:
- d. Whether a deliberate attempt will be made to obtain expression of a foreign gene, and if so, what protein will be produced:
- e. The containment conditions:

Part C.3: Safety – Physical Hazards Risk Assessment

YES

NO

If YES, please complete ALL items below:

1. Location where experiments will be carried out:

2. Hazardous materials/operations involved:

- ____ High voltage equipment (> 600V AC or 1.0 KV DC)
- ____ High pressure/vacuum condition
- ____ High temperature
- ____ Laser (Class III or above)
- ____ Ionizing radiation (e.g. isotopes, X-ray). Please list isotopes _____
- ____ Non-ionizing radiation (e.g. UV, microwave, other EM fields)
- ____ Others _____

3. Description of work processes involving hazardous operations, including the type of operation, operating conditions, maximum quantity, duration, and other relevant information:

4. Proposed controls:

a. Engineering control:

- ____ Proper shielding/enclose
- ____ Proper guarding
- ____ Others _____

- ____ Proper relief devices
- ____ Proper insulation
- ____ N/A

b. Administrative control:

- ____ Safety training ____ Approved operating procedures ____ RUA no. _____
- ____ Laser hazard control plan no. _____ ____ Access control
- ____ Proper waste management practice ____ Others _____
- ____ N/A

c. Personal protective equipment:

Safety glasses Face shield Lab coat
 Mask Respirator
 Appropriate gloves Others _____
 Specify: _____
 N/A _____

Part D: **Human Participants Research**

YES

NO

Note: If the research work will involve handling biological specimens of human participants, please also complete the appropriate sections of Part C.

1. Details of procedures to be used in the Research:
2. Participants involved in the research (describe the characteristics of the participant population, including anticipated number, age, sex, health status, identify the criteria for inclusion or exclusion, explain the rationale for the involvement of special classes of participants, if any, such as fetuses, pregnant women, children, human in vitro fertilization, prisoners, and others.):
3. Identify sources of research material obtained from individually identifiable living human participants in the form of specimens, records or data, etc.:
4. Answer each of the following questions by circling "Yes" or "No":
 Do your procedures expose your participants to any risk of:
 - a. physical harm Yes/No
 - b. pain Yes/No
 - c. stress Yes/No
 - d. fatigue Yes/No
 - e. noxious stimulation Yes/No
 - f. emotional distress Yes/No
 - g. invasion of privacy Yes/No
 - h. any other form of physical or psychological discomfort Yes/No
 - i. any other potential risks such as social and legal Yes/No
5. Estimate the degree of risk involved in your participants:
 - a. Describe the steps you will take to minimize the risk and to protect your participants from it, including risks of confidentiality, if necessary discuss provisions of ensuring medical or professional intervention in the event of adverse effects to the participants. Also when appropriate, describe provisions for monitoring the data collected to ensure the safety of participants.
 - b. How will you explain the risk to your participants?
 - c. How will you obtain their consent to take part in the research?

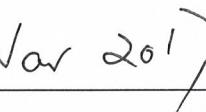
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6. Will you collect names, addresses or any other details which would make it possible to identify your participants?Yes/No. If "Yes":
- Describe the identifying data you will collect
 - How will you use these data?
 - How will you dispose of these data?
 - What procedures will you follow to make sure that your participants cannot be identified?

Part E: Declaration

The information supplied above is to the best of my/our knowledge and believed to be accurate and feasible. I/We shall take all possible precautions to ensure that the proposed research is (are) conducted in such a manner as described above in order to safeguard the welfare of, and minimize the pain suffered by, the human and animal participants involved in the study. Also, the procedures described, and material used in the research, are designed to minimize hazard and harmful results to the research workers, participants, and the natural environment.


Signature (Principal Investigator)


Date

To be completed by Head of Department

I hereby endorse this application and confirm that the Principal Investigator/Supervisor is appropriately qualified and the Department has adequate facilities for the experiment(s) to be conducted in such a way as to safeguard the welfare of the research workers, participants and the natural environment.


Signature (Head)


Date

17/11/2017