Modification Form for Permit BIO-RRI-0023

Permit Holder: Arthur Brown

Approved Personnel
(Please stroke out any personnel to be removed)

Adam Verhoeve

Anna Pniak

Kathy Xu

Nicole Geremia

Additional Personnel
(Please list additional personnel here)

William Monty Mckillop

Magdalena Dragan

Dr. Lynne Weaver AB

Dr. Feng Bao AB

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli	
Approved Cells	Human (primary), Rodent (primary and established), A7, Neu 7, DI-TNC1, HEK 293	Normal Human Astrocytes from Lonza
Approved Use of Human Source Material	Bone marrow	human blood (as per Weaver protocol) AB
Approved GMO	SV 40 Large T antigen, PGL 3, PGL4, pCMV, E1A oncogene	
Approved use of Animals	Mice (w spinal cord injury)	

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Jan 8, 2007

Signature of Permit Holder: Arthur Brown

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

Modification Form for Permit BIO-RRI-0023

Permit Holder: Arthur Brown

Approved Toxin(s)

These cells will be cultured with various media. The cells will be lysed and their RNA or protein harvested. The levels of different mRNAs will be assayed by quanitative PCR and the levels of proteins by immunocytochemistry or Western Blot analysis.

** PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
*** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Jan 8, 2007

Signature of Permit Holder: Arthur Brown

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

Lonza Walkersville, Inc.
www.lonza.com
scientific.support@lonza.com
Scientific Support: 800-521-0390
Document # TS-AA-1044-4 02/09
Walkersville, MD 21793-0127 USA

Clonetics® Normal Human Astrocytes

Instructions for Use

Receiving Instructions: Unpack immediately! Packages may contain components with various storage requirements!

Safety

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro procedures.

WARNING: CLONETICS® AND POIETICS® PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, Biosafety in Microbiological and Biomedical Laboratories, 1999. If you require further information, please contact your site Safety Officer or Scientific Support.

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.
3. For proliferating cells – swab down the flask of proliferating cells with 70% ethanol or isopropanol, then place the flask in 37°C, 5% CO_2, humidified incubator and allow to equilibrate for three to four hours. After cells have equilibrated, remove shipping medium from the flask and replace with fresh medium.
4. AGM™ BulletKit® Instructions: Upon arrival, store Astrocyte Basal Medium (ABM™) at 2°C to 8°C and SingleQuots® at -20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 2°C to 8°C and added to ABM™ within 72 hours of receipt. After SingleQuots® are added to basal medium, use within one month. Do not re-freeze.
5. ReagentPack™ Subculture Reagents are sterile-filtered and then stored at -20°C until shipment. Subculture reagents may thaw during transport. They may be refrozen once. If you plan to use within 3 days, store at 2°C to 8°C. Trypsin/ EDTA Solution has a limited shelf life or activation at 2°C to 8°C. If, upon arrival, Trypsin/EDTA is thawed, immediately aliquot and refreeze at -20°C. We recommend that the HEPES-BSS and the Trypsin Neutralizing Solution be stored at 2° to 8°C for no longer than one month.

   Note: To keep Trypsin/EDTA fresh and active after thawing, you may aliquot it into sterile centrifuge tubes and re-freeze at -20°C.

Using media or reagents other than what's recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

Preparation of Media

For a BulletKit®, perform the following steps:

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the entire amount to the basal medium with a pipette.
3. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
4. Transfer the label provided with each kit to the basal medium bottle being supplemented. Use it to record the date and amount of each supplement added. We recommend that you place the completed label over the basal medium label, allowing for the basal medium lot number and expiration date) to be visible.
5. Record the new expiration date on the label based on the shelf life.

Note: If there is concern that sterility was compromised during the supplementation process, the entire newly prepared growth medium may be refiltered with a 0.2 µm filter to assure sterility. Routine refiltration is not recommended.

Thawing of Cells / Initiation of Culture Process

1. The recommended seeding density for NHA is 5,000 cells/cm^2.
2. To set up cultures calculate the number of vessels needed based on the recommended seeding density of 5,000 cells/cm^2 and the surface area of the vessels being used. Do not seed cells into a well plate immediately out of cryopreservation. Add the appropriate amount of medium to the vessels (1 ml/5 cm^2) and allow the vessels to equilibrate in a 37°C, 5% CO_2, humidified incubator for at least 30 minutes.
3. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, then retighten. Quickly thaw the cryovial in a 37°C water bath by gently swirling the vial only until the ice disappears. Watch your cryovial closely; when the last sliver of ice melts remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.
4. Resuspend the cells in the cryovial and dispense them into the preincubated culture vessels at the calculated seeding density. Gently rock the culture vessel to evenly distribute the cells and return to the incubator.
5. Centrifugation should not be used to remove cells from cryopreservation medium. This action is more damaging than the effects of residual DMSO in the culture.

Subculturing

The following instructions are for a 25 cm^2 flask. Adjust all volumes accordingly for other size flasks.

Preparation for subculturing the first flask:

1. Subculture the cells when they are 70-80% confluent and contain many mitotic figures throughout the flask.
2. For each 25 cm^2 of cells to be subcultured:

- Thaw 2 ml of Trypsin/EDTA and allow to come to room temperature.
- Allow 7-10 ml of HEPES Buffered Saline Solution (HEPES-BSS) to come to room temperature.
- Allow 4 ml of Trypsin Neutralizing Solution (TNS) to come to room temperature.

3. Remove growth medium from 2°C to 8°C storage and allow to warm to room temperature.
4. Prepare new culture flasks by adding pre-warmed medium at a volume of 1 ml/5 cm^2 of surface area, label with cell strain, lot number, passage and date. Place the flasks in the incubator until cells are ready to be seeded.

In a sterile field:

1. Aspirate the medium from one flask.
2. Rinse the cells with 5 ml of room temperature HEPES-BSS. DO NOT forget this step. The medium contains trypsin inhibitors such as calcium or serum proteins.
3. Aspirate the HEPES-BSS from the flask.
4. Cover the cells with 2 ml of Trypsin/EDTA solution.
5. Place the flask in the 37°C incubator for 3 to 4 minutes.
6. Allow the trypsinization to continue until approximately 90% of the cells have rounded up. Examine the culture flask under the microscope to determine the extent of the detachment.
7. At this point, rap the edge of the flask against the palm of your hand to release the majority of cells from the culture surface. If only a few cells detach, return the flask to the incubator for 30 seconds to 1 minute.
8. After the cells have detached, neutralize the trypsin in the flask with 4 ml of room temperature Trypsin Neutralizing Solution.
9. If a majority of the cells do not detach within 5 minutes, the enzyme activity of the trypsin has been compromised by low temperature or an overextended shelf life. Proceed to harvest the cells from the flask as described above, and either re-trypsinize with fresh, warm Trypsin/EDTA solution or rinse with Trypsin Neutralizing Solution, add fresh, warm medium to the flask and return to the incubator until fresh trypsinization reagents are available.
10. Quickly transfer the detached cells to a sterile 15 ml centrifuge tube.
11. Rinse the flask with a final 2 ml of HEPES-BSS to collect residual cells, and add this rinse to the centrifuge tube.
12. Examine the flask under the microscope to make sure the harvest was successful by observing the number of cells remaining. This should be less than 5%.
13. Centrifuge the cell suspension at 160 x g to 200 x g for 5 minutes at 2°C to 8°C.

14. Aspirate the supernatant and resuspend the cell pellet in 2 ml of AGM™.
15. Determine cell count and viability using a hemacytometer and Trypan Blue.
16. Calculate the volume of the cell suspension needed to seed the flask at a density of 5,000 cells/cm^2 and add the appropriate volume of cell suspension to a tissue culture flask or dish (pre-filled at 1 ml/5cm^2 of surface area with pre-warmed medium). If seeding into well plates at this time, the recommended density is 10,000 cells/cm^2. Mix gently to evenly distribute the cells and return the flask to the incubator.

Maintenance

1. Change the growth medium the day after seeding and every other day thereafter. As the cells become more confluent, increase the volume of media as follows: under 25% confluence then feed cells 1 ml per 5 cm^2, 25-45% confluence then feed cells 1.5 ml per 5 cm^2, over 45% confluence then feed cells 2 ml per 5 cm^2.
2. Warm an appropriate amount of medium to 37°C in a sterile container. Remove spent medium from flask and replace it with the warmed, fresh medium and return the flask to the incubator.
3. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer and warm only the required volume to a sterile secondary container.

Ordering Information

Cryopreserved Cells (Single donor)		
CC-2565	NHA	≥1,000,000 cells
Proliferating Cells		
CC-2665	NHA	T-25 flask
CC-0297	NHA	T-75 flask
CC2565T150	NHA	T-150 flask
CC2565T225	NHA	T-225 flask
Proliferating Cells in PreSeeded Plates		
CC-2565W6	NHA	6 wells
CC-2565W12	NHA	12 wells
CC-2565W24	NHA	24 wells
CC-2565W48	NHA	48 wells
CC-0093	NHA	96 wells

Lonza

Related Products

Astrocyte Medium (Must be purchased separately):

CC-3186	AGM™ BulletKit®	Kit which contains a 500 ml bottle of ABM™, (CC-3187) and AGM™ SingleQuots® (CC-4123)
CC-3187	ABM™	Astrocyte Basal Medium (no growth factors) (500 ml)
CC-4123	AGM™ SingleQuots®	Supplements for a complete growth medium, developed especially for NHA (CC-2565)

Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza warrants its cells in the following manner only if Clonetics® Media and Reagents are used.

1. Clonetics® NHA Cryopreserved Cultures are assured for experimental use for at least ten population doublings.
2. Clonetics® NHA Proliferating Cultures are assured for experimental use for at least five population doublings.
3. Additional population doublings and subcultures are possible, but growth rate, biological responsiveness and function deteriorate with subsequent passage.
4. To avoid the loss of your cells and forfeiture of your warranty, subculture cells before they reach 80% confluence.

Quality Control

HIV-1, Hepatitis B and Hepatitis C are not detected for all donors and/or cell lots. Routine characterization of NHA includes positive immunofluorescence staining for Glial Fibrillary Acid Protein (GFAP) in the first passage out of cryopreservation; GFAP expression decreases with passaging. For detailed information concerning QC testing, please refer to the Certificate of Analysis.

When placing an order or for technical support, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics® Products, refer to the Lonza website or our current catalog. To obtain a catalog, additional information or technical support you may contact Lonza by web, e-mail, telephone, fax or mail.

After spinal cord injury (SCI), neutrophils and monocyte/macrophages enter the injured cord. causing intraspinal inflammation and secondary damage. Our laboratory has shown that the CD11d integrin, expressed by human and rat neutrophils and monocytes, plays a major role in this process. Our recent 10 papers (see CV of LCW) are the total bibliography on the role of this integrin in SCI and constitute the great majority of papers related to its functional relevance. We have investigated the anti-inflammatory effects of an anti-CD11d mAb using a clinically relevant clip-compression model of SCI. Rats given an anti-CD11d mAb, i.v. at 2, 24 and 48 h after clip compression SCI have ~80% fewer neutrophils in the lesion site at 72 h after injury and 50% fewer monocyte/ macrophages (**Mabon *et al*, 2000, Saville, *et al*, 2004**). In later studies (**Bao, *et al*, 2004 a,b, 2005**) we showed that this reduction of leukocyte influx into the cord was paralleled by 30-70% decreases, in the lesion area, of reactive oxygen species, of metabolic enzymes that generate them such as MPO, COX-2, iNOS and $gp91^{phox}$ (abbreviations in Research Plan) and of protein oxidation and nitration, lipid peroxidation and DNA and RNA oxidation. The treatment decreased caspase-3 expression and cell death (neuron counts) by 50%. Most of these effects were detected as early as 6 h after SCI and were long-lasting. Recently **Ditor *et al.*** (submitted to Exp. Neurol) showed significant neuroprotective effects of the anti-CD11d mAb when the treatment was delayed to 6 h after SCI, a clinically relevant onset of treatment. The blocking effects of the anti-CD11d mAb show that a large portion of oxidative damage can be attributed to the influx of haematogenous inflammatory cells. The 3-dose treatment also improved neurological outcomes assessed from 2-12 wk after SCI. In three studies (**Gris *et al.*, 2004;2005; Oatway *et al*, 2005**), using SCI at the 4^{th} (T4) or 12^{th} (T12) thoracic segment, the 3-dose treatment improved motor performance, autonomic function (decreased autonomic dysreflexia) and reduced neuropathic pain. These functional changes correlated with significantly greater amounts and increased organization of myelin, neurofilament and serotonergic axons in and near the lesion. **Gris *et al*, 2005 and Weaver *et al.*, 2005** showed that an acute treatment with methylprednisolone usually was less effective than the anti-CD11d mAb and that combining methylprednisolone with the mAb reversed the positive effects of the mAb. Finally, in ongoing studies we have shown that the CD11d integrin is expressed by neutrophils and monocytes of control and acutely cord-injured human subjects. These findings show the potential for translation of our studies to a clinical treatment. The proposed studies will further address mechanisms for the positive effects of the anti-CD11 mAb treatment and compare them to effects of treatment with a mAb to the $\alpha4\beta1$ integrin.

Another leukocyte integrin, the $\alpha4\beta1$, that is expressed by rat and human neutrophils, monocyte/ macrophages and lymphocytes, has important roles in the activation and migration of these cells into and within tissue. Because the $\alpha4\beta1$ integrin may be highly relevant to secondary damage after SCI, we have started studies of the role of this $\beta1$ integrin in the inflammatory response of the injured spinal cord. Like the CD11d/CD18, we have demonstrated expression of the $\alpha4\beta1$ by leukocytes of control and cord-injured human subjects. In rats after clip compression SCI at T4, the $\alpha4\beta1$ integrin is expressed by monocytes, lymphocytes and neutrophils in blood (by immunocytochemistry). Western blot analysis of leukocytes isolated from the blood revealed that expression of this integrin is slightly increased at 24 h after SCI. Expression of $\alpha4\beta1$ in the rat spinal cord homogenates was increased at 6 and 24 h after SCI, compared to that in uninjured control rats. Effects of blocking the $\alpha4\beta1$ integrin were assessed in rats treated i.v. with an anti-$\alpha4$ mAb or a control mAb at 6 and 48 h after SCI. The anti-$\alpha4$ mAb treatment decreased MPO activity and ED-1 expression in homogenates of the lesion by ~50% at 72 h after the SCI, consistent with blockade of neutrophil and monocyte/macrophage influx into the cord. Lipid peroxidation and the oxidative enzyme $gp91^{phox}$ in these homogenates were also reduced. Behavioural studies of treatment effects in rats with SCI at T12 have shown improvement in locomotor function and reduction in neuropathic pain at 3 wk after injury. These preliminary studies warrant the prediction that the anti-$\alpha4$ mAb may be an effective neuroprotective treatment for SCI. The treatment effects of the anti-$\alpha4$ mAb may be due to initial blockade of leukocyte migration into the cord and/or to mobilization of bone marrow cells that later enter the cord. We will examine these mechanisms. Our data suggest that blocking the CD11d/CD18 or the $\alpha4\beta1$ integrin, or both, will be an effective treatment for SCI.

A new interdisciplinary team at the Robarts Research Institute will develop innovative treatments for the immediate rescue and subsequent repair of the injured spinal cord, using a multidisciplinary approach. Five scientists, and a spinal cord neurosurgeon/scientist, have developed a research program with two major themes: 1) discovery and development of new ways to protect the traumatized spinal cord from damaging secondary injury ***(Rescue)*** and, 2) restoration of spinal cord function after injury by the use of human stem cells (SC) ***(Repair).*** Rescue and repair are inextricably linked as minimizing secondary damage by rescue tactics will foster repair by regeneration. Team members have the divergent expertise to address these problems and, in combination, will undertake approaches more innovative and complete than would be possible as individuals. The integrated research is underpinned by two questions.

1. Can we selectively modulate the inflammatory response to spinal cord injury (SCI), minimizing its cytotoxic consequences without disrupting its beneficial aspects?

2. Can delivery of human SC to cord-injured animals result in functionally significant repair?

We propose to employ rat and mouse models of a clinically relevant clip-compression spinal cord injury (clip-SCI) that closely replicates the key pathophysiology of human injury. These models mimic the primary mechanical injury and secondary damage by well-characterized mechanisms including inflammation, a powerful contributor. In addition to the animal studies, we will elucidate the poorly understood mechanisms for inflammation after human SCI, permitting us to ascertain if rodent studies can truly be translated to the human condition. The outcome of SCI depends initially upon axon survival at the injury site and, despite the awareness that early inflammation is significant, no clinically useful anti-inflammatory treatment has been developed. We offer an innovative neuroprotective, anti-inflammatory strategy. In contrast to neuroprotection, cell-based reparative strategies are in early stages of consideration. Diverse types of SC administered to animal SCI models yielded promising results, suggesting that they may be useful for repair. We will address that possibility using human SC, assuring the clinical relevance of our results and progress will be monitored by novel micro magnetic resonance imaging.

Objective 1.1 (*Rescue*). (FOSTER-GAREAU, DEKABAN AND WEAVER) As the first cells entering the injured spinal cord, neutrophils initiate the inflammatory cascade leading to secondary damage. The first objective of our neuroprotective strategy is to increase the barriers to neutrophil infiltration into the injured cord beyond that accomplished by our previous strategy, a mAb to the $\alpha D\beta 2$ integrin. We will determine potential synergistic effects of a combined blocking strategy using the anti-$\alpha D\beta 2$ mAb that impairs monocyte/macrophage and neutrophil infiltration plus an inhibitor of P-selectin, a molecule that permits neutrophil tethering to the endothelium. Treatment(s) will be administered to rats after clip-SCI to assess effects on autonomic and motor outcomes and chronic pain. ***Hypothesis***. By enhancing the blockade of neutrophils, the inflammatory cascade will be averted more effectively, leading to greater tissue sparing and neurological improvement. ***Experiments.*** We will assess neuroprotection and lesion sparing effects of these treatments with magnetic resonance microscopy time course studies and behavioural assessments of autonomic, locomotor and sensory neurological outcomes.

Objective 1.2 (*Rescue*). (DUGGAL, WEAVER AND DEKABAN) The profile of **1)** integrins on circulating leukocytes and of chemokines and cytokines in blood of **patients** within 72 hr of SCI and **2)** leukocytes, chemokines and cytokines in the acutely-injured human spinal cord will be ascertained to determine if our rat anti-inflammatory treatments will translate effectively to the clinical SCI. ***Hypotheses.*** Within 72 hr of SCI, neutrophils and monocytes are activated and upregulate expression of integrins. Most of the intraspinal leukocytes are neutrophils. Expression of pro-inflammatory chemokines and cytokines is increased in CSF and spinal cord. ***Experiments.*** Expression of integrins on the leukocytes will be examined by fluorescence-activated cell sorting (FACS). Blood and cerebrospinal fluid samples will be obtained at 24-72 hr after SCI and analyzed, by ELISA, for chemokines, pro-inflammatory cytokines, neutrophils and macrophages. With our Pathology Department, sections of spinal cord at the injury site will be obtained from patients who died within 72 hr of SCI and stained for chemokines and cytokines.

Objective 1.3 (*Rescue*). (DEKABAN, BROWN AND FOSTER-GAREAU) This study will identify

differences in gene expression profiles of macrophages derived from hematogenous monocytes (hMacs) and those derived from intraspinal microglia (mMacs) that account for the differing activities of these two types of macrophages. ***Hypothesis.*** The un-activated precursors (CNS microglia or blood monocytes) giving rise to the activated macrophages at a SCI site participate in the secondary injury and wound repair in different ways with respect to phagocytosis, oxidative burst and cytokine release. ***Experiments.*** Comparative gene expression profiles of hMacs and mMacs will be determined. Mice that express EGFP in cells of granulocyte/monomyelocyte lineage will be subjected to a severe clip-SCI. At 72 hr-14 days after SCI, blood and spinal cord samples will be purified into hMac and mMac populations using antibodies to specific markers and FACS analysis. Using subtraction-suppression hybridization PCR, subtracted cDNA from hMac and mMac populations will be isolated and cDNA libraries generated. The percentage of differentially expressed genes in each subtracted population will be assessed and cDNAs identified by sequencing individual clones. We will also hybridize the standard cDNA libraries to a spotted micro-array chip. Differences in the phagocytic capacity of hMacs and mMacs will be studied to develop methods for targeted imaging of specific cell populations. Using isolated cell populations, cellular uptake of iron oxide nanoparticles will be measured at 4 Tesla using a susceptometry technique. Confirming functional studies will assess phagocytic properties of each population by FACS analysis and oxidative burst by myeloperoxidase and NADPH oxidase assays.

Objective 2 (*Repair*). **(BROWN, BHATIA AND FOSTER-GAREAU)** These studies will ascertain the potential role of human SC in regeneration and restoration of structure and function after SCI. ***Hypothesis.*** Regeneration of spinal tracts and restoration of function after SCI can be effected by administering human SC. ***Experiments***. To evaluate the benefit of human SC after SCI we will use NOD/SCID mice, as they lack a functional immune system and will not reject the human cells. To ensure clinical translation, we use human SC because, whereas the pathophysiology of SCI is very similar in mice and man, the behaviour of human and mouse SC is significantly different. Human hematopoietic and embryonic SC will be studied to determine which type engrafts optimally and is most beneficial for recovery from SCI. First we will characterize responses of NOD/SCID mice to clip-SCI, evaluated by microimaging and histological/immunocytochemical analysis. Next the following effects of human SC will be assessed: 1) Different delivery approaches of SC will be evaluated for engraftment in SCI mice using immuno-staining for human-specific antibodies. 2) Locomotor recovery and autonomic function will be assessed. 3) The cellular makeup of the injured cord will be determined to estimate the amount and type of stem cell engraftment. 4) Effects on regeneration will be assessed using tract-tracing methods. 5) SC will be followed using novel cellular imaging and microimaging to assess the impact of stem cell therapy on repair of the injured spinal cords.

RELEVANCE. We are focussing on new strategies for spinal cord ***rescue*** and ***repair*** after injury, particularly the use of SC and nanosciences. Our research goal is to discover and develop innovative treatments, using a multidisciplinary approach. **CROSS THEME.** The proposed research extends from investigations of the human inflammatory response to SCI to the most basic aspects of SC regenerative medicine. The two major research themes both link biomedical and clinical research. **VALUE-ADDED.** By focusing all team members on the critical themes, ***Rescue and Repair***, the expertise and contributions of each individual team member are integrated in a manner that would not be matched if each team member were funded separately. **TRAINING.** The training of graduate students, postdoctoral and clinical fellows is a critical team objective. We have developed this program to provide new opportunities for multidisciplinary training of qualified scientists poised to take up SCI research, adding capacity to a clear research need. **RECRUITMENT.** We will recruit 2 new Scientists to this initiative in years 4 and 5 and are now recruiting a senior stem cell Scientist and contrast agent chemist/Scientist to the team. **TRANSLATION.** We will promote knowledge translation by developing an electronic web-based tool, establishing/maintaining partnerships with SCI consumer organizations, expanding our research partnership with clinicians in SCI treatment and continuing our ongoing relationships with SCI neurosurgeons and rehabilitation physicians, throughout Ontario and Canada.

Summary of Approvals for Permit BIO-RRI-0023

Permit Holder: Arthur Brown

Approved Personnel (Please stroke out any personnel to be removed

Allyson Tighe

Adam Verhoeve

Anna Pniak

Kathy Xu

Nicole Geremia

Additional Personnel

	Please stroke out any approved Biohazards* to be removed below	Write additional Biohazards for approval below.
Approved Microorganisms*	E. coli	
Approved Cells*	Human (primary), Rodent (primary and established), A7, Neu 7	DI- TNC1 HEK 293
Approved Use of Human Source Material*	Bone marrow	
Approved GMO*	SV 40 Large T antigen, PGL 3, PGL4, pCMV	E7A oncogene ql.
Approved use of Animals*	Mice (w spinal cord injury)	
Approved Toxin(s)*		

Date of last Biohazardous Agents Registry Form Jan 8, 2007

Signature of Permit Holder: Arthur Brown

BioSafety Officer(s): A Hurley March 28/08

Chair, Biohazards Subcommittee: G. M. Kidder 4 April 2008

robarts RESEARCH

BIO-RRI-0023

BIOHAZARDOUS AGENTS REGISTRY FORM

Reviewed by Biosafety Subcommittee: February 2006

This form must be completed by each Principal Investigator when completing a grant application or grant renewal to be administered by the Robarts Research Institute, if the use of biohazardous and/or infectious agents is proposed. For any proposed animal work involving the use of biohazardous agents or animals carrying zoonotic agents infectious to humans, this form must also be completed.

COMPLETED FORMS ARE TO BE RETURNED TO BIOSAFETY SUBCOMMITTEE CHAIR, ROOM 3-34.1.

*If there are any changes to the information on these forms (excluding grant title and funding agencies) a new form must be completed and sent to the Biosafety Subcommittee Chair **BEFORE** implementation of these changes can occur.*

If multi-team grants are being applied for, each individual Investigator of the team must submit a Biohazardous Agents Registry Form to the Biosafety Subcommittee Chair.

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

For questions regarding this form, please contact Biosafety Subcommittee Chair at ext. 34125.

1.0 Contact Information

PRINCIPAL INVESTIGATOR: Dr Arthur Brown

SIGNATURE: Arthur Brown

DATE: Jan 2 '07

DEPARTMENT: BTRG

ADDRESS:

TELEPHONE: x 34308

EMAIL: abrown@robarts.ca

Location of experimental work to be carried out.

Building(s):

Room(s): 3-36, 211

For work being performed at Institutions affiliated with the Robarts Research Institute, the Safety Officer for the Institution where experiments will take place must sign the form prior to it being sent to Robarts Research Institute, Biosafety Subcommittee Chair. See Section 13.0, Approvals

GRANT TITLE(S):

ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY(S) EXPLAINING THE BIOHAZARD(S) USED.

FUNDING AGENCY/AGENCIES: CIHR, ORF, Heart & Stroke

Anticipated Grant End Date:

Names of all personnel working under Principal Investigator's supervision in this location:

All current lab personnel - List named

Nicolae Gerenia
Kathy Xu
Anna Pniak
Adam Verhoeve
Allyson Tighe
Paul Gris
Irene Ngai-Kwan Ho

Note : A list of human pathogens categorized according to Risk Group can be obtained by calling the Office of Laboratory Security directly at (613) 957-1779 or accessing their Web site : http://www.phac-aspc.gc.ca/ols-bsl/index.html

2.0 Microorganisms

2.1 Does your work involve the use of microorganisms? YES ✓ NO ○
If **NO**, please proceed to Section **3.0**

2.2 Please complete the table below:

Name of Microorganism	Is microorganism a known human pathogen? YES/NO	Is microorganism a known animal pathogen? YES/NO	Is microorganism a known zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?	Health Canada or CFIA Containment Level (select one)
E. coli (competent, non pathogenic)	No	No	No	1L	1○ 2○ 3○
					1○ 2○ 3○
					1○ 2○ 3○

3.0 Cell Culture

3.1 Does your work involve the use of cell cultures? YES ⊘ NO ○
If **NO**, please proceed to Section **4.0**.

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

Cell Type	Is this cell type used in your work? YES / NO	Established or Primary *	Supplier of Primary Cell Culture Tissue
Human	Yes	Primary	Dr. A. Xenocostas
Rodent	Yes	Primary & Established	generated in lab ? ←
Non-human primate	No		
Other (specify)			

* i.e. derived from fresh tissue

establishe cones From J. Fawce

3.3 Complete the following table.

"Primary gene 2.11.

Specific Cell Line	Source / Supplier	HC or CFIA Containment Level (select one)
A7	Dr. J. Fawcett	1 ○ 2 ⊘ 3 ○
Neu 7	"	1 ○ 2 ⊘ 3 ○
		1 ○ 2 ○ 3 ○

see attach

4.0 Use of Human Source Materials

4.1 Does your work involve the use of human source materials? YES ⊘ NO ○
If **NO**, please proceed to Section **5.0**

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

Human Source Material	Specify Source, or Not Applicable (NA)	Is Human Source Material known to be infected with an infectious agent? YES/NO	Name of Infectious Agent	HC or CFIA Containment Level (select one)
Human Blood (whole) or other Body Fluid	Bone marrow From Healthy Donors	No		1 ○ 2 ⊘ 3 ○
Human Blood (fraction) or other Body Fluid				1 ○ 2 ○ 3 ○
Human Organs (unpreserved)				1 ○ 2 ○ 3 ○
Human Tissues (unpreserved)				1 ○ 2 ○ 3 ○

@ this is?? - work-up of healthy donors before transplant (Dr. A. Xenocostas)

5.0 Genetically Modified Organisms and Cell lines

5.1 Will genetic modifications be made to the organism, virus or cell line? YES ● NO ○
If **NO**, please proceed to Section 6.0

5.2 Will genetic sequences from any of the following be involved?

- HIV YES ○ NO ●

If **YES**, specify: _____

- HTLV 1 or 2 YES ○ NO ●

If **YES**, specify: _____

- Other human or animal pathogen and/or their toxins YES ○ NO ●

If **YES**, specify: _____

5.2 Will intact genetic sequences be used from:

- SV 40 Large T antigen YES ● NO ○
- Adeno E1A YES ○ NO ○
- Known or suspected oncogenes YES ○ NO ○

If **YES**, specify: A7 line carries SV40 LgT & Neu7 carries Neu oncogene.

5.4 Will a live vector(s) (viral or bacterial) be used for gene transduction? YES ○ NO ●

If **YES**, name vector: [illegible]

5.5 List specific vector(s) to be used: PGL3, PGL4, pCMV

5.6 Will vector be replication defective? YES ○ NO ●

5.7 Will vector be infectious to humans or animals? YES ○ NO ●

5.8 Will this be expected to increase the Containment Level required? YES ○ NO ●

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials using the vector(s) in 5.5 be conducted? YES ○ NO ●
If **NO**, please proceed to Section 7.0
If YES, attach a full description of the make-up of the virus.

6.2 Will vector be able to replicate in the host? YES ○ NO ●

6.3 How will the vector be administered? _____

6.4 Please give the Health Care Facility where the clinical trial will be conducted.

_____

6.5 Has human ethics approval been obtained? YES ○ NO ○

Approval # _____

7.0 Animal Experiments

7.1 Will any of the agents listed be used in live animals? YES ⊗ NO ○
If NO, please proceed to section 8.0

7.2 Name of animal species to be used: Mouse Bone Marrow-derived cells to be

7.3 AUS protocol # 2003-001-01 transplanted into Spinal Cord injured mice.

7.4 If using murine cell lines, have they been tested for murine pathogens? YES ⊘ NO ○

8.0 Use of Animal species with Zoonotic Hazards

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

- Pound source dogs YES ○ NO ⊘
- Pound source cats YES ○ NO ⊘
- Sheep or goats YES ○ NO ⊘
- Non- Human Primates YES ○ NO ⊘

If YES specify species _____

- Wild caught animals YES ○ NO ○

If YES specify species _____

9.0 Biological Toxins

9.1 Will toxins of biological origin be used? YES ○ NO ⊘
If NO, please proceed to Section 10.0
If YES, please name the toxin _____

9.2 What is the LD_{50} (specify species) of the toxin? _____

10.0 Import Requirements

10.1 Will the agent be imported? YES ⊘ NO ○
If NO, please proceed to Section 11.0
If YES, country of origin United Kingdom

10.2 Has an Import Permit been obtained from HC for human pathogens? YES ● NO ○

10.3 Has an import permit been obtained from CFIA for animal pathogens? YES ⊘ NO ○

10.4 Has the import permit been sent to Biosafety Subcommittee Chair? YES ● NO ○

If YES, Permit # PHAC P-13551, CFIA-A-2006-03344-4

??? In-Progress RN

11.0 Training Requirements for Personnel Named on Form

All personnel named in section 1.0 of this form who will be using any of the above named agents are required to attend the following training courses given by OH&S.

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS

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 2.0 to 10.0 have been trained as required.

SIGNATURE _____

12.0 Containment Levels

12.1 For the work described in sections **2.0** to **10.0**, select the highest HC or CFIA Containment Level required. 1○ 2◉ 3○

12.2 Has the facility been certified by Biosafety Subcommittee Chair for this level of containment?

YES ◉ NO ○

If **YES**, give date: February 02, 2007 and permit number: 2007-01-(2-11-1)

13.0 Approvals

G.M. Kelly 2 April 2008

Robarts Research Institute

Signature _____ Date Jan 8/07

Biosafety Officer for the Institution where experiments will take place

Signature _____ Date _____

Biosafety Officer of Robarts Research Institute (if different than above)

Signature _____ Date _____

Note: This permit will be in effect from _____ to _____,

subject to annual facility re-certification.