School of Engineering and Technology, IUPUI
Multidisciplinary Undergraduate Research Institute (MURI)

MURI Mentors Project Application Form

For fall 2005

- *To be completed by a Mentor Faculty Member or Researcher from the School of Engineering and Technology.*
- *To fill in this form place your cursor in the form field and type. Tab from field to field.*

Date: **August 9, 2005**

Name of Proposer: **Hazim El-Mounayri**[1]

Title of Proposer: **Associate Professor**

Department of Proposer: **Mechanical Engineering**

Proposed Project Title: **Biomechanics of smooth muscle cell differentiation: Experimental study using an innovative in vitro mechanical system**

Approximate Duration: **Six months**[2]

Number of Students Requested: **Two (one from Mechanical Engineering and one from the School of Medicine)**

Disciplines or Majors Involved (at least two disciplines): **Engineering & Medicine**

Support Needed from MURI for Supplies and Equipment Usage ($2,000 limit per team): **$1,924**

Project proposal with sections for the following information (please attach or cut and paste into this form):

1) Rationale & Objectives

In the human body, smooth muscle cells that are found in blood vessels are very different from those found in the bladder. This distinction is not seen when culturing the cells in a laboratory environment; rather the cells remain "generic" smooth muscle cells that have

[1] In collaboration with the department of physiology (O. El-Mounayri and Dr. P. Herring)
[2] Additional funding is available from the department of physiology to support the research project beyond the four months for which the MURI grant is pursued

not differentiated to become specific to any certain region within the human body. It is hypothesized that if the cells were cultured in an environment that is more similar to a specific region within the body, then they may differentiate to become specific to that region of the body. However, most of the current knowledge of smooth muscle cell biology comes from studies conducted on cells grown in stationary environment. On the other hand, previous studies have indicated an important role of environmental factors to maintain smooth muscle specific shape (phenotype) and there is growing evidence that the mechanical force perceived by smooth muscle cells is one of the determinants of smooth muscle cell phenotype and function. The development of a system that would allow the study of smooth muscle specific gene expression under dynamic condition is critical to the understanding of the mechanism of smooth muscle cell differentiation and provides the basis for elucidating smooth muscle cell related diseases such as atherosclerosis, restenosis and asthma.

The proposed work aims at developing such a system (or mechanical device) as well as using the latter to experimentally stimulate cell differentiation by applying dynamic (cyclic) loading with different intensities and at different frequencies. More specifically, the following objectives will be pursued.

1. To develop a device that is capable of stimulating smooth muscle cells in a manner that is similar to the stimuli within the human body. The device should be capable of creating a strain within the laboratory cultured cells that is similar to various regions within the body.

2. To use the device to study the biomechanics of smooth muscle cells differentiation. The substrates will be subjected to variable cyclic loading and the response in terms of the level of cell differentiation observed/measured.

2) Research Methodology

First, a design concept that was developed in the summer of 2005 (Figure 1) will be refined and the single-unit product expanded into a complete multi-well apparatus with control system and user interface.

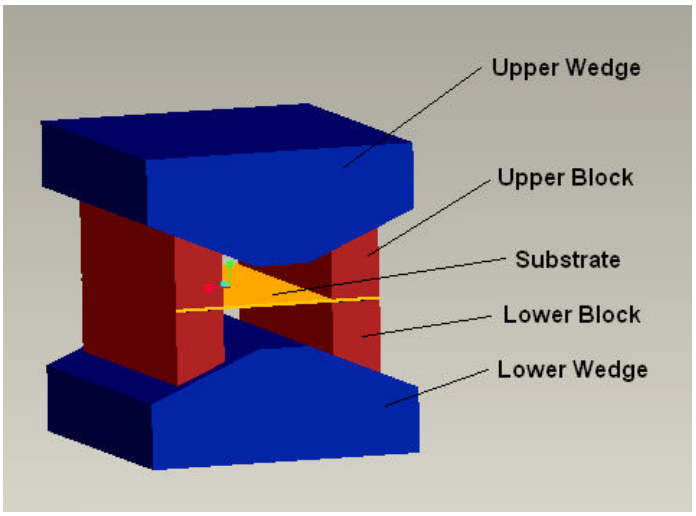

Figure 1: Concept developed in summer of 2005 [1]

Next, the following experimental study will be conducted:

1. Flexible substrates such as collagen or small intestinal submucosa also known as SIS will be first tested for uniform deformation using strain gages. SIS is an **acellular** biomaterial used to treat a wide range of conditions including dermal wounds and burns, reinforcement of many types of weakened tissue, and treatment of urinary incontinence (produced by Cook Company) and is also known to be a good substrate for smooth muscle cell.

2. Primary smooth muscle cells isolated from different mouse tissues in high glucose DMEM medium containing 5U/ml penicillin/ 50µg/ml streptomycin and 10% fetal bovine serum, will be seeded on flexible substrate.

3. Primary smooth muscle cells cultured on flexible substrate will be incubated at 37°C in static condition or under defined strains.

4. The level of smooth muscle specific proteins in both static and dynamic conditions will be determined by Western blotting and the change of smooth muscle specific gene induction will be determined by real time reverse transcription coupled with PCR.

The cyclic loading will be varied in terms of magnitude and frequency. In fact, the stresses that are placed on blood vessel smooth muscle cells would clearly be different than those placed on the cells that line the bladder. These will vary in frequency as well as magnitude. As for the difference in frequency, it can easily be seen that the blood vessel cells have stresses imposed upon them at a frequency that is equal to the pulse. On the other hand, the cells that can be found in the bladder may have a constant increasing stress as the bladder is filling. This would be followed by a quick release of stress as the bladder is relieved.

3) Team Organization

The team will consist of an undergraduate student from the Mechanical Engineering Department working under the supervision of Dr. H. El-Mounayri and an undergraduate student from the Department of Physiology who will be mentored by O. El-Mounayri (senior PhD student) and advised by Dr. P. Herring (Department of Celullar and Integrative physiology). The mechanical engineering department will work closely with the team members from the school of medicine to design, test and validate a cyclic loading device and establish its mechanical properties. Initially, the device will be used to apply defined strains such as static, cyclic or stepwise to cells grown on an elastic membrane. The team members from the school of medicine will isolate smooth muscle cells from different tissues, grow them on flexible substrate and analyze the phenotype of the cells exposed to different mechanical loading as well as examine the level of smooth muscle specific genes expression.

Specific Students Tasks:

A. Tasks to be completed by undergraduate student from Physiology department

a. Conducting pilot experiments to examine the efficiency by which different cultured cells (smooth muscle and nonmuscle cells) attach to various flexible substrates under static or mechanically loaded conditions.
b. Isolating primary smooth muscle and nonmuscle cells from mouse tissues. Cells will be seeded at various densities on flexible substrate in order to identify the optimal density required to achieve cell confluence 5-7 days following plating.
c. Culturing primary cells or cell lines (smooth muscle and nonsmooth muscle) on the substrate (once optimal plating density is determined) which are then maintained either under static conditions or subjected to mechanical loading. Mechanical loading will be performed at different magnitude and frequency.
d. Examining cultured smooth muscle and nonmuscle cells by immunohistochemistry and western blotting to assess morphology and proliferation both under static and variable cyclic loading conditions.
e. Conducting experiments 3 times for each condition and each time student will be comparing results from static condition to the dynamic conditions.

B. Tasks to be completed by undergraduate student from Mechanical Engineering department:

a. Extending the design concept that was developed in the summer of 2005 (Figure 1) from a single-unit product to a complete multi-well apparatus
b. Validating the device.
c. Measuring the deformation in the substrates (using strain gages) and establishing their mechanical properties.
d. Adding a control system and user interface
e. Wiring out the device to the incubator and testing the complete system

4) Expected Outcomes

1. A device that is capable of stimulating smooth muscle cells in a manner that is similar to the stimuli within the human body. The device would be capable of creating a strain within the laboratory cultured cells that is similar to various regions within the body.

2. A better understanding of the mechanism of smooth muscle cell differentiation; more specifically, the effect of dynamic loading on the differentiation process.

5) Benefits

The development of a system that permits the study of smooth muscle specific gene expression under dynamic condition is critical to the understanding of the mechanism of smooth muscle cell differentiation and *provides the basis for elucidating smooth muscle cell related diseases such as atherosclerosis, restenosis and asthma.*

6) Time-Table

Task / Month	*1*	*2*	*3*	*4*	*5*	*6*
Extending the single unit product into a complete multi-well apparatus						
Adding interface and control system.						
Testing the system						
Testing Flexible substrates for uniform deformation using strain gages						
Primary smooth muscle cells isolated and cultured						
Conducting testing under different dynamic conditions						
Final report						

7) Justification of Budget for Equipment and Supplies

Items	*Cost*	***Budget needed from MURI***	*Cost sharing from the department of cellular and integrative physiology*
Tooling, material (stainless steel), and labor for the six stretching units; Actuators	1266	**$1,266**	
Strain gauges	$60	**$60**	
Load cell	$598	**$598**	
Substrates, tissue culture, western blotting equipment and reagents, reagent to extract messenger RNA for realtime RT-PCR as well as realime RT-PCR supplies and reagent, and disposable plasticware	$5,000		$5,000
Total	$6,924	**$1,924**	$5,000

8) Short Resume. *See attachment.*

9) References:

B. Snyder, B. Donelson, O. El-Mounayri, P. Herring, and H. El-Mounayri, "Smooth Muscle Cell Stretching Device", Technical report, ME 91 – Engineering Project, ME department, IUPUI, Summer 2005.

Hazim A. El-Mounayri, Associate Professor of Mechanical Engineering
Principal Investigator

Dr. El-Mounayri has been conducting research in manufacturing process control, including modeling, simulating and optimizing the machining process. He developed empirical, semi-empirical and AI-based models for accurate prediction and optimization of milling. The developed models and optimization techniques are integrated into the current state-of-the-art CAD/CAM technology to produce enhanced systems that are readily available for industry. The developments include both physical models and geometric models. The geometric simulators are implemented using standard open architecture solid modeling kernels. CAD/CAM is also used to conduct applied R&D projects as well as training to the local industry. Dr. El-Mounayri has worked as consultant for a number of companies on the utilization and customization of CAD/CAM technology for process/product modeling and simulation, especially for manufacturing applications. He has published over 40 technical papers and gave presentations at various national and international conferences. Dr. El-Mounayri is currently conducting research in virtual manufacturing and intelligent machining which aims at developing the Advanced Virtual Manufacturing Laboratory for Training, Education and Research (AVML). The new initiative is supported by federal and state funds. Dr El-Mounayri's research interest include Advanced manufacturing, intelligent machining, manufacturing process modeling, simulation and control, Virtual manufacturing, CAD/CAM enhancement, and Solid modeling techniques and applications.

Selected Publications

1. H. El-Mounayri, H. Kishawy & J. Briceno, "Optimization of CNC Ball End Milling: A Neural Network-based Model", accepted for publication in the Journal of Materials Processing Technology, May 2004
2. H. El-Mounayri, J. Briceno, and M. Gadallah, "A New Artificial Neural Network Approach to Modeling Ball-End Milling", *International Journal of Advanced Manufacturing Technology*, 2003.
3. V. Tandon, H. El-Mounayri, and H. Kishawy, "NC End Milling Optimization Using Evolutionary Computation", *International Journal of Machine Tools and Manufacture*, v. 42, pp. 595-605, 2002.
4. H. El-Mounayri, H. Kishawy, and V. Tandon, "Optimized CNC End Milling: A Practical Approach", *International Journal of Computer Integrated Manufacturing*, v. 15, pp.453-470, 2002.
5. El-Mounayri, D. Aw, T. Wasy, and A. Wasfy "Virtual CNC machining: A comprehensive approach", Accepted for presentation at the ICC&IE 2005.
6. Hazim El-Mounayri, Daniel Aw, Tamer Wasfy and Ayman Wasfy, "Virtual Advanced Manufacturing Laboratory for Training and Education" , 2005 ASEE Annual Conference, Portland OR, June 12-14, 2005.
7. H. El-Mounayri, H. Deng, and S. Mukhopadhyay, "A Neural network Model for Integrated Modeling and Optimization of CNC End Milling", submitted to NAMRCXXXI, McMaster University, Hamilton, ON, Canada, May 20-23, 2003.
8. H. El-Mounayri, H. Deng, and S. Mukhopadhyay, "A Neural network Model for Integrated Modeling and Optimization of CNC End Milling", NAMRCXXXI, McMaster University, Hamilton, ON, Canada, May 20-23, 2003.
9. H. El-Mounayri, Z. Dugla and H. Deng, "Predicting Surface Roughness in End Milling Using an Innovative Technique from Evolutionary Computation", accepted for presentation in Symposium on Advances to Further the Automation of Metal Removal Processes at the International Mechanical Engineering Congress and Exposition (IMECE 2002), ASME, Nov. 2002.

Principal Research Projects

1. SBIR/STTR Phase II: Advanced Virtual Manufacturing Lab for Research, Training, and Education, NSF, $500,000, Academic Investigator (IUPUI share: 30%), 06/05-06/07.
2. SBIR/STTR Phase I: Advanced Virtual Manufacturing Lab for Research, Training, and Education, Indiana 21st Century Fund, $100,000, Academic Investigator (IUPUI share: 48%), 12/04-06/05
3. SBIR/STTR Phase I: Advanced Virtual Manufacturing Lab for Research, Training, and Education, NSF, $100,000, Academic Investigator (IUPUI share: 30%), 12/03-12/04

4. Basic assessment of the rotary tool performance during dry hard machining, SME (Society of Manufacturing Engineers), $15,000, PI, 06/03-06/04.
5. Introduction of Pro/ENGINEER and Implementation of Computer-Aided Manufacturing Post-processors for Raytheon, Raytheon Systems Company, Indianapolis, IN, $30,000, PI, 07/00-07/01.

Herring, Brian, Paul
Associate Professor
Department of Cellular and Integrative Physiology

Co-Investigator

Selected Publications

1. **Herring, B.P.** and England, P.J. The turnover of phosphate bound to myosin light chain-2 in perfused rat heart. Biochem. J. (1986) **260**, 205-214.
2. **Herring, B.P.,** Nunnally, M.H., Gallagher, P.J. and Stull, J.T. Molecular characterization of rat skeletal muscle myosin light chain kinases. Am. J. Physiol. (1989) **256**, C399-404.
3. **Herring, B.P.,** Stull, J.T. and Gallagher, P.J. Domain characterization of rabbit skeletal muscle myosin light chain kinase. J. Biol. Chem. (1990) **265**, 1724-1730
4. **Herring, B.P.,** Fitzsimons, D.P., Stull, J.T. and Gallagher, P.J. Acidic residues comprise part of the myosin light chain binding site on skeletal muscle myosin light chain kinase. J. Biol. Chem. (1990) **265**, 16588-16591
5. **Herring, B.P.** Basic residues are important for Ca^{2+}/calmodulin binding and activation but not autoinhibition of rabbit skeletal muscle myosin light chain kinase. J. Biol. Chem. (1991) **266**, 11838-11841
6. **Herring, B.P.** Gallagher, P.J. and Stull, J.T. Substrate specificity of myosin light chain kinases. J. Biol. Chem. (1992) **267**, 25945-25950
7. **Herring, B.P.** and Smith, A.F**.** Telokin expression is mediated by a smooth muscle cell-specific promoter. (1996) Am. J. Physiol. **270,** C1656-1665.
8. **Herring, B.P.** and Smith, A.F. Telokin expression in A10 smooth muscle cells requires serum response factor. (1997) Am. J. Physiol. **272**, C1394-C1404.
9. **Herring, B.P**., Hoggatt, A.M., Smith, A.F. and Gallagher, P.J. Targeted expression of SV40 large T-antigen to visceral smooth muscle induces proliferation of contractile smooth muscle cells and results in megacolon. (1999) J. Biol. Chem. **274**, 17725-17732
10. **Herring, B.P**., Dixon, S. and Gallagher, P.J. Smooth muscle myosin light chain kinase expression in cardiac and skeletal muscle. (2000) Am. J. Physiol:Cell Physiology **279,** C1656-C1664
11. **Herring, B.P**., Lyons, G.E., Hoggatt, A.M. and Gallagher, P.J. Telokin expression is restricted to smooth muscle tissues during mouse development. (2001). Am. J. Physiol. **280**, C12-C21
12. **Herring, B**.P. Kreigel, A.M. and Hoggatt, A.M. Identification of Barx2b, an SRF-associated homeodomain protein. (2001) J. Biol. Chem. **276**,14482-14489
13. El-Mounayri, O., Triplett, J.W., Yates, C.W. and **Herring, B.P**. Regulation of smooth muscle-specific gene expression by homeodomain proteins, Hoxa10 and Hoxb8. (2005) J. Biol. Chem. In press.

Research projects ongoing or completed in the last 3 years.

4/1/98-3/31/06. NIH RO1 HL75835
Telokin Gene Regulation in Smooth Muscle.
Annual direct costs $200,000 (PI)

9/30/01 - 8/31/06 NIH RO1 DK61130
Regulation of visceral smooth muscle-specific gene expression during development.
Annual Direct Costs $200,000 (PI)

02/01/04 – 01/33/07 NIH RO1 DK065644
Synthetic smooth muscle cell-selective promoters.
Annual Direct Costs $190,000 (PI)

Omar El-Mounayri, Senior PhD Student
Department of Cellular and Integrative Physiology

Co-Investigator

Publications
El-Mounayri, Omar, Evaluation of Left Ventricular Compliance and Stiffness during Pacing Induced Heart Failure and Recovery, M.S.B.M.E. Thesis, Purdue University, May 2002

Omar El-Mounayri, Jason Triplett, Charles W.Yates and B. Paul Herring, Regulation of telokin promoter activity by homeodomain proteins, HoxA10 and HoxB8 (2005) J. Biol. Chem

B. Paul Herring, Ketrija Touw, April Hoggatt, Julia Azriel, Jiliang Zhou, **Omar El-Mounayri**. Smooth muscle cell-restricted expression of telokin. Endothelium (2004), 10, 357 (Abstract)

Omar El-Mounayri, Jason Tripplet, B. Paul Herring. Regulation of telokin promoter activity by homeobox proteins. Endothelium (2004), 10, 357 (Abstract)

Paul Herring, Ketrija Touw, April Hoggatt, Julia Azriel, **Omar El-Mounayri,** Jiliang Zhou and Feng Yin. Mechanisms Of Smooth Muscle Cell-Restricted Gene Expression. Cardiovascular Pathology (2004) 13, Issue 3, Supplement 1, p12 (Abstract)

Applications for external fellowships
2004 Travel fellowship from depart of cellular and integrative physiology, IU medical

Meetings attended and oral / poster presentations
2003 Vascular Biology Retreat (Poster).
2003 Annual department of physiology retreat (Oral).
2004 North America Vascular Biology Organization Developmental Biology Workshop

2004 Annual department of physiology retreat (Oral).
2004 PhD thesis proposal defense
2004 Annual retreat poster: Molecular mechanism of GATA-6 dependent regulation of SM-specific telokin expression. F. Yin, J. Zhou**, O. El-Mounayri**, A. Hoggatt, P. Herring .

Distinctions and awards
2003 Moenkhaus physiology graduate fellowship.
Abstract selected for oral presentation in 2003 annual depart of physiology retreat (Cash

Abstract selected for oral presentation in 2004 annual depart of physiology retreat (Cash

2005 SigmaXi oral presentation selected for 2nd place (Cash Prize)

Teaching/seminars and Presentations
Department of cellular and Integrative Physiology, research in progress 2/18/04.
2004 SigmaXi oral presentation.

External Grants
7/1/05-6/30/07. AHA. Regulation of smooth muscle-specific genes by homeodomain proteins. $52,000 (26,000 annual direct cost), Omar El-Mounayri , Principal Investigator