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CENTER FOR RESEARCH AND LEARNING

INDIANA UNIVERSITY–PURDUE UNIVERSITY INDIANAPOLIS

Multidisciplinary Undergraduate Research Institute

# MURI Project Proposal Form

## Section I: Proposal Cover Page

Date of submission: _March 10, 2011_____

Proposed project title: __Interactions of Human Oral Cells with Oral Bacterial Cells___

| Principal Mentor | |
|---|---|
| Name: Richard L. Gregory, Ph.D. | Title: Professor |
| Phone number: 274-9949 | Email: rgregory@iupui.edu |
| Department: Oral Biology/Microbiology | School: Dentistry and Medicine |

| Co-mentor | |
|---|---|
| Name: L. Jack Windsor | Title: Associate Professor |
| Phone number: 274-1448 | Email: ljwindso@iupui.edu |
| Department: Oral Biology/Cell Biology | School: Dentistry |

| Co-mentor | |
|---|---|
| Name: Fengyu Song | Title: Assistant Professor |
| Phone number: 274-2415 | Email: fesong@iupui.edu |
| Department: Oral Biology/Regenerative Biology | School: Dentistry and Science |

Please note that preference will be given to projects that include mentors from multiple disciplines.

The Department of Oral Biology is a department with faculty from many different disciplines. Dr. Gregory is a microbiologist, Dr. Windsor is a cell biologist and Dr. Song works in the area of regenerative biology with dental pulp cells. This project calls upon each of these three distinct disciplines.

The project will be carried out and completed (check only one):

☐ Summer Program (June 1-July 29, 2011)

☒ Academic Year Program (October 5, 2011-April 30, 2012)

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## Section II: Student Request Page

Total number of students requested: _____5_____
(Note: The total number of students must exceed the number of mentors by two)

Total Number of freshmen and/or sophomores to be recruited: _____2-3_____

Disciplines or majors of students (preference will be given to projects that include at least two disciplines or majors): _Biology, Chemistry, Pre-Dental, Pre-Medical, SPEA, Liberal Arts__

Skills expected from students: __Good analytical skills, persistence and strong attention to detail
_____

_____

Names of students you request to work on this project.

(Mentors are invited to recommend students that they would prefer to work on the proposed project. Please provide an email address and a rationale; for example, a student may have an essential skill, may already be working on a similar project, or may be intending to apply to graduate school to pursue the same area of research.)

The Center for Research and Learning will consider the students requested below, but cannot guarantee placement of specific students on teams.

| **Name of Student:** | **Student's Email:** | **Rationale:** |
|---|---|---|
| 1) **Jessica Morgan** | **morgan28@umail.iu.edu** | **Already working on a similar project** |
| 2) **Charla McGough** | **cmcgough@imail.iu.edu** | **Already working on a similar project** |
| 3)_____ | _____ | _____ |
| 4)_____ | _____ | _____ |
| 5)_____ | _____ | _____ |
| 6)_____ | _____ | _____ |

## Section III: Body of Proposal

***(A maximum of 5 pages is allowed for answers to questions 1-10.)***

***Objectives:*** Smokers have increased periodontal disease (caused by the anaerobic *Porphyromonas gingivalis*) and dental caries (caused by the facultative anaerobe *Streptococcus mutans*). These bacteria are known to bind to or interact with gingival epithelial cells and gingival fibroblast cells-*P. gingivalis* contributing to periodontal disease and dental pulp cells-*S. mutans* causing dental root caries and endothelial cells-*S. mutans* associated with atherosclerosis. However, little is known about the effects of tobacco directly on these bacteria on their ability to affect these human cells and to cause disease. It is our hypothesis that tobacco up-regulates the expression of certain virulence genes and proteins to increase the pathogenic potential of *P. gingivalis* and *S. mutans*. **The objectives of this research are to investigate the effects that nicotine and tobacco-treated <u>bacterial cells</u> have on these human cells with respect to their release of pro-inflammatory cytokines and matrix metalloproteinases (MMP).** This will contrast with our 2011 summer proposal where students will focus on the effects of virulence factors from tobacco-treated bacteria secreted into **<u>culture supernatant</u>** on several human cell types and the subsequent release of pro-inflammatory cytokines and MMP. In addition, we will add analysis of endothelial cells. Each student will grow bacteria in the Gregory lab and then move after about 2-3 months to either the Windsor or Song labs to assess the effects of the bacterial cells on human cells.

***Significance:*** The project will begin to address the important roles these bacteria play in periodontal disease and caries of smokers. The long term goal is to develop treatment modalities to reduce the effects of smoking on periodontal disease and caries.

***Student Opportunity:*** Students will have the opportunity to be present at the beginning of this exciting project. They will be able to experience at least two different laboratories. We have not examined the effects of these bacterial cells on human oral cells previously and expect that this will lead to information that may elucidate additional mechanisms that the bacteria use in causing disease.

***Research Methodology:*** *S. mutans* UA159 and *P. gingivalis* ATCC 33277 will be incubated in Tryptic Soy broth (TSB) or modified Schaedler broth, respectively, with optimal concentrations of cigarette smoke condensate (CSC), nicotine and dissolvable smokeless tobacco (DST) in the Gregory lab. These optimum concentrations will be determined from previous student data by using the concentrations that increase certain bacterial virulence factors such as *S. mutans* growth, biofilm formation, and antigen I/II expression, and proteolytic activity for *P. gingivalis*. Typically, these concentrations are 0.25 mg of CSC/ml, 0.5 mg of nicotine/ml and 3.3% for DST. The cultures will be grown in the presence of the tobacco products for 24 h at 37$^{o}$C in 5% $CO_2$ and centrifuged at 10,000 x g for 15 min.

The cell pellet will be washed three times with sterile saline and resuspended in saline containing 0.05% formaldehyde to kill the bacteria. The cells will remain in formaldehyde for 3 days at room temperature and then washed three times to remove the formaldehyde. The cells will be standardized to 0.5 absorbance at 540 nm and stored at 4$^{o}$C until used.

The various *S. mutans* cells will be used in Dr. Song's lab to assess the ability of the *S. mutans* cells to affect human pulp cell release of pro-inflammatory and anti-inflammatory cytokines, as well as MMP expression. In addition, the effects of the *S. mutans* cells will be examined for the ability to affect pulp cell growth. It is hypothesized that *S. mutans* cells treated with tobacco will increase pro-inflammatory cytokines and MMPs over that untreated with tobacco. This will provide further confirmation of the effect of *S. mutans* and smoking on dental caries and periapical infections.

Human pulp cells were isolated from sound teeth extracted for orthodontic purpose with IUPUI IRB approval. The cells were maintained as cell lines in the laboratory and will be used between passage 3 to 8 for the following experiments. After the various number of *S. mutans* are incubated with 7.5 x $10^4$ human pulp cells/well of 6-well tissue culture plates for 72 h, the human pulp cells will be evaluated for their proliferation utilizing water-soluble tetrazolium salt-1 assay (WST-1, Roche Allied Science, Indianapolis, IN) following the manufacturer's instructions. The conditioned media will then be collected and resolved on zymogram gels containing 1 mg/ml of gelatin with or without any specific inhibitors in order to analyze the MMP expressions and activities under the influence of *S. mutans* cells. The cytokine expression will be evaluated by semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) after the RNA is collected from treated or untreated human pulp cells. The interaction between *S. mutans* and dental pulp cells will be evaluated by the Bactect staining (Invitrogen, Fazzi, 2002) following the manufacture protocol. The intensity of the fluorescent signal will be measured and analyzed under the microscope. The number of bacterial cells adhering to the human cells will be counted. The expression of major pro-inflammatory (IL1, TNFα, IL6, IL8, MIP-1, GRO and GROα) and anti-inflammatory (IL4, IL10, and IL13) cytokines will be examined by RT-PCR after collecting the total RNA from the treated and untreated pulp cells and using cytokine microarrays (RayBiotech). Each experiment will be performed at least three times and One-way ANOVA will be used to detect statistical differences between different treatment groups.

The various *P. gingivalis* cells will be used in Dr. Windsor's lab to assess the ability of the *P. gingivalis* cells to affect human gingival epithelial and gingival fibroblast cell release of pro-inflammatory and anti-inflammatory cytokines, as well as MMP expression. In addition, the effects of the *P. gingivalis* cells will be examined for the ability to affect epithelial and fibroblast cell growth. It is hypothesized that *P. gingivalis* cells treated with tobacco will increase pro-inflammatory cytokines and MMPs over that untreated with tobacco. This will provide further confirmation of the effects of *P. gingivalis* and smoking on periodontal disease. Identical methodology as described above for pulp cells will be used for human gingival epithelial, gingival fibroblast and

human umbilical vein endothelial cells (HUVEC). In addition, the effects of the tobacco-treated *P. gingivalis* cells on cell-mediated collagen degradation will be assessed.

***Team Communication and Organization:***

a. The Principal Mentor will take the lead in supervising students to initially identify the specific project they will work on and to coordinate the team meetings and ensure student attendance at all required research seminars and programs;
b. The Principal and Co-Mentor(s) will be available to provide students assistance in design of specific studies and experiments, to meet regularly with each student to plan experiments and analyze data and prepare research reports and presentations;
c. Graduate students and postdocs will train students in some of the laboratory assays;
d. All students in the project will grow bacteria in the Gregory lab and assess the effects of the bacterial cells in the Windsor or Song labs on cytokine and matrix metalloproteinase expression and measure their attachment.
e. All students will prepare their research for presentation in a poster or oral format.

These studies will complement others ongoing in the Tobacco Cessation and Biobehavioral Group. The proposed project seeks the pathophysiology of tobacco and its related chemicals on human oral tissues from multiple perspectives, such as microbiology, cell biology, molecular biology, biochemistry, immunology, and biobehavior and enables us to begin to identify the effects of blocking these tobacco-associated pathologies. Students will work closely with the three MURI faculty (Drs. R.L. Gregory, L.J. Windsor and F. Song), as well as with postdoctoral and Ph.D. students working on other tobacco-related projects. Students will meet individually with their MURI faculty at least 2-3 times a week as well as regular lab meetings to discuss experimental design and research data. MURI students working in the same lab are expected to collaborate on their projects as some of the same skills will be used in their projects. Many MURI mentors host social outings at their house to foster a greater feeling of teamwork. In addition, all students and MURI faculty will meet together once a month for each student to present his/her project and data. It is expected that all five MURI students will have a basic understanding of each others' projects. This will build camaraderie among the MURI students and provide them with a certain level of comfort in discussing their project to a group. All MURI students, dental students and PhD students meet every other Tuesday evening in our Student Research Presentation Program (SRPP) to take turns presenting their work in front of their peers as well as a few faculty members. This gives the MURI students feedback in an informal setting that is preferred over a larger format. Students approved for this project will be given background readings specific to the laboratories they will primarily be working in (see references 1-9 as examples) before their MURI project begins. Students from various backgrounds (Science-Biology, Chemistry, Pre-Dental and Pre-Medical, SPEA, Liberal Arts) are desired as it allows a diversification of ideas. All of the specific projects to be conducted by MURI students are multidisciplinary in nature. Many SPEA students would benefit because of their potential interest in environmental and health affairs

connected with a focus on tobacco in this project. Accepting students into this project from non-science backgrounds (Liberal Arts) allows them to understand a different area.

***Expected Outcomes and Benefits:*** Attempts have been made to understand the mechanism(s) of how tobacco influences human oral cells and microorganisms. The project is designed to systemically analyze those effects from different perspectives and be able to combine the results of the oral cells and bacteria together to have a complete picture regarding the tobacco's effects on the oral cavity. The results from this proposed project will be helpful to support ongoing projects in Tobacco Cessation and Biobehavioral Group, which are pilot studies needed for NIH grant applications in the near future. In addition, students will acquire sufficient knowledge to be conversant in their specific field of research and master the necessary skills to conduct research and present their work with the platform provided from this proposed project in the dental school's research environment. All students will be expected to present their research at all required MURI and/or UROP workshops, as well as our school's annual Research Day and the IUPUI campus Research Day held every April. We will ask the students to participate in the two Research Days even though they would have completed their research in the summer prior to Research Day because many of the students applying to our laboratories for research projects are pre-dental students. All students conducting research at our school participate in a biweekly SRPP and the MURI Fellows would be expected to present their work at the end of the summer research period. The projects conducted during the summer will significantly differ from our previous MURI projects in that these individual experiments will measure the effect of bacterial cells directly on the human cells. With the knowledge gained from this proposed project, information for understanding how tobacco affects the oral cavity can be obtained. Building on this knowledge, future strategies for intervention with tobacco on oral pathological conditions can be constructed. This proposed project will also help to build the base for future NIH grants. Moreover, students in this program would benefit by gaining important research skills such as enhancing their knowledge of the scientific method and other skills specific to their projects as well as interact with the MURI faculty and other associated research personnel (graduate students and technicians). One of the tangible benefits will be that each student will be able to record at least one published abstract (MURI poster presentation and/or IUPUI and IUSD Research Days) on their resume and able to verbally present their work before a group of faculty and other students.

***Time Table:*** In the first week, students would be introduced to all personnel in the laboratory, department and to other MURI faculty and students, assigned desk space and a lab coat and take required online training courses offered by IUPUI in laboratory safety and blood borne disease without regard to the type of research to be conducted (see time line). Also in the first week, students will be given additional reading material on their topic and receive detailed instruction in the basic aspects of the laboratory procedures. Subsequent weeks will be devoted to experimental design and pilot experiments. By the second month of the project, students should be fairly independent

in the experimental procedures they have been instructed on and conducted preliminary pilot experiments. It is anticipated that the bulk of the research data that will be reported will be obtained in the latter half of the 9 month program. The last month of the program will be devoted to completion of any remaining experiments and writing the final report of the project. In addition, students will prepare a poster throughout the entire program with the introduction and methods sections being written early in the project and the results and discussion sections being prepared at the end of the program.

| | **ACTIVITIES DURING EACH WEEK of PROGRAM** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ACTIVITY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Introduction to Lab Proc. | X | X | | | | | | | |
| Learn Exp. Procedures | | X | X | | | | | | |
| Experiments in Gregory Lab | X | X | X | X | | | | | |
| Experiments in Windsor and Song Labs | | | | X | X | X | X | X | X |
| Completion of Exp. | | | | | | | | | X |
| Written Report | | | | | | | | | X |

***Itemized Budget:*** Total $2,000. The supply budget consists of an equal division of the funds among each student (for 5 students, each would be able to utilize up to $400/each for laboratory supplies, reagents or small equipment).

***Sustainment of Research Project:*** We are currently developing an NIH grant in consultation with NIGMS officers to provide support after this MURI project. We will use data generated from the MURI students as preliminary data for this grant submission.

***Risk Management:***

Please check any risk assurances that apply to this proposal:

☐ Animals (IACUC Study #): _____

☒ Human Subjects (IRB Study #): _0304-58, NS0805-human fibroblast and epithelial cells and NS0905-human pulp cell studies)__

☐ r-DNA (IBC Study #): _____

☐ Human Pathogens, Blood, Fluids, or Tissues must be identified if used: _____

## Section IV: References/Bibliography (insert 1-2 pages as needed)

1. Zheng, C., and R. Gregory. 2008. The effect of nicotine and cotinine on the growth of *Streptococcus mutans* and the protein expression. Abstracts of the American Society for Microbiology.
2. Zheng, C. and R.L. Gregory. In vitro effect of cigarette smoking condensate on the growth and sucrose-dependent adherence of *Streptococcus mutans* and *Streptococcus gordonii*. Caries Res. (manuscript in revision).
3. Windsor, L.J., E. Allam and R.L. Gregory. Cigarette smoke toxicity and oral health. In: *Cigarette Smoke Toxicity - Linking Individual Chemicals to Human Diseases*. D. Bernhard (ed.). Wiley-VCH Verlag GmbH & Co. KGaA (pub.) (submitted).
4. Gregory, R.L., J.C. Kindle, L.C. Hobbs, T. VanTo and H.S. Malmstrom. 1990. Effects of smokeless tobacco on the ability of secretory component to bind to the IgA/J chain complex. Human Antibodies and Hybridomas 1:126-131.
5. Gregory, R.L., J.C. Kindle, L.C. Hobbs and H.S. Malmstrom. 1991. Effect of smokeless tobacco use on mucosal immune factors. Archs. Oral Biol. 36:25-31.
6. Zhou, J., B.L. Olson, and L.J. Windsor. 2007. Nicotine increases the collagen-degrading ability of human gingival fibroblasts. J. Periodont. Res. 42:228-235.
7. Almasri, A., K. Wisithphrom, L.J. Windsor, and B.L. Olson. 2007. Nicotine and lipopolysaccharide affect cytokine expression from gingival fibroblasts. J. Periodont. 78:533-541.
8. Song, F., A.S. Bergdoll, and L.J. Windsor. 2006. Temporomandibular joint synovial fibroblasts mediate serine proteinase dependent Type I collagen degradation. Biochim. et Biophys. Acta. 1760:1521-1528.
9. Song, F., K. Wisithphrom, J. Zhou, and L.J. Windsor. 2006. Matrix metalloproteinase dependent and independent collagen degradation. Frontiers in Bioscience. 11:3100-3120.
10. Rainey, C.L., P.A. Condor and J.V. Goodpaster. 2011. Chemical characterization of dissolvable tobacco products promoted to reduce harm. J. Agricultural and Food Chemistry (in press).
11. Fazii, P., E. Ciancaglini and G. Riario Sforza, Differential Fluorescent Staining Method for Detection of Bacteria in Blood Cultures, Cerebrospinal Fluid and Other Clinical Specimens, European Journal of Clinical Microbiology & Infectious Diseases 21:373-378

## Section V: CVs/Resumes (insert 2 pages per mentor for a maximum of 6 pages)

See attached 2 page NIH-style biosketches for Drs. Richard Gregory, L. Jack Windsor and Fengyu Song.

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| Richard L. Gregory | Professor of Oral Biology and Director of PhD Program |
| eRA COMMONS USER NAME<br>RGREGORY | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| Eastern Illinois University, Charleston, IL | B.S. | 1976 | Med. Tech. & Chemistry |
| Southern Illinois University, Carbondale, IL | Ph.D. | 1982 | Microbiol. & Immunology |
| University of Alabama at Birmingham | Postdoc | 1984 | Mucosal Immunology |

### A. Positions and Honors

**Positions and Employment**

1975-76 Medical Technologist, Decatur Memorial Hospital, Decatur, IL
1984 Visiting Instructor, Department of Microbiology, University of Alabama, Tuscaloosa, AL
1984-91 Assistant-Associate Prof. of Oral Biology, School of Dentistry, Emory University, Atlanta, GA
1991-2001 Associate Professor of Oral Biology, School of Dentistry, Indiana Univ.
1992-2001 Associate Professor of Pathology & Laboratory Medicine, Sch. of Medicine, Indiana Univ.
1996 Visiting Scientist (Sabbatical), Dept. of Microbiol. and Mol. Genetics, Univ. of Vermont
1996-2003 Director, Short-Term Health Professional Student Research Program, School of Dentistry
1999-present Director, PhD Dental Science & Student Res. Programs, Sch. of Dentistry, Indiana Univ.
2001-present Professor of Oral Biology, School of Dentistry, Indiana Univ.
2001-present Professor of Pathology & Laboratory Medicine, School of Medicine, Indiana Univ.
2001-2002 Acting Director of Research, School of Dentistry, Indiana Univ.
2008-present Member, IU Center for Environmental Health and Simon Cancer Center

**Other Experience and Professional Memberships**

1987-present NIH Reviewer's Reserve
1993-present Editorial Board, *Clinical & Diagnostic Laboratory Immunology*

**Honors**

1994 Indiana University School of Dentistry Distinguished Faculty Award for Research
1996-00, 2002-3,8 Indiana University Teaching Excellence Recognition Awards
2003 Indiana University School of Dentistry Outstanding Faculty Member of the Year

### B. Selected peer-reviewed publications (from 89 peer-reviewed publications and 213 abstracts).


1. Gregory, R.L. and S.J. Filler. 1987. Protective secretory IgA antibodies in humans following oral immunization with *Streptococcus mutans*. Infect. Immun. 55:2409-2415. PMCID: PMC260722.
2. Gregory, R.L., J.C. Kindle, L.C. Hobbs, S.J. Filler, H. Malmstrom. 1990. Function of anti-*S. mutans* antibodies: inhibition of virulence factors and enzyme neutralization. Oral Micro. Immun. 5:181-188.
3. Gregory, R.L., J.C. Kindle, L.C. Hobbs, T. VanTo and H.S. Malmstrom. 1990. Effects of smokeless tobacco on the ability of secretory component to bind to the IgA/J chain complex. Human Antibodies and Hybridomas 1:126-131.
4. Gregory, R.L., J.C. Kindle, L.C. Hobbs and H.S. Malmstrom. 1991. Effect of smokeless tobacco use on mucosal immune factors. Archs. Oral Biol. 36:25-31.
5. Gregory, R.L., L. Gfell, H.S. Malmstrom. 1995. Differences in sIgA and serum antibodies to *S. mutans* isolates from caries-resistant and caries-susceptible subjects. Adv. Exp. Med. Biol. 371B:1149-1152.
6. Fontana, M., L.E. Gfell, R.L. Gregory. 1995. Characterization of preparations enriched for *S. mutans* fimbriae: salivary IgA antibodies in caries- and caries-active subjects. Clin. Diagn. Lab. Immunol. 2:719-725. PMCID: PMC170228.
7. Perrone, M., L.E. Gfell, M. Fontana, R.L. Gregory. 1997. Antigenic characterization of fimbriae preparations from *Streptococcus mutans* isolates from caries-free and caries susceptible subjects. Clin. Diagn. Lab. Immunol. 4:291-296. PMCID: PMC170521.
8. Gregory, R.L., A.M.A. Rahman and D.R. Avery. 1998. Effect of restorative treatment on mutans streptococci and IgA antibodies. Ped. Dent. 20:273-277.

9. Ray, C.A., L.E. Gfell, T.L. Buller and R.L. Gregory. 1999. Interactions of *Streptococcus mutans* fimbrial-associated surface proteins with salivary components. Clin. Diagn. Lab. Immunol. 6:400-404. PMCID: PMC103730.
10. Fontana, M., A.J. Dunipace, G.K. Stookey and R.L. Gregory. 1999. Intranasal immunization against dental caries with *Streptococcus mutans* enriched fimbrial preparation. Clin. Diagn. Lab. Immunol. 6:405-409. PMCID: PMC103731.
11. Fontana, M., T.L. Buller, A.J. Dunipace, G.K. Stookey and R.L. Gregory. 2000. An in vitro microbial-caries model used to study the efficacy of antibodies to *Streptococcus mutans* surface proteins in preventing dental caries. Clin. Diagn. Lab. Immunol. 7:49-54. PMCID: PMC95821.
12. Gregory, R.L. 2001. Modified immunogenicity of a mucosally administered antigen. Clin. Diagn. Lab. Immunol. 8:540-544. PMCID: PMC96097.
13. Ge, J., D.M. Catt and R.L. Gregory. 2004. *Streptococcus mutans* surface $\alpha$-enolase binds salivary MG2 and human plasminogen. Infect. Immun. 72:6748-6752. PMCID: PMC523000.
14. Catt, D.M. and R.L. Gregory. 2005. Identification and analysis of a *Streptococcus mutans* murein hydrolase. J. Bact. 187: 7863-7865. PMCID: PMC 1280295.
15. Sanui, T. and R.L. Gregory. 2009. Analysis of *Streptococcus mutans* biofilm proteins recognized by salivary IgA. Oral Microbiol. Immunol. 24:361-368.
16. Weng, Y., X. Guo, R.L. Gregory and D. Xie. 2010. A novel antibacterial dental glass-ionomer cement. Eur. J. Oral Sci. 118:531-534.
17. Weng, Y., X. Guo,J. Zhao, R.L. Gregory and D. Xie. 2010. A PQAS-containing glass-ionomer cement for improved antibacterial function. J. Biomedical Science and Engineering. 3:956-963.
18. Allam, E., W. Zhang, C. Zheng, R.L. Gregory, and L.J. Windsor. 2011. Smoking and oral health. In: *Cigarette Smoke Toxicity - Linking Individual Chemicals to Human Diseases*. D. Bernhard (ed.). Wiley-VCH Verlag GmbH & Co. KGaA (pub.) pp. 257-280.

**C. Research Support**

**Ongoing Research Support**

Indiana University Windsor (PD) 7/1/07-6/30/10
Indiana University Tobacco Cessation and Biobehavioral Center
The major goal of this project is to investigate the effect of tobacco on mucosal biology.
No overlap with the proposed application.
Role: Co-director

Wm. Wrigley Jr. Company (several studies) Gregory (PI) 2007-present
Study on the MIC/MBC and time killing analysis of oral bacteria.
The major goal of this project is to investigate novel antimicrobial compounds for use in chewing gum.
No overlap with the proposed application.
Role: PI

NIH CTSI Fellowship Grant McCarlie (Student trainee) 2008-10
Relationship of MHC alleles with caries
The major goal of this project is to provide a predoctoral student stipend in the area of biomedical research.
No overlap with the proposed application.
Role: Co-Mentor

NIH/NHLBI UO1 grant Twigg (PI) 9/23/09-7/31/14
Lung Microbiome and Pulmonary Inflammation/Immunity in HIV Infection
The major goal of this project is to investigate the microbiome of the respiratory tract in HIV subjects.
No overlap with the proposed application.
Role: Co-investigator

NIH/NIDCR RC1 grant Xie (PI) 10/1/09-9/30/11
High-performance Biocompatible GIC System with Permanent Antibacterial Function
The major goal of this project is to investigate the ability of a high-performance biocompatible GIC system to provide long-lasting antimicrobial activity.
No overlap with the proposed application.
Role: Co-investigator

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME<br>Windsor, L. Jack | POSITION TITLE<br><br>Associate Professor of Oral Biology |
|---|---|
| eRA COMMONS USER NAME<br>LJWINDSO | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| Samford University, Birmingham, AL | B.S. | 1981 | Biology |
| University of Alabama at Birmingham, Birmingham, AL | Ph.D. | 1993 | Biochemistry |

**A. Positions and Honors.**

**Positions and Employment**

1981-1983 Graduate Teaching Assistant, Samford University, Birmingham, AL.
1983-1987 Research Assistant, University of Alabama at Birmingham, Birmingham, AL.
1987-1993 Graduate Student, University of Alabama at Birmingham, Birmingham, AL.
1993-1994 Research Associate, University of Alabama at Birmingham, Birmingham, AL.
1994-1995 Research Instructor, University of Alabama at Birmingham, Birmingham, AL.
1995-1998 Research Assistant Professor, Univ. of Alabama at Birmingham, Birmingham, AL.
1995-1998 Associate Scientist, Cell Adhesion and Matrix Research Center, University of Alabama at Birmingham, Birmingham, AL.
1997-1998 Associate Scientist, Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL.
1997-1998 Graduate Faculty, University of Alabama at Birmingham, Birmingham, AL.
1999-2005 Assistant Professor of Oral Biology, Indiana University, Indianapolis, IN.
2000-2005 Adjunct Assistant Professor of Anatomy and Cell Biology, Indiana University, Indianapolis, IN.
2000- Indiana University School of Dentistry Graduate Faculty, Indiana University, Indianapolis, IN.
2000- Indiana University Graduate Faculty, Indiana University, Indianapolis, IN
2005- Associate Professor of Oral Biology, Indiana University, Indianapolis, IN.
2005 - Adjunct Associate Professor of Anatomy and Cell Biology, Indiana University, Indianapolis, IN.

**Honors**

IUPUI 2007 Glenn W. Irwin, Jr., M.D., Experience Excellence Recognition Award
American Dental Education Association Leadership Institute Fellow, 2008-2009
President IUSD Faculty Council 2008-2009
Indiana Dental Association Outstanding Teacher of the Year, 2008
Indiana University Trustee's Teaching Award, 2009
Indiana University Purdue University Indianapolis Best MURI Team, 2009
Omicron Kappa Upsilon (OKU), elected member of the Theta Theta Chapter 2009

**B. Selected peer-reviewed publications (in chronological order).**

(Publications selected from 60 publications and 97 Abstracts)

1. Song, F. and Windsor, L. J.: Non-Matrix Metalloproteinases (MMP) Mediated Collagen Degradation, *Biochim. Biophys. Acta*, **1721**:65-72, 2005.
2. Zhou, J. and Windsor, L. J.: *Porphyromonas gingivalis* affects Host Collagen Degradation by Affecting Expression, Activation, and Inhibition of Matrix Metalloproteinases, *J. Periodont. Res*., **41**:47-54, 2006.

3. Wisithphrom, K. and Windsor, L. J.: Interleukin-1a Alters the Expression of Matrix Metalloproteinases and the Collagen Degradation by Pulp Fibroblasts, *J. Endod*., **32**:186-192, 2006.
4. Song, F., Wisithphrom, K., Zhou, J., and Windsor, L. J.: Matrix Metalloproteinase Dependent and Independent Collagen Degradation, *Front. Biosci*., **11**:3100-3120, 2006.
5. Wisithphrom, K. and Windsor, L. J.: The Effects of Tumor Necrosis Factor-□, Interleukin-1□, Interlukin-6, and Transforming Growth Factor-$\alpha$1 on Pulp Fibroblast Mediated Collagen Degradation, *J. Endod*., **32**:853-861, 2006.
6. Song, F., Bergdoll, A. S., and Windsor, L. J.: Temporomandibular Joint Synovial Fibroblasts Mediate Serine proteinase Dependent Type 1 Collagen Degradation, *Biochim. Biophys. Acta*, **1760**:1521-1528, 2006.
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8. Zhou, J., Olson, B. L., and Windsor, L. J.: Nicotine Increases the Collagen Degrading Ability of Human Gingival Fibroblasts, *J. Periodont. Res*., 42:228-235, 2007.
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12. Gregson, K., O'Neill, J. T., Platt, J. A., and Windsor, L. J.: In vitro induction of hydrolytic activity in human gingival and pulp fibroblasts by triethylene glycol dimethacrylate and monocyte chemotatic protein-1, *Dent. Mater.*, **24**:1461-1467, 2008.
13. Zhang, W., Song, F., and Windsor, L. J.: Cigarette smoke condensate affects the collagen- degrading ability of human gingival fibroblasts, *J Periodontal Res.* 2009 Dec;44(6):704- 13. Epub 2009 May 18.
14. Ghoneima, A. A., Allam, E. S., Zunt, S. L., and Windsor, L. J.: Bisphosphonate treatment and orthodontic considerations, *Orthod. Craniofac. Res*., **12**:1-10, 2009.
15. Gregson, K. S., Windsor, L. J., and Platt, J. A.: Biodegradation of a dental resin material by fibroblast conditioned media, *Dent. Mater.*, **25**:1358-1362, 2009.
16. Allam, E., Draz, A., Hassan, A., Neamat, A., Galal, M., and Windsor, L. J.: RANKL Expression in Ligature-Induced Periodontitis in Osteoporotic and Nonosteoporotoc Rats, *J. Periodont. Res*., in press, 2009
17. Zhang, W., Song, F., and Windsor, L. J.: Tobacco and *P. gingivalis* effects on gingival fibroblasts, *J Dental Res.,* in press, 2009.
18. Allam, E., W. Zhang, C. Zheng, R.L. Gregory, and L.J. Windsor. 2011. Smoking and oral health. In: *Cigarette Smoke Toxicity - Linking Individual Chemicals to Human Diseases*. D. Bernhard (ed.). Wiley-VCH Verlag GmbH & Co. KGaA (pub.) pp. 257-280.

**C. Research Support**

Research Support

R29-AR44701 Windsor (PI) 9/01/1997-8/31/03
NIAMS/NIH
Acivation of Collagenase in Rheumatoid Arthritis.
The goal of this project is to study the mechanisms by which human fibroblast collagenase is activated by synovial fibroblast cell lines established from patients with rheumatoid arthritis.
Role: PI

Research Support Funds Grant 07/01/05-06/30/06
Indiana University Purdue University at Indianapolis
The Relationship between Dental Resins and Pulp Cell Activity
The goal of this project is to determine the effects that dental resins has on pulp cell activities such and extracellular matrix degradation and cytokine production.
Role: PI

Signature Center 7/01/07-6/30/10
Indiana University Purdue University at Indianapolis
Tobacco Cessation and Biobehavioral Center
Role: Director/PI

**Fengyu Song, D.D.S., M.S., Ph.D.**

**EDUCATION**:

**B. S. (D.D.S.) in Dentistry,** West China University of Medical Science, Chengdu, Sichuan, P. R. China, June, 1998

**M. S. in Prosthodontics,** West China University of Medical Science, Chengdu, Sichuan, P. R. China, June, 1998

**Ph.D. in Oral Biology,** Indiana University, Indianapolis, Indiana, August, 2006

**ACADEMIC APPOINTMENTS**:

**Graduate Faculty**, Indiana University, 2009-present

**Graduate Faculty**, Indiana University School of Dentistry, 2008-present

**Assistant Professor**, Indiana University School of Dentistry, Department of Oral Biology, August 2007 –present

**Adjunct Assistant Professor**, Indiana University-Purdue University Indianapolis, School of Science, Department of Biology, January 2008 -present

**Assistant Research Scientist**, Center for Regenerative Biology and Medicine, Department of Biology, School of Science, Indiana University-Purdue University Indianapolis (IUPUI), Indianapolis, Indiana, March 2007 – July 2007

**PROFESSIONAL ORGANIZATIONS:**

| | |
|---|---|
| International Association for Dental Research | 2004-present |
| Society for Developmental Biology | 2008-present |
| American Association for Dental Research | 2004-present |
| American Dental Education Association | 2006-present |
| American Association of Oral Biologist | 2008-present |

**PROFESSIONAL SERVICE**:

**Grant Reviewer:**

Israeli Ministry of Science and Technology, Life Sciences Division, Israel, ad hoc reviewer, 2008

**Scientific Advisory Board**

Journal of Endodontics

**Manuscript reviewed for:**

Journal of Dentistry
International Endodontic Journal
Journal of Periodontology

**GRANT SUPPORTS:**

**IUPUI Research Support Funds Grant (RSFG),** PI**,** $22,800. Title: Qualitative and Quantitative Study of Repair in Segmental Defects of the Limb and Mandibular Bones of the Axolotl, March 2010-Feburary 2011.

**Chinese Scholar Fellowship, Chinese Government Scholar Administration, PI,** $45,000 (for appointed postdoc only), Titled: "The Extracellular Matrix Remodeling Ability of Human Osteoblast under Fluid Sheer Stress", January 2010-December 2011

**IUPUI Multidisciplinary Undergraduate Research Institute Grant**, Co-Investigator, IUPUI Center for Research and Learning**,** Summer 2008, 2008-2009, Summer 2009, 2009-2010, and summer of 2010

**RC1 NIH /NIDCR,** Co-Investigator**,** $797,375, Titled: "High-performance Biocompatible Cement System with Permanent Antibacterial Function", October 2009-September 2011

**2010 Special Congressional Appropriation Project** (Pending), Co-PI, $1.0M, Titled: Regenerative Medicine for Battlefield Injuries, July 2011-June 2014

**NIH R03, PI,** $150,000 (Pending), Titled: "Amphibian Cell Dedifferentiation and Cell Cycle Extension", July 1, 2011-June 30, 2013, pending

**RECENT PUBLICATIONS**:

Labban N. Y, **Song F.**, Al-Shibani N., Windsor L. J., Journal Prosthodontics Research, 2008, 100: 390-397.

Zhang W., **Song F.**, Windsor L. J., Journal of Periodontal Research, 2009, 44(6):704-13.

Rao N.**,** Jhamb D.**,** Milner D. J.**,** Li B.**, Song F.,** Wang M.**,** Voss S. R.**,** Palakal M., King M. W.**,** Saranjami B.**,** Nye H. L. D.**,** Cameron J. and Stocum D. L., BMC Biology 2009, **7:**83.

Zhang W., **Song F.**, Windsor L. J., Journal of Dental Research, 2010, 89(5):527-531.

**Song F.,** Li B., Stocum D. L., Amphibians as Research Models for Regenerative Medicine, Organogenesis, 2010, 6:141-150.

Huang J., Qiu L., Ding L., Wang S., Wang J., Zhu Q., **Song F.** and Hu J., International Immunopharmacology, 2010, 10(10): 1279-1283.

Santosh N., Windsor L. J., Mahmoudi B., Li B., Zhang W., Chernoff E., Rao N., Stocum D. L. and **Song F.**, Developmental Dynamics, (In press).

Jhamb D., Rao N., Milner D. J. and **Song F.**, Cameron J., Stocum D. L. and Palakal M. J., BMC Bioinformatics (In press).

Sun J., Weng Y., Song F. and Xie D., Journal of Biomaterial Science and Engineering (In press).

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## Section VI: Support Letters (insert 1- 2 pages as needed)

None, no collaborative arrangements.

## Section VII: Appendix (Title of and information on the status and outcomes of past MURI projects received by the Principal Mentor. Please insert 1 page summary per previous MURI project as needed according to template below. Maximum - 5 pages.)

**Because of the 5 page limit, only MURI reports for Summer 2009, Fall/Spring 2009-10 and Summer 2010 are included.**

***Title of Past MURI Project:*** Ability of tobacco to increase the virulence of oral disease by affecting human cells and bacteria.

***Date Awarded: 05/2009***

***Date Completed: 07/2009***

***Brief Description:*** Student research projects will focus on the effects of nicotine on: 1) adhesins, metabolism and biofilm formation of *S. mutans* (Gregory lab); 2) expression of proinflammatory cytokines from human gingival fibroblasts (Windsor lab); and 3) release of MMP from human pulp fibroblasts and ability of green tea to inhibit (Song lab).

***Outcomes: Each student completed their project and presented a poster at either the Dental School Research Day and/or a local scientific meeting.***

***Poster presentations:*** Inessa Levitt, Anna Jouravlev, Danielle Cory, Elizabeth Smith, Mia Recupito, Boniface Nganga, and Milan Patel. All presented posters.

**Gregory:** The three projects conducted by the 3 MURI students in my laboratory were separate but related studies that allowed us to progress in our overall lab goal of understanding the mechanism of tobacco/nicotine on oral bacteria. Specifically, Inessa Levitt studied the effects of nicotine on *Streptococcus mutans* phosphotransferase (PTS) and acid production from glucose. Her results indicated that nicotine at physiological levels enhance PTS and acid production of this bacterium. Anna Jouavlev and Danielle Cory worked on related projects on *S. mutans* growth and expression of a cell surface adhesin with Anna studying the long term effects of nicotine exposure to *S. mutans* mimicking a long term smoker. Her data indicated that *S. mutans* develops tolerance to nicotine and in fact she produced 2 mutants of *S. mutans* that are resistant to high nicotine levels. Danielle studied how long the effect of nicotine lasted once the nicotine pressure was removed from the *S. mutans* culture mimicking a smoker undergoing cessation. Her data demonstrates that the nicotine effect remains for at least 10 passages of the bacterium after removal of nicotine. All three students have done oral and poster presentations regarding their work in the Student Research Presentation Program (SRPP) at the dental school and in the CRL summer research presentation program, respectively.

**Windsor:** The two projects conducted by the students on our MURI team in my laboratory produced data that advanced our understanding of the effects of nicotine/tobacco on cells in the oral cavity. Mia Recupito worked on determining the effects of nicotine, cigarette smoke condensate (CSC), *Porphyromonas gingivalis* (*P. gingivalis*), nicotine/*P. gingivalis*, or CSC/*P. gingivalis* on the expression of chemoattractant protein-1 (MCP-1). She evaluated the effects utilizing a MCP-1 ELISA kit. She showed that levels of MCP-1 from human gingival fibroblasts increased with nicotine, *P. gingivalis*, and *P. gingivalis* with nicotine, whereas they decreased with CSC and *P. gingivalis* with CSC. However, the reason why MCP-1 concentrations in cell supernatants from cells treated with CSC decreased is unclear at the present time. Multiple attempts at confirming these results utilizing MCP-1 Western Blotting have not been successful because of antibody sensitivity issues. Mia has remained in the laboratory to complete some experiments, which will include utilizing an AlphaLISA kit to confirm the data from the regular MCP-1 ELISA kit. Elizabeth Smith worked on further studying the effects that nicotine and CSC has on osteoblasts in regard to cytokine/growth factor, matrix metalloproteinases (MMP), and type I collagen expression. She examined the changes in cytokine/growth factor and MMP protein expression utilizing RayBiotech Protein arrays, while she utilized Polymerase Chain Reaction (PCR) to detect changes in type I collagen mRNA expression. She found that nicotine was toxic to the osteoblasts at 1000 μg/ml and CSC was toxic at 200 μg/ml particulate matter. Nicotine (250 μg/ml) caused an increase in expression of IL-7 and MMP-1 by osteoblasts and a decrease in expression of IFN-γ and MCP-1. CSC (100 μg/ml) caused an increase in expression of GM-CSF, IFN-γ, and RANTES by osteoblasts and a decrease in expression of IL-3, IL-13, MMP-9, MMP-10, and TIMP-4. Nicotine and CSC caused a decrease in expression of Type I collagen by osteoblasts. Elizabeth is now working on writing all her results up in a manuscript for submission.

**Song:** The two projects conducted by two students, Boniface Nganga and Milan Pater, have been progressed successfully. The data generated from the projects will lead to future research investigations and can be used for manuscript construction. Human pulp fibroblasts, also known as human pulp cells, have the potential to be involved in dentin formation since they are able to produce mRNA of dentin specific proteins such as dentin sialoprotein (DSP) and dentin matrix acidic phosphoprotein 1(DMP1). Both nicotine and cigarette smoke condensate at various concentrations were able to affect the mRNA productions of DSP and DMP1. Further investigations will be to detect the changes of DSP and DMP1 at the protein level under the influence of nicotine and cigarette smoke condensate. Both students have done oral and poster presentations regarding their work in the Student Research Presentation Program (SRPP) at the dental school and in the CRL summer research presentation program, respectively.

***Title of Past MURI Project:*** Molecular and cellular changes in oral human and bacterial cells after exposure to tobacco.

***Date Awarded: 10/2009***

***Date Completed: 05/2010***

***Brief Description:*** Student research projects will focus on the effects of tobacco and/or nicotine on: 1) adhesins, metabolism and biofilm formation of *S. mutans* (Gregory lab); 2) expression of MMP and proinflammatory cytokines from human endothelial cells and periodontal fibroblasts (Windsor lab); and 3) expression of extracellular matrix genes and proteins in response to tobacco and nicotine (Song lab).

***Outcomes: Each student completed their project and presented a poster at either the Dental School Research Day and/or a local scientific meeting.***

***Poster presentations:*** Inessa Levitt, Jessica Morgan, Mallory Wilson, Anna Jouravlev, Delnaaz Daruwala, Eric Grow and Larry Voiles. All presented posters.

**Gregory:** The goals of the project in this lab were met as Inessa Levitt, Mallory Wilson, Jessica Morgan and Anna Jouravlev worked extremely hard on their projects and identified several important aspects of tobacco effects on oral bacteria. They worked well together and with others in the lab as a team. The organization of this group worked very well and I would not change any of the procedures that we used. Some expected research data did develop in that they demonstrated that nicotine upregulates nicotinic receptors on *Streptococcus mutans* (Jouravlev) and that nicotine accumulates in a *S. mutans* biofilm implying that nicotine will also accumulate in dental plaque of smokers (Levitt). Furthermore, Wilson demonstrated that nicotine causes planktonic *S. mutans* to decrease while biofilm attached bacteria increase and Morgan established that nicotine causes a significant increase in hydrophobicity of *S. mutans* and a concomitant increase in biofilm formation. These results were all hypothesized but as with all research the data raises additional questions that will be pursued in future projects.

**Windsor:** Periodontal disease is characterized by host inflammatory responses to bacterial infections. Tobacco is considered a risk factor for periodontal disease. The purpose of one of the team members' study (Daruwala) was to determine the effects that bacteria and tobacco alone, as well as in combination, have on the expression of growth-regulated oncogene alpha (Gro-α), which is a chemoattractant for inflammatory cells. The study demonstrated that the bacteria increased Gro-α expression and that the tobacco decreased Gro-α expression from human gingival fibroblasts. In addition, the combination decreased Gro-α expression. Future experiments will examine different gingival fibroblast cell lines, since it is known that they can have differential responses.

Collagen is a major connective tissue protein and also is constantly being remodeled, degraded, and synthesized. Chemicals from tobacco are found in their highest concentrations in

the oral cavity where intake occurs. This can lead to multiple diseases including periodontal disease, which involves the degradation of collagen. This team members' study (Voiles) examined the effects that nicotine and cigarette smoke condensate (CSC) have on type I collagen mRNA expression in human gingival fibroblasts (HGFs) by Polymerase Chain Reaction (PCR). Collagen expression was slightly increased in the nicotine and the CSC exposed HGFs in comparison to the untreated control. Future experiments will examine additional HCF cell lines in order to confirm these results.

**Song:** Eric Grow has studied on the effect of smoke condensate on human pulp cells for one and half years. His latest research activity with our MURI team is to detect and analysis the cytokine expression under the influence of cigarette smoke condensate using cytokine array kit from Bio-Ray technology. Due to the time limitation, the study using the green tea extract against smoke condensate is initiated but need more time to advance further. Eric will volunteer to work in the lab this coming summer and finish up the project. A manuscript regarding this research effort is under construction.

***Title of Past MURI Project:*** Effect of Nicotinic Acetylcholine Receptor Agonists/Antagonists on Human Cells and Oral Bacteria.

***Date Awarded: 05/2010***

***Date Completed: 07/2010***

***Brief Description:*** Tobacco affects all of the cells in the oral cavity. Student research projects will focus on the effects of nicotine agonists/antagonists on: 1) nicotine's ability to alter adhesins, metabolism and biofilm formation of *S. mutans* (Gregory lab); 2) expression of proinflammatory cytokines and MMPs from human gingival epithelial cells and fibroblasts (Windsor lab); and 3) release of MMPs and dentin-specific proteins from human dental pulp fibroblasts (Song lab). Agonists include: epibatidine and acetylcholine, while antagonists include: mecamylamine. In addition, other agents such as cotinine, trimetaphan camsylate, d-turbocurarine, doxacurium, succinylcholine, and 18-methoxycoronaridine should bind based on similar amine groups and will be assayed.

***Outcomes: Each student completed their project and presented a poster at either the Dental School Research Day and/or a local scientific meeting.***

***Poster presentations:***

***Title:*** **EFFECT OF NICOTINE ON *STREPTOCOCCUS MUTANS* BACTERIOCIN PRODUCTION AGAINST NONCARIOGENIC BACTERIA**
***Date: 07/2010***
***Students Involved: Kendra Heeke***

***Title:*** **EFFECTS OF FIVE NICOTINIC AGONISTS AND ANTAGONISTS ON NICOTINIC ACETYLCHOLINE RECEPTORS IN HUMAN GINGIVAL FIBROBLASTS**
***Date: 07/2010***
***Students Involved: Jordan Jenkins***

***Title:*** **NICOTINE EFFECTS HUMAN PULP CELLS**
***Date: 07/2010***
***Students Involved: Emily Thurston***

***Title:*** **EFFECTS OF CIGARETTE SMOKE CONDENSATE ON HUMAN PULP CELLS**
***Date: 07/2010***
***Students Involved: Sheila Ngetich***

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## Section VIII: Signature

**Name and Signature of the Principal Mentor:**

*(typing the full name suffices as signature for electronic copies)*

| Richard L. Gregory | | 3/10/11 |
|---|---|---|
| Name | Signature | Date |