Modification Form for Permit BIO-UWO-0003

Permit Holder: Daniel Belliveau

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

~~Mandeep Sidhu~~

~~Vishal Gupta~~

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	~~Mouse retrovirus (VSV-G), Adenovirus (recombin. deficient), E.coli: dH5alpha, JM109~~	
Approved Primary and Established Cells	~~Rodent (primary): nervous tissue. Human (established): SHSY5Y, IMR-32, SKNMC, HEK 293. Rodent (established): PC12, N2A.~~	mouse or rodent tissues (human later)
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	~~[plasmids]: pCMV-6. [viral vector]: Retrovirus (VSV-G)~~	
Approved Use of Animals	~~Rat~~	
Approved Biological Toxin(s)		

** 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 STORED, USED AND DISPOSED OF..*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: _____

Current Classification: 2 **Containment Level for Added Biohazards:** _____

Date of Last Biohazardous Agents Registry Form: Feb 18, 2011

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____ **Date:** _____

Research do be performed in the labs MSB475 and MSB479
While there have been significant advances in developing 3D teaching tools for anatomy, the teaching of histology on the basis of 3D reconstructions has not received equal attention. The work to be done in the lab involves the processing of perfusion-fixed animal tissues for serial histological sectioning and staining. Although human tissues will be used in the future the initial project will use tissues obtained from small mammals. Mouse or rat tissues will be obtained from collaborators that use these animals for other research purposes. Tissues will be fixed by perfusion in a solution containing 2% formaldehyde and 2.5% glutaraldehyde and processed for embedding in Epon 812 (lab M475). This will involve the use of serial dilutions of ethanol and propylene oxide. Since tissue integrity is essential for 3D reconstruction from serial sections, complete semi-thin (1 μm thick) serial sections will be obtained with a Histo Jumbo diamond knife and a Reichert/Jung Ultracut E microtome (lab M479). Sections will be transferred to microscope slides and stained with Methylene blue and azure II, a polychromatic stain that has been shown to give outstanding differential staining of various cellular components in plastic embedded tissues (lab M475). Stained sections will be photographed using a 40X lens on a Light Microscope equipped with a digital color camera. Cellular structures of interest will be marked and outlined and 3D volume rendering will be performed using the digital animation software in Amira.

Alcohols and organic solvents, as well as fixatives such as glutaraldehyde and formaldehyde, will be stored and disposed of according to WHMIS and Biosafety Guidelines.

The lab facility in DSB00060 has been vacated by Drs. Belliveau and Sandig.