

*The University of Western Ontario*  
**BIOLOGICAL AGENTS REGISTRY FORM**  
Approved Biohazards Subcommittee: October 14, 2011  
Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://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, 1<sup>st</sup> edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to [jstanle2@uwo.ca](mailto:jstanle2@uwo.ca)) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or [biosafety@uwo.ca](mailto: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/](http://www.uwo.ca/humanresources/biosafety/).

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

**If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.**

|                            |                                                             |
|----------------------------|-------------------------------------------------------------|
| PRINCIPAL INVESTIGATOR:    | <b>Hiran Perinpanayagam</b>                                 |
| DEPARTMENT:                | <b>Dentistry</b>                                            |
| ADDRESS:                   | <b>DSB 0079</b>                                             |
| PHONE NUMBER:              | <b>ext. 82230</b>                                           |
| EMERGENCY PHONE NUMBER(S): | <b>519-317-1018</b>                                         |
| EMAIL:                     | <b><a href="mailto:hperinpa@uwo.ca">hperinpa@uwo.ca</a></b> |

Location of experimental work to be carried out :

|            |            |          |             |
|------------|------------|----------|-------------|
| Building : | <b>DSB</b> | Room(s): | <b>0031</b> |
| Building : |            | Room(s): |             |
| Building : |            | Room(s): |             |

**\*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: **DENTSPLY Tulsa Dental Specialties: Research Contract Agreement**  
GRANT TITLE(S): **Research on the Cellular Response to MTA**  
UNDERGRADUATE COURSE NAME(IF APPLICABLE):

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

| <u>Name</u>         | <u>UWO E-mail Address</u>                               | <u>Date of Biosafety Training</u> |
|---------------------|---------------------------------------------------------|-----------------------------------|
| <b>Nicholas Hou</b> | <b><a href="mailto:nhou2@uwo.ca">nhou2@uwo.ca</a></b>   | <b>27-9-11</b>                    |
| <b>Merwa Amer</b>   | <b><a href="mailto:mamer3@uwo.ca">mamer3@uwo.ca</a></b> | <b>6-2-12</b>                     |
|                     |                                                         |                                   |
|                     |                                                         |                                   |
|                     |                                                         |                                   |
|                     |                                                         |                                   |

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

Experimental surfaces will be rinsed twice with ethanol (70%), washed thrice with phosphate buffered saline (PBS) and then placed into individual wells of a 24-well tissue culture plate. The entire plate of disks will then be placed into a tissue culture hood and exposed to UV light (30 min) on each side to ensure sterility. Human embryonic palatal mesenchymal cells (HEPM, ATCC CRL-1486) will be seeded onto PPC and cpTi disks in multiple 24-well tissue culture plates (50,000 cells/well). The cultures will be maintained in Dulbecco's modified eagles medium (DMEM) supplemented with fetal bovine serum (FBS, 10%), L-glutamine (2  $\mu$ mol/ml), penicillin G (100 U/ml), streptomycin sulfate (100  $\mu$ g/ml) and amphotericin B (0.25  $\mu$ g/ml).

The tissue culture plates will be decontaminated by autoclaving according to the UWO Standard Operating Procedures posted at each autoclave. The steam must be saturated, penetrate the load and the temperature must reach a minimum of 121C (250F) for a minimum of 15 minutes. The material will then be disposed of as solid waste, by placing the autoclave bag in a clear garbage bag specifying biohazard waste or an opaque garbage bag with a label affixed indicating that the bag contains treated biomedical waste.

Changes to this page



**Please include a ONE page research summary or teaching protocol in lay terms.  
Forms with summaries more than one page will not be reviewed.**

Titanium implant osseointegration is dependent on the cellular response to surface modifications and coatings. Titania-enriched nano-composite polymeric resin coatings were prepared through the application of advanced ultrafine powder coating technology. Their surfaces were readily modified to create nano-rough (<100nm) surface nano-topographies that supported human embryonic palatal mesenchymal (HEPM) cell responses. Energy dispersive x-ray spectroscopy (EDX) confirmed continuous and homogenous coatings with a similar composition and even distribution of titanium. Scanning electron microscopy (SEM) showed complex micro-topographies, and atomic force microscopy (AFM) revealed intricate nano-features and surface roughness. Cell counts, mitochondrial enzyme activity (MTT), SEM and inverted fluorescence microscopy showed a marked increase in cell attachment, spreading, proliferation and metabolic activity on the nano-structured surfaces. RT-PCR analysis showed that type I collagen and Runx2 expression were induced, and Alizarin red staining showed that mineral deposits were abundant in the cell cultures grown on nano-surfaces. This enhancement in human mesenchymal cell attachment, growth and osteogenesis were attributed to the nano-sized surface topographies, roughness and moderate wetting characteristics of the coatings. Their dimensional similarity to naturally occurring matrix proteins and crystals, coupled with their increased surface area for protein adsorption, may have facilitated the response. Therefore, this application of ultrafine powder coating technology affords highly biocompatible surfaces that can be readily modified to accentuate the cellular response.

## 1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☐ YES ☒ 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? ☐ YES ☐ NO

If YES, please give the name of the species \_\_\_\_\_

What is the origin of the microorganism(s)? \_\_\_\_\_

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

*Please attach the CFIA permit.*

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

| Full Scientific Name of Biological Agent(s)*<br>(Be specific) | Is it known to be a human pathogen?<br>YES/NO               | Is it known to be an animal pathogen?<br>YES/NO             | Is it known to be a zoonotic agent?<br>YES/NO               | Maximum quantity to be cultured at one time? (in Litres) | Source/ Supplier | PHAC or CFIA Containment Level                                                                                  |
|---------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|----------------------------------------------------------|------------------|-----------------------------------------------------------------------------------------------------------------|
|                                                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |                                                          |                  | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |
|                                                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |                                                          |                  | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |
|                                                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |                                                          |                  | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |
|                                                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |                                                          |                  | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |
|                                                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |                                                          |                  | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |
|                                                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |                                                          |                  | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |
|                                                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |                                                          |                  | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |

*\*Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:*  
[http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA\\_Ecoli\\_list.pdf](http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf)

Additional Comments: \_\_\_\_\_

## 2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ 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             | <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No |                                       | Not applicable      |
| Rodent            | <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No |                                       |                     |
| Non-human primate | <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No |                                       |                     |
| Other (specify)   | <input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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             | <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No | CRL-1486 HEPM          | 1                                   | ATCC                              |
| Rodent            | <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No |                        |                                     |                                   |
| Non-human primate | <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No |                        |                                     |                                   |
| Other (specify)   | <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No |                        |                                     |                                   |

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

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

Additional Comments: \_\_\_\_\_

## 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 Infected With An Infectious Agent?<br>YES/UNKNOWN | Name of Infectious Agent (If applicable) | PHAC or CFIA Containment Level (Select one)                                                                     |
|--------------------------------------------|-------------------------------|----------------------------------------------------------------------------|------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| Human Blood (whole) or other Body Fluid    |                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> Unknown           |                                          | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |
| Human Blood (fraction) or other Body Fluid |                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> Unknown           |                                          | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |
| Human Organs or Tissues (unpreserved)      |                               | <input type="checkbox"/> Yes<br><input type="checkbox"/> Unknown           |                                          | <input type="checkbox"/> 1 <input type="checkbox"/> 2<br><input type="checkbox"/> 2+ <input type="checkbox"/> 3 |
| Human Organs or Tissues (preserved)        |                               | Not Applicable                                                             |                                          | Not Applicable                                                                                                  |

Additional Comments: \_\_\_\_\_



#### 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 Transformed or Transfected | Will there be a change due to transformation of the bacteria? | Will there be a change in the pathogenicity of the bacteria after the genetic modification? | What are the consequences due to the transformation of the bacteria? |
|-----------------------------|---------------|-------------------|---------------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
|                             |               |                   |                                 |                                                               |                                                                                             |                                                                      |

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

*\*\* Please attach a plasmid map.*

*\*\*\*No Material Safety Data Sheet is required for the following strains of E. coli:*

[http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA\\_Ecoli\\_list.pdf](http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf)

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

☐ YES, complete table below ☐ 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.*

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☐ NO

#### 5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: \_\_\_\_\_

## 6.0 Human Gene Therapy Trials

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

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

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

## 7.0 Animal Experiments

7.1 Will live animals be used? ☐ YES ☒ NO If NO, please proceed to section 8.0

7.2 Name of animal species to be used

7.3 AUS protocol #

7.4 List the location(s) for the animal experimentation and housing.

7.5 Will any of the agents listed in section 4.0 be used in live animals  
☐ NO ☐ YES, specify:

7.6 Will the agent(s) be shed by the animal:  
☐ YES ☐ NO, please justify:

## 8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If NO, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☐ NO

8.3 If YES, please specify the animal(s) used:

|                             |                                                  |                             |
|-----------------------------|--------------------------------------------------|-----------------------------|
| ◆ Pound source dogs         | <input type="checkbox"/> YES                     | <input type="checkbox"/> NO |
| ◆ Pound source cats         | <input type="checkbox"/> YES                     | <input type="checkbox"/> NO |
| ◆ Cattle, sheep or goats    | <input type="checkbox"/> YES, species            | <input type="checkbox"/> NO |
| ◆ Non-human primates        | <input type="checkbox"/> YES, species            | <input type="checkbox"/> NO |
| ◆ Wild caught animals       | <input type="checkbox"/> YES, species & colony # | <input type="checkbox"/> NO |
| ◆ Birds                     | <input type="checkbox"/> YES, species            | <input type="checkbox"/> NO |
| ◆ Others (wild or domestic) | <input type="checkbox"/> YES, specify            | <input type="checkbox"/> NO |

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

## 9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☐ YES ☒ NO If **NO**, please proceed to Section 10.0

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

9.3 What is the LD<sub>50</sub> (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time\*?

9.5 How much of the toxin or hormone is stored\*?

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☐ NO  
If **YES**, Please provide details:

\*For information on biosecurity requirements, please see:

[http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity\\_Requirements.pdf](http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf)

Additional Comments: \_\_\_\_\_

## 10.0 Insects

10.1 Do you use insects? ☐ 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:  
☐ "One-time" use, give location:

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

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



## 11.0 Plants

- 11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0
- 11.2 If YES, please give the name of the species.
- 11.3 What is the origin of the plant?
- 11.4 What is the form of the plant (seed, seedling, plant, tree...)?
- 11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use
- 11.6 Do you do any modifications to the plant? ☐ YES ☐ NO  
If yes, please describe:
- 11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
- 11.8 Is the CFIA permit attached? ☐ YES ☐ NO  
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

## 12.0 Import Requirements

- 12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO  
If **NO**, please proceed to Section 13.0
- 12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO
- 12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? ☐ YES ☐ NO
- 12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

## 13.0 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.

**An X in the check box indicates you agree with the above statement... ☒**  
**Enter Your Name** Hiran Perinpanavagam **Date:** February 1, 2012

#### 14.0 Containment Levels

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

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

☐ YES, location and date of most recent biosafety inspection:

☐ NO, please certify

☒ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **B10-UWO-0240**

#### 15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO

If YES please describe:

15.2 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:

**1. Secure the scene. 2. Provide medical First Aid. 3. Complete Accident/Incident Report Form. 4. Identify contributing factors & corrective actions. 5. Distribute Form to OHS, Chair, Student/Employee, Supervisor.**

15.3 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.shs.uwo.ca/workplace/workplacehealth.html>

**An X in the check box indicates you agree with the above statement...** ☒

**Enter Your Name** Hiran Perinpanayagam **Date:** Feb 1<sup>st</sup>, 2012

15.4 Additional Comments: \_\_\_\_\_

#### 16.0 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:



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Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's [Material Transfer Agreement](#) or, in certain cases, an MTA specified by the depositing institution.

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## Cell Biology

|                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                 |                                               |            |
|----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|-----------------------------------------------|------------|
| ATCC® Number:                    | CRL-1486™                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | <a href="#">Order this Item</a> | Price:                                        | \$363.00   |
| Designations:                    | HEPM                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                 | Depositors:                                   | M Macy     |
| <a href="#">Biosafety Level:</a> | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                 | Shipped:                                      | frozen     |
| Medium & Serum:                  | <a href="#">See Propagation</a>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                 | Growth Properties:                            | adherent   |
| Organism:                        | Homo sapiens (human)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                 | Morphology:                                   | fibroblast |
| Source:                          | Tissue: palatal mesenchyme                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                 |                                               |            |
| Permits/Forms:                   | In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                 |                                               |            |
| Isolation:                       | Isolation date: July, 1979                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                 | <a href="#">Related Cell Culture Products</a> |            |
| Applications:                    | transfection host( <a href="#">Roche FuGENE® Transfection Reagents</a> )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                 |                                               |            |
| Receptors:                       | epidermal growth factor (EGF)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                 |                                               |            |
| DNA Profile (STR):               | Amelogenin: X<br>CSF1PO: 10,11<br>D13S317: 8,12<br>D16S539: 11,12<br>D5S818: 11,13<br>D7S820: 8,10<br>TH01: 6,9,3<br>TPOX: 8,11<br>vWA: 17,18                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                 |                                               |            |
| Cytogenetic Analysis:            | This is a human diploid cell line with the 46,XX karyotype. The modal chromosome number was 46, occurring in 76% of cells. The rate of cells with polyploidies was 4.2%. No consistent chromosome aberrations were detected.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                 |                                               |            |
| Age:                             | fetus                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                 |                                               |            |
| Gender:                          | female                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                 |                                               |            |
| Comments:                        | The cells are highly responsive to epidermal growth factor (EGF).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                 |                                               |            |
| Propagation:                     | <b>ATCC complete growth medium:</b> 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%.<br><b>Temperature:</b> 37.0°C                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                 |                                               |            |
| Subculturing:                    | <b>Protocol:</b><br><ol style="list-style-type: none"><li>1. Remove and discard culture medium.</li><li>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.</li><li>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).<br/>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.</li><li>4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.</li><li>5. Add appropriate aliquots of the cell suspension to new culture vessels.</li><li>6. Incubate cultures at 37°C.</li></ol><br><b>Subcultivation Ratio:</b> A subcultivation ratio of 1:3 to 1:6 is recommended<br><b>Medium Renewal:</b> 2 to 3 times per week |                                 |                                               |            |
| Preservation:                    | <b>Freeze medium:</b> Complete growth medium supplemented with 5% (v/v) DMSO<br><b>Storage temperature:</b> liquid nitrogen vapor phase                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                 |                                               |            |
| Related Products:                | Recommended medium (without the additional supplements or serum described under ATCC Medium): <a href="#">ATCC 30-2003</a>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                 |                                               |            |



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recommended serum: ATCC [30-2020](#)

**References:**

1067: Yoneda T, Pratt RM. Mesenchymal cells from the human embryonic palate are highly responsive to epidermal growth factor. Science 213: 563-565, 1981. PubMed: [7017936](#)  
1068: Yoneda T, Pratt RM. Interaction between glucocorticoids and epidermal growth factor in vitro in the growth of palatal mesenchymal cells from the human embryo. Differentiation 19: 194-198, 1981. PubMed: [6458523](#)  
58036: Yoneda T, Pratt RM. Glucocorticoid receptors in palatal mesenchymal cells from the human embryo: relevance to human cleft palate formation. J. Craniofacial Genet. Dev. Biol. 1: 411-423, 1981.  
58037: Pratt RM, et al. Prescreening for environmental teratogens using cultured mesenchymal cells from the human embryonic palate. Teratog. Carcinog. Mutagen. 2: 313-318, 1982. PubMed: [6130630](#)

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----- Original Message -----

**Subject:**Re: Biological Agents Registry Form: Perinpanayagam

**Date:**Wed, 14 Mar 2012 08:37:38 -0400

**From:**Hiran Perinpanayagam <hperinpa@uwo.ca>

**To:**Jennifer Stanley <jstanle2@uwo.ca>

New Info

Jennifer

I've added the following paragraph to page 2 (attached):

**The tissue culture plates will be decontaminated by autoclaving according to the UWO Standard Operating Procedures posted at each autoclave. The steam must be saturated, penetrate the load and the temperature must reach a minimum of 121C (250F) for a minimum of 15 minutes. The material will then be disposed of as solid waste, by placing the autoclave bag in a clear garbage bag specifying biohazard waste or an opaque garbage bag with a label affixed indicating that the bag contains treated biomedical waste.**

Please let me know if that satisfies the requirements, or if there's anything else that I need to address.

Hiran

**Subject:** Re: Bernards BARF

**From:** Jennifer Stanley <jstanle2@uwo.ca>

**Date:** 3/19/2012 4:47 PM

**To:** skoval@uwo.ca, John Millar <jsmillar@uwo.ca>

**CC:** Sintia Xhiku <sxhiku@uwo.ca>

Thanks! We'll sign it for approval!  
Jennifer

On 3/19/2012 4:46 PM, Susan Koval wrote:

Hi Jennifer,

I reviewed Mark Bernard's BARF - the revised section on use and storage of biological agents. The revised version is well-written and clearly explains what the lab intends to do/does with bacteria and fungi.

Regards,  
Susan

Sintia  
Please include  
e-mail info from Dr.  
Koval in the  
minutes...  
Jen