THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 2009
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 ~~also~~ be updated at least every 3 years or when there are changes to the biohazards 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 Biohazard 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	Martin Sandig
SIGNATURE	
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Location of experimental work to be carried out: Building(s) DSB Room(s) 00060

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: HSFO: Integrin signaling in monocyte transendothelial migration
GRANT TITLE(S): HSFO: Vascular smooth muscle cell phenotype switching and elastin synthesis in 3D tissue engineered coronary artery substitutes
NSERC: Tumor cell transendothelial migration

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Ying Xia, Ph.D.

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? O YES ⊠ NO
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)*	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 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 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 in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	⊠ Yes O No	Coronary Arteries Umbilical Veins Lung Microvasculature	Not applicable
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

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2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☒ Yes ○ No	THP-1, PC3, DU145, LnCAP 293	ATCC
Rodent	○ Yes ○ No		
Non-human primate	○ Yes ○ No		
Other (specify)	○ 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 ☒ 1 ○ 2 ○ 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☒ 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 Known to Be 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	Healthy donors/ LHSC UH	○ Yes ☒ No		○ 1 ○ 2 ○ 3
Human Blood (fraction) or other Body Fluid		○ Yes ○ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (unpreserved)		○ Yes ○ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (preserved)		○ Yes ○ No		○ 1 ○ 2 ○ 3

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 ○ NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E-coli	pcDNA3.1, pIRES2-EGFP	Invitrogen Clontech	RhoA, Rac1 cDNAs excised from pcDNA3.1 and cloned into pIRES2-EGFP	Construction of viral vectors

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

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
Phoenix helper-free packaging cells	pLNCX2	Clontech	RhoAG14V, RhoAT19N, Rac1G12V, Rac1T17N, Cdc42G12V, Cdc42T17N	defects in microfilament assembly dynamics and cell motility

* 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? ⊗ YES O NO

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ⊗ NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

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

5.3 How will the virus 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 ⊗ 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 be used in live animals O YES, specify: _____ O NO

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

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

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? 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 lifestage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

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10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? 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

10.9 Please 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_____
If no, please proceed to Section 10.0 ⊗ NO

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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

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13.0 Containment Levels

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

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

⊗ YES, permit # if on-campus BIO-UWO-0023
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: April 3, 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Name Martin Sandig
Nom

5. A complete informative summary of the proposal on this page.
Présentez sur cette page un résumé complet du projet de recherche.

Integrin signaling in monocyte transendothelial migration

Atherosclerosis involves the extravasation of monocytes into the intima of arterial vessels, a process known as transendothelial migration or diapedesis. Cellular adhesion and motility during diapedesis is regulated largely by the monocyte integrins VLA-4 and LFA-1, binding to endothelial VCAM-1 and ICAM-1, respectively. Under the regulation of the chemokines MCP-1 and the intracellular signaling molecule PI3K these integrins activate signaling complexes that link integrin-mediated adhesion to the regulation of the actin cytoskeleton and cell motility. Specific actin assemblies are nucleated by the Arp2/3 protein complex. During leukocyte chemotaxis filopodia are formed through Cdc42/WASP activation of Arp2/3, while lamellipodia form through activation of Arp2/3 by Rac1/WAVE2. Our recent work demonstrated in monocytes that PI3K in the absence of MCP-1 induced the activation of VLA-4, enhanced adhesion and spreading on VCAM-1, and promoted diapedesis without affecting ICAM-1 dependent interactions. In the presence of MCP-1, in contrast, LFA-1/ICAM-1 interactions were stimulated in a PI3K-dependent manner, while VLA-4/VCAM-1 interactions were rendered PI3K independent. These experiments strongly suggested that activation of LFA-1 and VLA-4 is differentially regulated, involving both MCP-1 and PI3K. It is not known, however, how following ligand binding specific signaling pathways link LFA-1 and VLA-4 to specific changes in cytoskeletal assemblies resulting in coordinated motility during diapedesis. We hypothesize that during adhesion, motility and diapedesis VLA-4/VCAM-1 interactions largely regulate Arp2/3 activation through Cdc42/WASP initiating filopodia formation, while LFA-1/ICAM-1 interactions largely mediate lamellipodia formation through Rac1/WAVE2. To address this hypothesis we will use substrates coated with VCAM-1 and ICAM-1 protein, a well estabilshed culture model of diapedesis, and a hypercholesterolemic rat model of atherogenesis to dissect spatio-temporal changes in the association of monocyte integrins with key cytoskeletal regulators during monocyte adhesion, motility and diapedsis. We will focus on the following specific aims:

Aim 1: Characterize signaling complexes associated with VLA-4 and LFA-1 in response to MCP-1 in unbound monocytes and following binding to their ligands VCAM-1 and ICAM-1, respectively. We will expose untreated or MCP-1 treated monocytes to a) soluble VCAM-1 and ICAM-1 protein, b) VCAM-1 and ICAM-1 coated surfaces, and c) endothelial monolayers to examine at various times by immunoprecipitation and western blot analysis the composition and state of phosphorylation of signaling complexes associated with VLA-4 and LFA-1. We will focus on WASP, WAVE2, Arp3, RhoA, Rac1 Cdc42 and PI3K using commercially available antibodies. Experiments will be done in the absence and in the presence of chemical crosslinking to capture transient molecular complexes. The role of PI3K will be explored by pretreating monocytes with the PI3K inhibitor LY294002 or its inactive analogue LY303511. Epitope specific antibodies and flow cytometry will be employed to determine the relative activtion state of VLA-4 and LFA-1

Aim 2: Determine whether VLA-4 and LFA-1 specifically colocalize with Cdc42/WASP or Rac1/WAVE2 in filopodia or lamellipodia, respectively, during adhesion to VCAM-1 or ICAM-1, and during diapedesis through endothelium in culture and in situ. Using multi-label immunocytochemistry and laser scanning confocal microscopy we will colocalize PI3K, CDc42/WASP/Arp3 and Rac1/WAVE2/Arp3, as well as molecular components identified under Aim 1 with LFA-1 and VLA-4 in monocytes adhering to protein carpets, during diapedesis through endothelial monolayers in culture, and during atherogenesis in a hypercholesterolemic rat model in situ. Changes in adhesion, morphology, motility, and diapedesis will be quantified by imaging following F-actin staining and by time-lapse video microscopy.

Aim 3: Determine the effects of WASP and WAVE2 inhibition on monocyte interactions with VCAM-1, ICAM-1 and endothelial monolayers. Pharmacological inhibition of WASP using the WASP inhibitor Wiskostatin and siRNA inhibition of WASP and WAVE2 expression will be used to determine their respective roles during adhesion and spreadind on VCAM-1 and ICAM-1 as well as during diapedesis through endothelial monolayers. Following F-actin labeling monocyte filopodia and lamellipodia formation will be scored as well as spatio-temporal changes in morphology during diapedesis. This aim will explore to what extent

The proposed experiments will allow us to gain a better understanding of the molecular mechanisms that regulate monocyte motility during extravasation and may help us to design novel therapeutic strategies to treat pathogenic inflammatory responses such as

5. A complete informative summary of the proposal on this page.
Présentez sur cette page un résumé complet du projet de recherche.

Vascular smooth muscle cell phenotype switching and elastin synthesis in 3D tissue engineered coronary artery substitutes.

Background: Diseases of the cardiovascular system are the major causes of mortality in Canada. Current surgical interventions to replace diseased blood vessels using prosthetic materials often fail due to host immune rejection. Other alternatives such as xenografts face host rejection and inter-species disease transmission. Tissue engineering has emerged as a promising technology in the design of responsive living blood vessels with properties similar to that of the native tissue. In tissue engineering of blood vessels, biodegradable porous 3D scaffolds are seeded with vascular cells and cultured in a bioreactor to remodel with extracellular matrix proteins. Vascular smooth muscle cells (VSMCs) and elastin constitute the main components of the tunica media of arterial vessels. In arteries, elastin confers elasticity preventing dynamic tissue creep by stretching under load and recoiling to their original configurations after the load is released. In addition to the mechanical responsiveness, elastin is a potent autocrine regulator of VSMC activity for preventing fibrocellular pathology. Elastin induces VSMC actin stress fiber organization, inhibits proliferation and regulates migration. Indeed elastin knockout studies and clinical observations have revealed an essential regulatory function since, in the absence of extracellular elastin, VSMC proliferation stenoses arteries. Thus to ensure appropriate mechanical function of the vessel and to prevent vessel stenosis, successful engineered vascular tissues must incorporate an elastic component and as such, represents a critical design goal. In spite of its critical role in vessel integrity, elastin is conspicuously absent from previous tissue-engineered vascular substitutes. Although progress has been made towards understanding the underlying principles of in vivo elastin biosynthesis and incorporation into fibers, many of the conditions and mechanisms required to form viable, elastin-containing engineered vessels are still elusive. Our main goal is to induce VSMC phenotype shifts to engineer coronary artery substitutes. We hypothesize that under specific biochemical and biomechanical signals, VSMCs seeded into porous 3D biodegradable polyurethane scaffolds will modulate their phenotype. In a synthetic phenotype, they will proliferate and produce elastin while in a subsequent contractile phenotype, they will express contractile markers similar to that observed in vivo. In order to test the above hypothesis we propose to focus on the following specific objectives: (i) Establish the role of fibronectin immobilization on 3D porous biodegradable polyurethane scaffolds on attachment, proliferation and synthetic phenotypic expression of human coronary artery smooth muscle cells (HCASMCs). (ii)Elucidate the effects of TGF-betta and retinoic acid on elastin biosynthesis by HCASMCs grown on 3D biodegradable polyurethane scaffolds under static and dynamic (pulsatile distension) culture conditions. (iii)Determine the effect of serum withdrawal on the induction of the contractile phenotype of HCASMCs grown on 3D biodegradable polyurethane scaffolds. Research plan: Porous biodegradable polyurethane scaffolds will be fabricated and fibronectin immobilization will be carried out by a known ethylcarbodiimide (EDC)/N-hydroxy succinimide (NHS) activation method. ELISA methods will be used to ascertain fibronectin immobilization. Following HCASMC seeding, cell attachment and proliferation will be studied by fluorescence microscopy. Cellular differentiation will be evaluated by specific VSMC differentiation markers (h-caldesmon and smoothelin). We anticipate to promote long-term attachment of HCASMCs and modulate the synthetic phenotype. The effect of exogenous biomechanical signals (TGF-betta and retinoic acid) on elastin synthesis will be assessed by Laser Scanning Confocal Microscopy (LSCM) and Western blot analyses. Further quantification of elastin, following alkali extraction, will be determined by colorimetric absorbance. We anticipate HCASMCs to retain their synthetic phenotype and enhance elastin synthesis in response to these signals. We will also investigate the effect of biomechanical signals on cell phenotype and elastin synthesis by subjecting the scaffold to pulsatile distention. After a period of elastin production, we will induce the contractile phenotype of HCASMCs by serum withdrawal. The switching of media conditions in the bioreactor can easily been done since our bioreactor is capable of exchanging media under flow conditions. Following serum starvation, we will analyze expression of contractile proteins using Western blots. We anticipate upregulation of contractile proteins in response to serum withdrawal which is vital for engineered vessels to function properly.

Significance: Key information regarding vascular smooth muscle cell phenotype and elastin synthesis in 3D culture is important for understanding the factors that regulate tissue-engineered vessel maturation. The proposed study may lead to the fabrication of elastin containing vascular tissues.

Sandig, M (Martin)

SUMMARY OF PROPOSAL / RÉSUMÉ DE LA PROPOSITION

In the space provided below, state the objectives of the proposed research program and summarize the scientific approach, highlighting the novelty and expected significance of the work.

Dans l'espace prévu ci-dessous, énoncez les objectifs du programme de recherche proposé et résumez la démarche scientifique, en soulignant l'originalité et l'importance prévue des travaux.

Tumor metastasis follows a cascade of events that involves the departure of individual malignant cells from the primary tumor, their dissemination through the vasculature and their extravasation at preferred secondary sites in distant organs, where subsequently metastases form and impede normal organ function. This complex process is largely regulated by adhesive interactions of tumor cells between themselves, with the extracellular matrix and with vascular endothelial cells during transendothelial migration (diapedesis). These interactions are functionally linked to the ability of tumor cells to change shape, to migrate through extracellular matrix and to extend motile processes that breach the endothelial barrier during diapedesis. This research proposal focuses on understanding the molecular mechanisms that regulate interactions between breast tumour cells and endothelial cell, and specifically addresses the question whether and how communication via gap junctions between tumor cells and endothelial cells regulates cellular motility during diapedesis. Using a well characterized in vitro assay, that combines high-resolution laser scanning confocal microscopy (LSCM), and time-lapse video microscopy, with quantitative analysis of diapedesis our previous work revealed that introducing connexin 43 (Cx43) into communication-deficient HBL100 breast tumor cells increased diapedesis. This increase was dependent on functional Cx43 and gap junction intercellular communication between tumor cells and endothelial cells. The main goal of this proposal is to explore the molecular mechanisms controlling gap junction dependent regulation of diapedesis and to explore the role of RhoGTPases in tumor cell diapedesis. We hypothesize that connexin expression and/or heterocellular gap junction dependent signalling modulates the expression and activation of specific RhoGTPases that influence tumor cell motility and interactions with the endothelium required for diapedesis, and that different connexins may play differential roles in this process. In Aim 1 we will determine if ectopic expression of the endothelial connexins Cx43, Cx37, and Cx40 in HBL100 tumor cells changes the expression levels and state of activation of RhoA, Rac-1 and CDC42. We will use western blot analysis and pull-down assays to quantify levels of total and active forms of these RhoGTPases. Our in vitro diapedesis assay will be used to correlate connexin expression, RhoGTPase levels and activity with changes in cellular behaviour at different stages during diapedesis. To confirm that observed changes in cellular behaviour is due to changes in RhoGTPase activity we will in Aim 2 differentially activate or inhibit RhoA, Rac-1 and CDC42 by expressing constitutively active and dominant negative forms of the GTPases in HBL-100 cells and by using pharmacological inhibitors. The effects on cell motility, cell shape and diapedesis will be examined by time-lapse microscopy and LSCM following labelling for F-actin and VE-cadherin. Cell adhesion to endothelium and extracellular matrix components will be quantified using adhesion assays and by quantifying focal contacts and cellular shape changes. To examine if tumour cell diapedesis is mediated by contact dependent modulation of interendothelial cell adhesion and motility through endothelial Rho GTPases we will in Aim 3 explore if tumor cells secrete factors that modulate RhoGTPase activity in endothelial cells and lead to modulation of endothelial cell-cell junctions. We will treat endothelial monolayers with tumour cell conditioned media and examine, using biochemical approaches, the composition and state of activation of adherens junctions and complexes known to affect endothelial contractility such as MLCK and RhoGTPases. In Aim 4 we will examine if Rho GTPases participate in endothelial responses required for tumour cell diapedesis. We will express constitutively active and dominant negative forms of the small GTPases RhoA, Rac-1 and Cdc42 in endothelial monolayers and examine the effect on connexin expression, gap junctional communication and tumor cell diapedesis. Using inhibitors and activators of MLCK, PI3-kinase and src kinases under these conditions will dissect signalling pathways that play a role in the endothelial response during diapedesis. Combining state of the art imaging techniques with molecular biology, biochemical and functional single cell analysis the experiments proposed focus on molecular events that regulate adhesion, motility, and diapedesis of breast tumor cells. We believe that these studies will therefore further our understanding of the molecular mechanisms that regulate tumour cell transendothelial migration and may point to novel avenues to design treatment regimen during early stages of cancer metastasis.

Cell Biology

ATCC® Number:	**CRL-1740™** Order this Item	Price:	**$256.00**
Designations:	LNCaP clone FGC	Depositors:	JS Horoszewicz
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent, single cells and loosely attached clusters
Organism:	*Homo sapiens* (human)	Morphology:	epithelial 
Source:	**Organ:** prostate **Disease:** carcinoma **Derived from metastatic site:** left supraclavicular lymph node		
Cellular Products:	human prostatic acid phosphatase; prostate specific antigen [21889]		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

Related Cell Culture Products

Restrictions:	Distribution of this material for commercial purposes will require execution of a Non-exclusive License Agreement. At the time of placing an order, customers must send a request to licensing@ATCC .org. Orders will be shipped when Customer Service receives confirmation from our Licensing officer.
Isolation:	**Isolation date:** 1977
Applications:	transfection host (technology from amaxa Roche FuGENE® Transfection Reagents)
Receptors:	androgen receptor, positive; estrogen receptor, positive [23045]
Tumorigenic:	Yes
Cytogenetic Analysis:	This is a hypotetraploid human cell line. The modal chromosome number was 84, occurring in 22% of cells. However, cells with chromosome counts of 86 (20%) and 87 (18%) also occurred at high frequencies. The rate of cells with higher ploidies was 6.0%.
Age:	50 years adult
Gender:	male
Ethnicity:	Caucasian

LNCaP clone FGC was isolated in 1977 by J.S. Horoszewicz, et al., from a needle aspiration biopsy of the left supraclavicular lymph node of a 50-year-old Caucasian male (blood type B+) with confirmed diagnosis of metastatic prostate carcinoma. [21889]
These cells are responsive to 5-alpha-dihydrotestosterone (growth modulation and acid phosphatase production). [23045]
The cells do not produce a uniform monolayer, but grow in clusters which should

be broken apart by repeated pipetting when subcultures are prepared.
They attach only lightly to the substrate, do not become confluent and rapidly acidify the medium.
Growth is very slow.
The cells should be allowed to incubate undisturbed for the first 48 hours after subculture.

Comments: When flask cultures are shipped, the majority of the cells become detached from the flask and float in the medium.
Upon receipt, incubate the flask (in the usual position for monolayer cultures) for 24 to 48 hours to allow the cells to re-attach.
The medium can then be removed and replaced with fresh medium.
If desired, the contents of the flask can be collected, centrifuged at 300 X g for 15 minutes, resuspended in 10 ml of medium and dispensed into a single flask.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels. Maintain cultures at a cell concentration between 1 X 10(4) and 2 X 10(5) cells/cm2.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: Twice per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time: about 34 hours

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001
recommended serum:ATCC 30-2020
derivative:ATCC CRL-10995
purified DNA:ATCC CRL-1740D

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References:

22410: Gibas Z, et al. A high-resolution study of chromosome changes in a human prostatic carcinoma cell line (LNCaP). Cancer Genet. Cytogenet. 11: 399-404, 1984. PubMed: 6584201

23045: Horoszewicz JS, et al. LNCaP model of human prostatic carcinoma. Cancer Res. 43: 1809-1818, 1983. PubMed: 6831420

32283: Hu SX, et al. Development of an adenovirus vector with tetracycline-regulatable human tumor necrosis factor alpha gene expression. Cancer Res. 57: 3339-3343, 1997. PubMed: 9269991

33090: Boffa LC, et al. Invasion of the CAG triplet repeats by a complementary peptide nucleic acid inhibits transcription of the androgen receptor and TATA-binding protein genes and correlates with refolding of an active nucleosome containing a unique AR gene sequence. J. Biol. Chem. 271: 13228-13233, 1996. PubMed: 8662737

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Cell Biology

ATCC® Number:	**HTB-81™** Order this Item	Price:	**$264.00**
Designations:	DU 145	Depositors:	KR Stone
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent
Organism:	*Homo sapiens* (human)	Morphology:	epithelial

Source:	**Organ:** prostate **Disease:** carcinoma **Derived from metastatic site:** brain
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications:	transfection host (technology from amaxa Roche FuGENE® Transfection Reagents)
Tumorigenic:	YES
Antigen Expression:	Blood Type O; Rh+
Cytogenetic Analysis:	This is a hypotriploid human cell line. Both 61 and 62 chromosome numbers had the highest rate of occurrence in 30 metaphase counts.The rate of higher ploidies was 3%. The t(11q12q), del(11)(q23), 16q+, del(9)(p11), del(1)(p32) and 6 other marker chromosomes were found in most cells. The N13 was usually absent. The Y chromosome is abnormal through translocation to an unidentified chromosomal segment. The X chromosome was present in single copy.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 1-2 PGM1, 1 PGM3, 2
Age:	69 years
Gender:	male
Ethnicity:	Caucasian
Comments:	The line is not detectably hormone sensitive, is only weakly positive for acid phosphatase and isolated cells form colonies in soft agar. The cells do not express prostate antigen. Ultrastructural analyses of both the cell line and original tumor revealed microvilli, tonofilaments, desmosomes, any mitochondria, well developed Golgi and heterogenous lysosomes.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium, 95%; DMSO, 5%
Storage temperature: liqid nitrogen vapor temperature

Related Products:

recommended serum:ATCC 30-2020
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
purified DNA:ATCC HTB-81D
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

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22858: Stone KR, et al. Isolation of a human prostate carcinoma cell line (DU 145). Int. J. Cancer 21: 274-281, 1978. PubMed: 631930
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32460: Carter RE, et al. Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase. Proc. Natl. Acad. Sci. USA 93: 749-753, 1996. PubMed: 8570628
32486: Nupponen NN, et al. Genetic alterations in prostate cancer cell lines

detected by comparative genomic hybridization. Cancer Genet. Cytogenet. 101: 53-57, 1998. PubMed: 9460501
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Cell Biology

ATCC® Number:	**CRL-1435™** Order this Item	Price:	**$256.00**
Designations:	PC-3	Depositors:	ME Kaighn
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent (The cells form clusters in soft agar and can be adapted to suspension growth)
Organism:	*Homo sapiens* (human)	Morphology:	epithelial 

Source:	**Organ:** prostate **Tumor Stage:** grade IV **Disease:** adenocarcinoma **Derived from metastatic site:** bone
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications:	transfection host (technology from amaxa Roche FuGENE® Transfection Reagents)
Tumorigenic:	YES
Antigen Expression:	HLA A1, A9
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 11 D16S539: 11 D5S818: 13 D7S820: 8,11 THO1: 6,7 TPOX: 8,9 vWA: 17
Cytogenetic Analysis:	The line is near-triploid with a modal number of 62 chromosomes. There are nearly 20 marker chromosomes commonly found in each cell; and normal N2, N3, N4, N5, N12, and N15 are not found. No normal Y chromosomes could be detected by Q-band analysis.
Age:	62 years adult
Gender:	male
Ethnicity:	Caucasian
Comments:	The PC-3 was initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old male Caucasian. [22363] The cells exhibit low acid phosphatase and testosterone-5-alpha reductase activities.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Preservation:

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020

References:

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various human tumors transplanted into nude mice. Anticancer Drug Des. 13: 35-45, 1998. PubMed: 9474241
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ATCC® Number:	**TIB-202™** Order this Item	Price:	**$264.00**
Designations:	THP-1	Depositors:	S Tsuchiya
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	suspension monocyte
Organism:	*Homo sapiens* (human)	Morphology:	

Source:	**Organ:** peripheral blood **Disease:** acute monocytic leukemia **Cell Type:** monocyte;
Cellular Products:	lysozyme [58053]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications:	transfection host (technology from amaxa Roche FuGENE® Transfection Reagents)
Receptors:	complement (C3), expressed [58053] Fc, expressed
Antigen Expression:	HLA A2, A9, B5, DRw1, DRw2 [58053]
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,13 D13S317: 13 D16S539: 11,12 D5S818: 11,12 D7S820: 10 THO1: 8,9.3 TPOX: 8,11 vWA: 16
Age:	1 year infant
Gender:	male
Comments:	The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin. [58053] Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). [22193]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: 2-mercaptoethanol to a final concentration of 0.05 mM; fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%

	Temperature: 37.0°C
Subculturing:	**Protocol:** Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 2-4 X 10(4) viable cells/ml. Subcultutre when cell concentration reaches 8X105) cells/ml.Do not allow the cell concentration to exceed 1 X 10(6) cells/ml. **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	approximately 26 hrs
Related Products:	purified DNA:ATCC TIB-202D purified RNA:ATCC TIB-202R
References:	22193: Tsuchiya S, et al. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. Cancer Res. 42: 1530-1536, 1982. PubMed: 6949641 22285: Skubitz KM, et al. Human granulocyte surface molecules identified by murine monoclonal antibodies. J. Immunol. 131: 1882-1888, 1983. PubMed: 6619543 32286: Cuthbert JA, Lipsky PE. Regulation of proliferation and Ras localization in transformed cells by products of mevalonate metabolism. Cancer Res. 57: 3498-3504, 1997. PubMed: 9270019 32351: Huang S, et al. Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. J. Virol. 70: 4502-4508, 1996. PubMed: 8676475 32395: Clark RA, et al. Tenascin supports lymphocyte rolling. J. Cell Biol. 137: 755-765, 1997. PubMed: 9151679 32466: Hambleton J, et al. Activation of c-Jun N-terminal kinase in bacterial lipopolysaccharide-stimulated macrophages. Proc. Natl. Acad. Sci. USA 93: 2774-2778, 1996. PubMed: 8610116 33031: Hsu HY, et al. Inhibition of macrophage scavenger receptor activity by tumor necrosis factor-alpha is transcriptionally and post-transcriptionally regulated. J. Biol. Chem. 271: 7767-7773, 1996. PubMed: 8631819 33088: Lucas M, Mazzone T. Cell surface proteoglycans modulate net synthesis and secretion of macrophage apolipoprotein E. J. Biol. Chem. 271: 13454-13460, 1996. PubMed: 8662812 33134: Sando GN, et al. Induction of ceramide glucosyltransferase activity in cultured human keratinocytes. J. Biol. Chem. 271: 22044-22051, 1996. PubMed: 8703011 33141: Ollivier V, et al. Elevated cyclic AMP inhibits NF-kappaB-mediated transcription in human monocytic cells and endothelial cells. J. Biol. Chem. 271: 20828-20835, 1996. PubMed: 8702838 58053: Tsuchiya S, et al. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int. J. Cancer 26: 171-176, 1980. PubMed: 6970727

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ATCC® Number:	**CRL-1573™** Order this Item	Price:	**$256.00**
Designations:	293 [HEK-293]	Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent epithelial
Organism:	*Homo sapiens* (human)	Morphology:	PHOTO

Source:
Organ: kidney
Cell Type: transformed with adenovirus 5 DNA

Permits/Forms:
In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Restrictions:
These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.

Applications:
efficacy testing [92587]
transfection host (technology from amaxa
Roche FuGENE® Transfection Reagents)
viruscide testing [92579]

Receptors:
vitronectin, expressed

Tumorigenic:
YES

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11,12
D13S317: 12,14
D16S539: 9,13
D5S818: 8,9
D7S820: 11,12
THO1: 7,9.3
TPOX: 11
vWA: 16,19

Cytogenetic Analysis:
This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Age:
fetus

Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses.

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92587: Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 2197-02.

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