THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 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	Dr. Kibret Mequanint
DEPARTMENT	Chemical & Biochemical Engineering
ADDRESS	Thompson Engineering Building, Room Number 439
PHONE NUMBER	519-661-2111 Ext. 88573
EMERGENCY PHONE NUMBER(S)	519-850 4820 (home); 519-851-4825 (mobile)
EMAIL	kmequani@eng.uwo.ca

Location of experimental work to be carried out: Thompson Engineering Building Room 420/420A

*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: **Heart and Stroke Foundation; NSERC**
GRANT TITLE(S): **Vascular tissue engineering**

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

Name	UWO E-mail Address	Date of Biosafety Training
Darryl Kenneth Knight	dknight9@uwo.ca	17 Dec. 2010
Dawit Seifu	dseifu@uwo.ca	09 Feb. 2011
Aparna Bhattacharyya	abhatta2@uwo.ca	18 Aug 2009
Shigang Lin	slin45@uwo.ca	20 Oct. 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.

Human Coronary artery Smooth Muscle Cells - from Lonza Walkersville, Inc, MD, USA

Human Coronary artery Endothelial Cells - from Lonza Walkersville, Inc, MD, USA

Human Umbilical Vein Cells - from Lonza Walkersville, Inc, MD, USA

...and Primary and secondary antibodies specific to the above 3 cells.

Storage: These cells are delivered frozen in dry ice. In the lab, cells are stored in liquid nitrogen dewars (-196 °C). for short term storage we use -86 °C freezer. Cells are thawed when there are needed and cultured in a standard culture facility for our purpose.

Disposal: Any unwanted or contaminated cells are disposed as a waste after cells are destroyed by means of 50% bleach solution. Consumable culture products exposed to cells are collected into an approved biohazards bag "commonly called the orange bag" and are autoclaved before disposed as waste. Glass products are also autoclaved before collection by a collector.

Please include a one page research summary or teaching protocol.

When studying human vascular diseases and therapeutics, a realistic model is a human tissue, but the inability to experiment directly on human subjects limits progress. Thus engineered human vascular tissue models to close this gap are of vital importance. Their significance is far-reaching as these "made to order" vascular tissues comprising human cells (smooth muscle and endothelial cells) and ECM (elastin and collagen) could serve as a powerful high-content tool to study disease and develop therapeutics. However, two interrelated unsolved challenges - regulation of vascular smooth muscle cell (VSMC) phenotype and synthesis of elastin in engineered vascular tissues - significantly hinder progress in this area. Given the structural and signaling roles of elastin in vascular stability, engineered human vascular tissues must incorporate elastin that is notably absent in currently engineered vascular tissues. Recently we have made technological and conceptual breakthroughs in biodegradable scaffold design and elastin synthesis. We showed that 3D fibrous geometry is a neccessary condition for elastin synthesis. In this application, we propose to study elastin synthesis and the regulation of VSMC phenotype at successive phases of engineered vascular tissue development to eventually create functional human vascular substitutes. In the short-term, our objective is to engineer a model tissue that can be used to study disease conditions, and cardiovascular and smooth muscle pharmacology. As we learn from these engineered model tissues and optimize their functions, the ultimate goal is to use these tissues clinically as a vascular replacement. We propose to test the following 2 specific hypotheses.

#1) Biodegradable 3D fibrous scaffold and mechanical forces regulate the phenotype of VSMCs and elastin synthesis.
#2) Endothelial cells (ECs) induce the contractile phenotype of VSMCs grown on biodegradable 3D fibrous scaffolds through JAGGED1/NOTCH3 signaling.

RESEARCH PLAN: 1) Human coronary artery smooth muscle cells (HCASMC) will be seeded on biodegradable 3D fibrous scaffolds that recapitulate key molecular features of the ECM and, cell-seeded scaffolds will be placed in pulsatile bioreactor systems. Cell attachment, migration, proliferation, differentiation and ECM production will be studied by confocal microscopy, real-time PCR, immunoblot, and immunohistochemistry. We will investigate the effects of TGF-beta-1 on cell phenotype and elastin synthesis in 3D. Western blot analysis of specific VSMC differentiation markers SM-alpha-actin, calponin, h-caldesmon

and smoothelin will be used to further examine cellular differentiation. We anticipate to promote long-term attachment of HCASMCs and to modulate their synthetic phenotype in 3D culture. Elastin synthesis will be assessed by immunocytochemistry, confocal microscopy, and Western blot analysis. Elastin will be quantified by colorimetric absorbance following alkali extraction. We anticipate HCASMCs will produce elastin in response to TGF-beta-1 and a supra-physiological biomechanical signal. To gain understanding of signal transduction differences between 2D and 3D cultures and, also to understand how different mechanical forces influence pathway activation, we will investigate the FAK/Src/Grb2/ERK1/2 pathway.

2) After a period of VSMC proliferation and elastin production, we will examine the role of ECs in the regulation of the contractile phenotype of HCASMCs. Human coronary artery endothelial cells (HCAECs) will be seeded onto engineered tissues. Using microscopy and Western blot analysis, we will study the extent of EC monolayer formation and the expression of HCASMC contractile proteins under different culture conditions and times. In separate experiments, the mechanism by which ECs induce the contractile phenotype of VSMC will be studied, focussing on NOTCH signalling in response to JAGGED1. We anticipate enhanced expression of contractile proteins in response to EC co-culture.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O 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 **O 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)* (Be specific)	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
	O Yes O No	O Yes O No	O Yes O No			O 1 O 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
	O Yes O No	O Yes O No	O Yes O No			O 1 O 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? **O 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** O No	Lonza Walkersville, Inc, MD, USA	Not applicable
Rodent	O Yes **O No**		
Non-human primate	O Yes **O No**		
Other (specify)	O Yes **O 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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	**O Yes** O No	Primary cultures	1/2	Lonza Walkersville, Inc, MD, USA
Rodent	O Yes **O No**			
Non-human primate	O Yes **O No**			
Other (specify)	O Yes **O 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** O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES **O 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/UNKNOWN**	**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? O 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 O 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) of bacteria and/or cells involving viral vectors be made?
O 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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES **O NO**
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
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? O YES **O NO** If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NO

6.5 Will the agent(s) be shed by the animal: O 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 **O No** If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

7.3 If yes, 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

7.4 If no live animals are used, please specify the source of the specimens:

_____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES **O 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 **O 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 **O 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? O YES, please give country of origin_____ **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 O NO

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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:

TEB 420A inspection done Jan 13, 2011 (JS) - BIO-UWO-0213 -

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** O 2+ O 3

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

O YES, date of most recent biosafety inspection: **2008**
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-0213**

14.0 Procedures to be Followed

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

We are using well-established human cells purchased and screened for disease. These cells will be handled as per supplier recommendations and standard safety. No special risk is known and hence do not require extraordinary steps in handling.

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash: Standard firs aid -such as washing. We do not anticipate needlestick injury but if that happens, medical attention will be sought.

14.3 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: **July 20, 2011**

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:

Info on Cells

-------- Original Message --------
Subject:RE: Biological Agents Registry Form - Mequanint
Date: Thu, 21 Jul 2011 16:56:12 -0400
From: Kibret Mequanint <kmequani@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: John S Millar <jsmillar@uwo.ca>

https://shop.lonza.com/shop/prd/primary-cells-cell-culture/lonza_b2b/7.0-7_2_86_69_76_10_13/2/DF36996BA21958F18852001A4B525E10/

Smooth Muscle: CC-2583

Endothelial: CC-2585 (Artery) and CC-2517 (Vein)

Fibroblast: Multiple tissue source (please see https://shop.lonza.com/shop/prd/fibroblasts/lonza_b2b/7.0-7_2_86_69_76_10_13/2/DF36999591CFE0F18852001A4B525E10/

Sincerely, Kibret

CASMC-Human Coronary Artery Smooth Muscle Cells

Clonetics® are performance tested and test negative for mycoplasma, bacteria, yeast and fungi. HIV-1, hepatitis B and hepatitis C are not detected for all donors and/or cell lots. A Certificate of Analysis (CoA) is provided for each cell lot purchased.

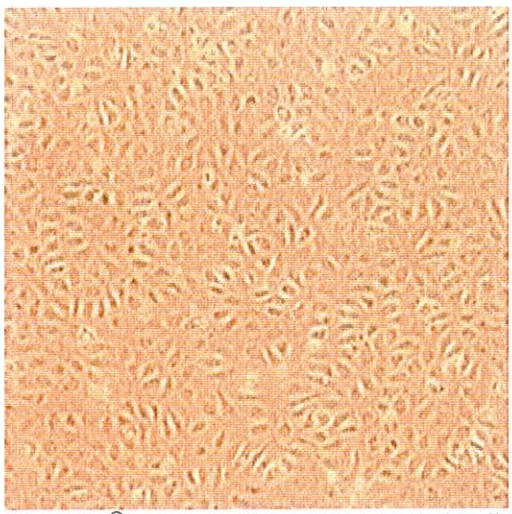
Cell Specifications: Clonetics® Cells are guaranteed to perform as indicated when used with Clonetics® Media and Reagents.

CC-2583	CASMC-Coronary Artery SM Cells,SmGM-2, cryo amp	678.00	AMP	1

Related Products
Supplemental Info

CC-3182	SmGM-2 BulletKit (CC-3181 & CC-4149)	129.00	
CC-5034	ReagentPack Subculture Reagents	64.00	

HCAEC-Human Coronary Artery Endothelial Cells



Clonetics® Coronary Artery Endothelial Cells and Media contain Normal Human Coronary Artery Endothelial Cells and optimized media for cell growth. Each system can quickly generate HCAEC cultures for experimental applications in cardiovascular pharmaceutical development and vascular pathology, including atherosclerosis. Clonetics® HCAEC-Coronary Artery Endothelial Cells and Media are convenient and easy to use, allowing the researcher to focus on results.

Cryopreserved... read more

CC-2585	HCAEC-Coronary Art. Endo Cells, EGM-2MV, cryo amp	690.00	AMP	1

Related Products
Supplemental Info

CC-3202	EGM-2 MV BulletKit (CC-3156 & CC-4147)	125.00	
CC-5034	ReagentPack Subculture Reagents	64.00	

Endothelial Cell Media Tech Sheet
Endothelial Cell Instructions
Cell Warranty and Hazard Statement
Intended Use Statement
Cryopreservation Instructions
HCAEC Coronary Artery Endothelial Cells - Tech Sheet
ReagentPack™ Subculture Reagents Tech Sheet
Endothelial Cell References

HUVEC-Human Umbilical Vein Endothelial Cells

Clonetics® HUVEC-Umbilical Vein Endothelial Cells and Media contain Normal Human Umbilical Vein Endothelial Cells and optimized media for cell growth. Each system can quickly generate HUVEC cultures for experimental applications in cardiovascular pharmaceutical development and vascular pathology, including atherosclerosis. Clonetics® HUVEC-Umbilical Vein Endothelial Cell Systems are convenient and easy to use, allowing the researcher to focus on results.... read more

CC-2517	HUVEC-Umbilical Vein Endo Cells,EGM,cryo amp	259.00	AMP 1	

Related Products
Supplemental Info

CC-3024	EGM Complete Medium 500 ml	116.00	
CC-3124	EGM BulletKit (CC-3121 & CC-4133)	118.00	
CC-5034	ReagentPack Subculture Reagents	64.00	

Endothelial Cell Instructions
Intended Use Statement
Cell Warranty and Hazard Statement
ReagentPack™ Subculture Reagents Tech Sheet
Endothelial Cell Media Tech Sheet
HUVEC Cell Reference
Cryopreservation Instructions
HUVEC Use in Cancer Study

Legal Statement and Privacy Policy

Fibroblast: Multiple tissue source (please see https://shop.lonza.com/shop/prd/fibroblasts/lonza_b2b/7.0-7_2_86_69_76_10_13/2/DF36999591CFE0F18852001A4B525E10/ Product Information

Fibroblasts

Products > Research Products > Primary Cells & Cell Culture > Clonetics™ Human Cells & Media

NHDF-Ad-Adult Human Dermal Fibroblasts

→ NHDF-Ad-Adult Human Dermal Fibroblasts pricing

NHDF-Neo-Neonatal Human Dermal Fibroblasts

→ NHDF-Neo-Neonatal Human Dermal Fibroblasts pricing

NHLF-Normal Human Lung Fibroblasts

→ NHLF-Normal Human Lung Fibroblasts pricing

AoAF-Human Aortic Adventitial Fibroblasts

→ AoAF-Human Aortic Adventitial Fibroblasts pricing

HPdLF-Human Periodontal Ligament Fibroblasts

→ HPdLF-Human Periodontal Ligament Fibroblasts pricing

Human Cardiac Fibroblasts from Atrium & Ventricle tissue

→ Human Cardiac Fibroblasts from Atrium & Ventricle tissue pricing

Human Intestinal Myofibroblasts

→ Human Intestinal Myofibroblasts pricing