THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Qingping Feng
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Location of experimental work to be carried out: Building(s) MSB Room(s) 253

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR, HSFO
GRANT TITLE(S): 1. Rac1 signaling in myocardial TNF-alpha expression in sepsis
2. Heart development in diabetes: Role of NO
3. Cardioprotection by erythropoietin: Role of NO

List all personnel working under Principal Investigators supervision in this location:

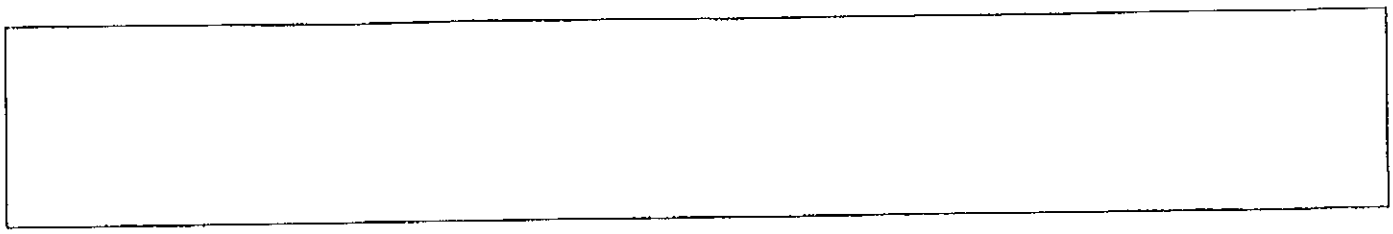
Name	UWO E-mail Address	Date of Biosafety Training
Sharon Lu	Sharon.lu@schulich.uwo.ca	July 14, 2008
Lily Xiang	Fxiang2@uwo.ca	May 26, 2008
Yin Liu	Yliu258@uwo.ca	Registered for refresher training
Ting Zhang	Tzhang53@uwo.ca	May 26, 2008
Carmen Leung	Cleung73@uwo.ca	Registered for refresher training
Hoda Moazzen	hmoazzen@uwo.ca	Registered for refresher training
Yan Wu	Ywu287@uwo.ca	Registered for refresher training
Murong Liu	Mliu223@uwo.ca	July 14, 2008

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Adenovirus' are stored in DMEM media with 2.5% glycerol at -80°C. Amplification of adenovirus is performed using HEK 293 cells by infecting the cells with 10 µl of the adenovirus in a 60 mm dish. The collection of cells are frozen and thawed three times and the supernatant is separated from cell debris for infection of target cells, which are neonatal cardiomyocytes. Any solution or containers that handled adenovirus are washed with bleach and autoclaved before disposal.

Lentivirus will be produced by the company Applied Biological Materials in Richmond, BC. The lentivirus will be purified and concentrated through ultracentrifugation by the company to produce a high titer. Lentivirus' are stored in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 buffer at -80°C. Cocktails containing a combination of 6 lentivirus', packaged in VSVG envelope, (Tbx5, Gata4, Baf60C, Nkx2.5, Oct3/4, Nanog) are infected into isolated fibroblasts from one of four cell sources: mouse neonatal skin, mouse embryo E14.5, mouse neonatal heart and the mouse adult heart. The isolated fibroblasts are grown until confluent and transfected at passage 2 or 3. Cells are incubated with the lentivirus for 24 hours at 37°C and 5% CO2 and each virus is used at a range of multiplicity of infections (MOIs) or 5-20. After 24 hours the lentivirus is removed from the cells and inactivated with 10% bleach. Cells are then monitored for viability and characteristic changes of differentiation from fibroblasts into cardiomyocytes. Following our *in vitro* studies with the lentivirus, we will establish which combination of lentivirus (Tbx5, Gata4, Baf60C, Nkx2.5, Oct3/4 and/or Nanog) will be most effective in reprogramming fibroblasts into cardiomyocytes. Upon approval of the protocol from the UWO animal subcommittee, we will inject infected cells that demonstrate characteristic changes into live animals for heart failure therapy. Specifically, the protocol will follow the details used in our *in vitro* work to develop viable cells with characteristic changes. The cells will then be injected into the pericardium cavity of mice and their ability to differentiate into cardiomyocytes will be evaluated using immunohistostaining. The mice will be sacrificed and their hearts will be fixed in paraformaldehyde and imbedded in paraffin. The hearts will then be sectioned onto slides and stained for cardiomyocyte-specific markers like α-actinin or troponin-I. After the successful injection of infected cells into the heart of mice has been shown, the established combination of lentivirus from our *in vitro* studies will be directly injected into the pericardium cavity of mice to demonstrate reprogramming of cells into cardiomyocytes. Immunohistostaining will be used to evaluate the ability of cells to differentiate into cardiomycotes. The mice will be sacrificed and their hearts will be fixed in paraformaldehyde and imbedded in paraffin. The hearts will then be sectioned onto slides and stained for cardiomyocyte-specific markers like α-actinin or troponin-I. Injection of lentiviral infected cells or direct injection of lentivirus into the cardiac region of mice will demonstrate *in vivo* reprogramming of cells into cardiomyocytes, which will be beneficial for heart function following myocardial infarction. Any solution or containers that handled lentivirus are washed with bleach and autoclaved before disposal.

The E.Coli bacteria will be stored at -80 degrees Celsius. 2.5x10^7 E. coli bacteria/ g body weight of the mouse will be injected into the penis vein of mice to induce sepsis and their survival will be monitored. At 3 days after bacterial injection the mice will undergo hemodynamic analysis and sacrificed and incinerated after. The E. coli bacteria will be inactivated with 10% bleach. Any solution or containers that handled the E. coli bacteria are washed with 10% bleach and autoclaved before disposal.



Please include a one page research summary or teaching protocol.

Directed cellular reprogramming of cardiac fibroblasts to cardiomyocytes

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With cardiovascular disease as the global leading cause of death, the development of cellular therapies to regenerate a damaged heart is imperative. Following myocardial infarction (MI), large numbers of cardiomyocytes are lost and fibroblasts proliferate rapidly, leading to impaired heart function. Many autologous cell types have been studied for their innate cardiogenic potential but none have been truly successful. It has been demonstrated that cardiomyocytes can be derived from human embryonic stem cells and induced pluripotent stem cells. However, these cell types can form teratomas when transplanted due to their original pluripotent nature. Studies on transdifferentiation or cellular reprogramming using defined factors have been promising because an abundant cell type can be directly reprogrammed into another important cell type without first becoming pluripotent. The present study proposes that cardiac fibroblasts, which proliferate extensively after MI, could be directed to transdifferentiate into cardiomyocytes with cardiac specific genes, therefore potentially improving cardiac function. The objectives of this study are to evaluate the ability of cardiac fibroblasts to reprogram into cardiomyocytes and to investigate if transplantation of these reprogrammed fibroblasts can improve heart function post-MI in a mouse model. Different combinations of lentiviral transductions with *Baf60c, Gata4, Tbx5, Nkx2.5, Oct3/4* and *Nanog* genes will be performed on cultured fibroblasts and monitored for spontaneous beating. Subsequently, immunofluorescence and western blot analysis will be carried out to analyze cardiomyocyte specific protein expression. The reprogrammed cells will be transplanted into the peri-infarct region of the heart after MI and heart function will be measured by Millar pressure-volume relationships. It is expected that transplantation of the lentiviral transduced fibroblasts will lead to cardiomyocyte regeneration, improve cardiac repair and cardiac function post-MI. This study may have a major impact on the regenerative medicine and treatment of MI.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? • YES O NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES • NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
Adenovirus	• Yes O No	O Yes • No	O Yes • No	0.1L	Applied Biomedical Materials	O 1 O 2 • 2+ O 3
Lentivirus	• Yes O No	O Yes • No	O Yes • No	0.1L	Applied Biomedical Materials	O 1 O 2 • 2+ O 3
E. coli (055iB5)	• Yes O No	• Yes O No	• Yes O No	0.2L	ATCC	O 1 • 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? • YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes • No		Not applicable
Rodent	• Yes O No	Mice heart, skin	2007-011-03
Non-human primate	O Yes • No		
Other (specify)	O Yes • No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	• Yes O No	HEK 293	ATCC (already have)
Rodent	O Yes • No		
Non-human primate	O Yes • No		
Other (specify)	O Yes • No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 O 2 • 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES • NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? • YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below • NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

4.3 Will genetic modification(s) involving viral vectors be made? • YES, complete table below O NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Adenovirus	*Adenoviral expression system*	*ABM*	***Cre** (not oncogenic gene) Catalogue #:* 000198A	*Gene knockdown of specifically floxed genes; cells remain healthy and do not turn cancerous.*
Lentivirus	*Lentivirus expression system*	*ABM*	***Oct ¾** (Cat. #:* LV010061), ***Tbx5** (Cat. #:* LV010060), ***Gata4** (Cat. #:* LV010058), ***Baf60c** (*LV010062), ***Nkx2.5** (Cat. #:* LV010059), ***Nanog** (Cat. #:* LV010063) *(All from ABM and not oncogenic genes)*	*Gene upregulation; cells change phenotype to cardiomyocyte-like; cells remain health and do not turn cancerous.*

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ • NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ • NO
- SV 40 Large T antigen O YES • NO
- E1A oncogene O YES •NO
- Known oncogenes O YES, please specify _____ • NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ • NO

4.5 Will virus be replication defective? • YES O NO

4.6 Will virus be infectious to humans or animals? BOTH • YES O NO

4.7 Will this be expected to increase the containment level required? O YES • NO

E1A - Yes

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES • NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? • YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used C57BL/6 mice
6.3 AUS protocol # 2007-011-03

6.4 Will any of the agents listed in section 4.0 be used in live animals • YES, specify: Lentivirus O NO
We are going to inject mice with lentivirus and lentivirus infected cells into mice.

6.5 Will the agent(s) be shed by the animal: • YES O NO, please justify:

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES • No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES • NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? O YES • NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:

_____

_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

10.0 Plants

10.1 Do you use plants? O YES • NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____

_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

_____

_____

_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? • YES, please give country of origin USA O NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES • NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES • NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ • NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 O 2 • 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?

• YES, permit # if on-campus BIO-UWO-0089
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: March 24, 2011

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

_____
_____
_____

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

The procedure according to Section 3.5 Medical Procedures and Incident Reporting of the UWO Biosafety Guidelines and Procedures Manual for Containment Level 1 and 2 Laboratories will be followed.

_____

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

-------- Original Message --------
Subject: Re: Biological Agents Registry Form (Feng)
Date: Thu, 24 Mar 2011 11:58:03 -0400
From: Carmen Leung <cleung73@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: Qingping Feng <Qingping.Feng@schulich.uwo.ca>

Hi Jennifer,

Please find attached an updated version of the Feng lab biological agents registry form with the requested changes.

Regarding Section 4.4, I am unsure what you mean by updating this section to reflect the use of HEK cells since this section does not ask about the use of HEK cells. Please clarify and I will be happy to answer any questions regarding this.

To confirm section 6.0, yes, we will be injecting lentivirus into live animals as well as cells.

Regards,

Carmen

New Info