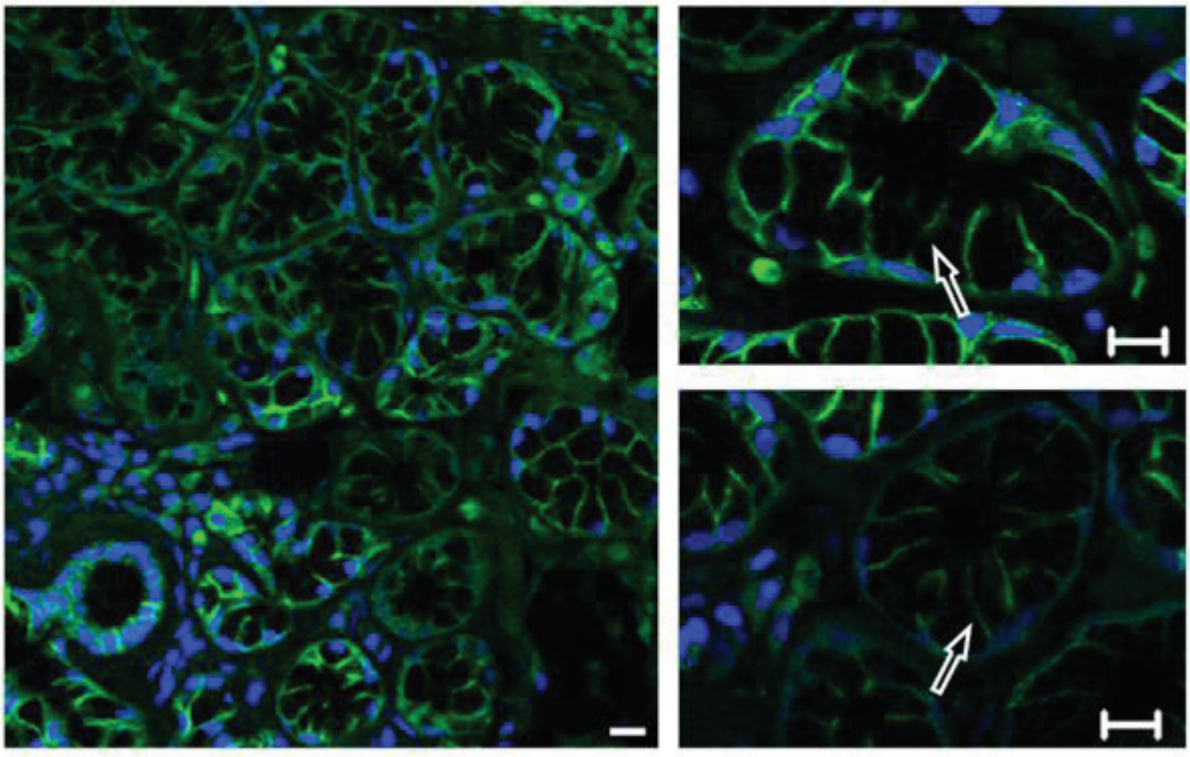
RESEARCH HANDBOOK 2011-2012



Salivary tissues of individuals diagnosed with SS display significant changes in immunolocalization of E-cadherin, which is frequently diminished at the lateral-apical regions (unfilled white arrows). Size bar: 10 mm. Courtesy of Dr. Maria Kukuruzinska's laboratory.

Boston University Henry M. Goldman
School of Dental Medicine

BOSTON UNIVERSITY

Research is an integral component of Boston University Henry M. Goldman School of Dental Medicine (GSDM)'s mission, goals, and objectives. The School's mission statement begins: "The Boston University Henry M. Goldman School of Dental Medicine will be the premier academic dental institution promoting excellence in dental education, research, oral health care, and community service to improve the overall health of the global population". In addition, the mission states: "We will shape the future of the profession through scholarship, creating and disseminating new knowledge, developing and using innovative technologies and educational methodologies, and by promoting critical thinking and lifelong learning."

What is "dental research"?

Dental research involves the use of scientific analysis, observation, and experimentation to acquire new knowledge in the field of dental medicine.

Deadline to apply
First-year research 2 x 3 : Jan 2, 2012
IREC 1: Feb 1, 2012
IREC 2: ongoing during second-year
IREC 3: ongoing during third-year

The benefits of research:

- become trained in the design and execution of scientific studies;
- enhance analytical thinking abilities;
- bring breadth and depth to their dental education;
- have a better understanding of innovative dental techniques, materials, and tools;
- become more informed dental clinicians.
- contribute to the dental literature by publishing the results; and
- improve eligibility for postgraduate specialty training programs and academic appointments.

The research environment at GSDM:

- Department of Molecular and Cell Biology, Evans 4, 72 East Concord St. http://dentalschool.bu.edu/research/molecular/index.html;
- Department of Periodontology and Oral Biology, CABR Building, 700 Albany Street, http://dentalschool.bu.edu/research/perio/index.html;
- Clinical Research Center, 100 East Newton Street,

- Departments of Endodontics, General Dentistry, Oral and Maxillofacial Surgery, Orthodontics and Dentofacial Orthopedics, and Oral Diagnosis and Radiology, 100 East Newton Street (http://dentalschool.bu.edu/departments/ortho/index.html), (http://dentalschool.bu.edu/treatment-policies/oral-diagnosis.html);
- Department of Health Policy and Health Services Research, 560 Harrison Avenue http://dentalschool.bu.edu/research/health-policy/index.html;
- Department of Restorative Sciences/Biomaterials, 801 Albany Street, (http://dentalschool.bu.edu/research/biomaterials/index.html);

Other research sites:

- Boston University School of Medicine (BUSM), http://www.bumc.bu.edu/busm/;
- any other research facility approved by the Predoctoral Research Committee. (Early application is necessary to complete the process of executing an affiliation agreement prior to start of research.)

Research faculty mentors:

GENERAL DENTISTRY

- Judith Jones, DDS, MPH, professor and chair. Research Area: Health outcomes research, oral-systemic relationships
- Paula Friedman, DDS, MSD, MPH, professor associate dean for administration and director, Geriatric Fellowship Program. Research Area: Geriatric dentistry
- Anita Gohel, BDS, CAGS, PhD, associate professor & director of oral diagnosis & radiology. Research Area: Radiology imaging and interpretation
- Ying Wu, DDS, MSD, PhD, assistant professor. Research Area: Oral mucosal disease, orofacial pain, management of medical compromised patient, oral cancer and salivary gland disease

ENDODONTICS

- George Huang, DDS, MSD, DSc, professor. Research Area: Stem cells regeneration

HEALTH POLICY AND HEALTH SERVICES RESEARCH

- Raul Garcia, DMD, MMS, professor and chair. Research Area: Epidemiology
- Michelle Henshaw, DDS, MPH, assistant professor and assistant dean for community practice. Research Area: Public health
- Elizabeth Kaye, MPH, PhD, associate professor. Research Area: Public health
- Woosung Sohn, DDS, PhD, Dr.PH, associate professor. Research Area: Cariology and oral health disparities

MEDICINE/INFECTIOUS DISEASES

- Frank Gibson, PhD, associate professor. Research Area: Microbiology

MOLECULAR AND CELL BIOLOGY

- David Levin, PhD, professor and chair. Research Area: Biochemistry/molecular biology
- Maria Kukuruzinska, PhD, professor and associate dean for research. Research Area: Molecular and cell biology/development
- Carlos Hirschberg, PhD, professor. Research Area: Biochemistry/molecular biology
- Phillips Robbins, PhD, professor. Research Area: Molecular and cell biology
- Miklos Sahin-Toth, MD, PhD, professor. Research Area: Biochemistry
- John Samuelson, MD, PhD, professor. Research Area: Microbiology

ORAL AND MAXILLOFACIAL SURGERY

- Pushkar Mehra, associate professor and chair. Research Area: Trauma, fracture, maxillofacial management and orthognathic surgery.
- Richard D'Innocenzo, associate clinical professor. Research Area: Trauma, fracture, maxillofacial management and anesthesia

- Andrew Salama, DDS, MD, assistant professor. Research Area: Evaluating tongue motion and speech following reconstructive surgery and developing novel chemo-preventive medications for oral cancer
- Wael Youseff, DDS, DMD, CAGS, assistant professor. Research Area: Inferior alveolar and lingual nerve repair, facial pain syndrome – temporomandibular joint disease

ORTHODONTICS
- Leslie Will, DMD, MSD, professor and chair. Research Area: Normal & abnormal growth, treatment outcomes & diagnostic tools
- Bo Ho, DDS, MS, CAGS, PhD, assistant Professor. Research Area: Cellular & molecular mechanisms that control orthodontic tooth movement

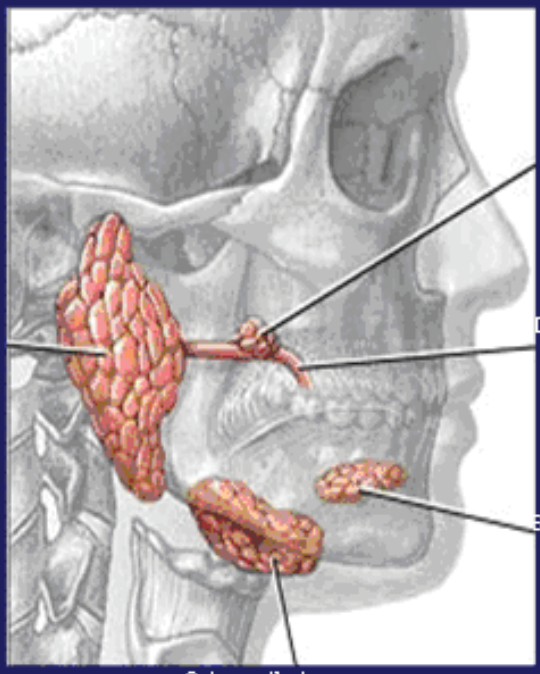
ORTHOPEDIC SURGERY
- Louis Gerstenfeld, PhD, professor. Research Area: Cell biology/bone

PEDIATRIC DENTISTRY
- Christopher Hughes, DDS, PhD, associate professor and chair. Research Area: Oral microbiology

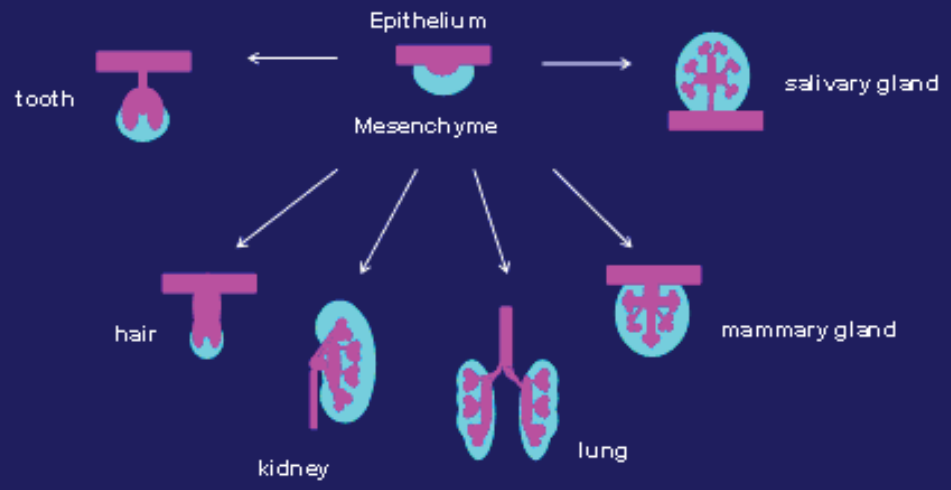
PERIODONTOLOGY AND ORAL BIOLOGY
- Salomon Amar, DMD, PhD, professor and director of inflammatory center. Research Area: Cell biology
- Serge Dibart, DDS, DMD, professor and program director. Research Area: Gingival epithelial cells
- Robert Gyurko, DDS, PhD, associate professor. Research Area: Periodontology/ immunology/bone physiology
- Eva Helmerhorst, MS, PhD, associate professor. Research Area: Biochemistry
- Cataldo Leone, DMD, DMSc, professor and associate dean for academic affairs. Research Area: Biochemistry/periodontology
- Yoshiyuki Mochida, DDS, PhD, associate professor. Research Area: Molecular biology
- Frank Oppenheim, DMD, PhD, professor. Research Area: Biochemistry
- Philip Trackman, PhD, professor. Research Area: Cell biology

RESTORATIVE SCIENCES/BIOMATERIALS
- Dan Nathanson, DMD, MSD, professor and chair. Research Area: Biomaterials
- Laisheng Chou, DMD, PhD, professor. Research Area: Cell biology/oral medicine
- Russell Giordano, DMD, DMSc, associate professor. Research Area: Biomaterials
- Gurkan Goktug, DDS, assistant professor and director of prosthodontics residents' lab. Research Area: Biomechanics of implants, immediate loading, soft tissue management around immediate implants, material consideration of implant supported over-dentures

Research in Oral Medicine

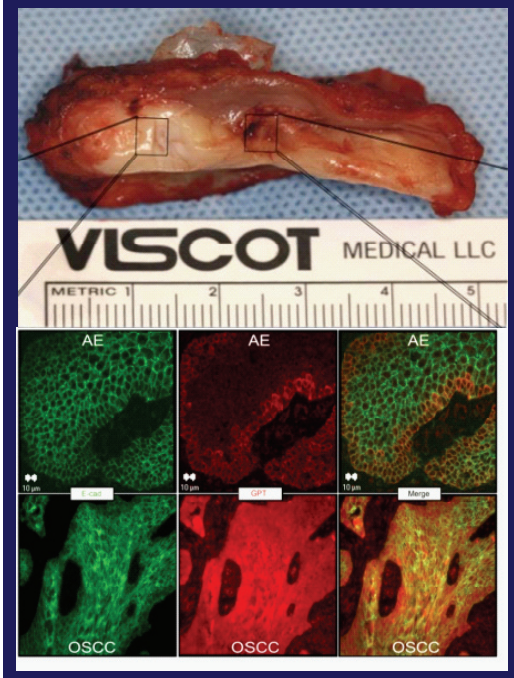


Salivary Glands and Teeth Develop through Branching Morphogenesis

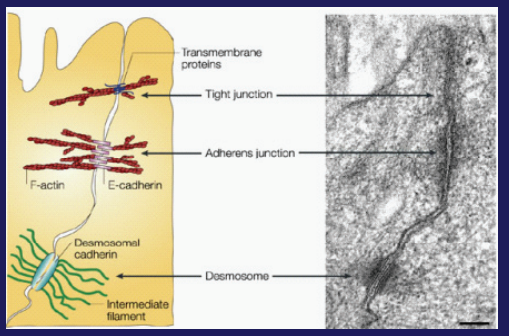


The following images represent selected research by GSDM faculty.

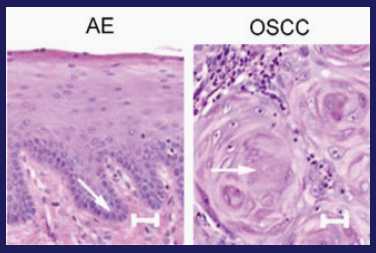
Maria Kukuruzinska, PhD



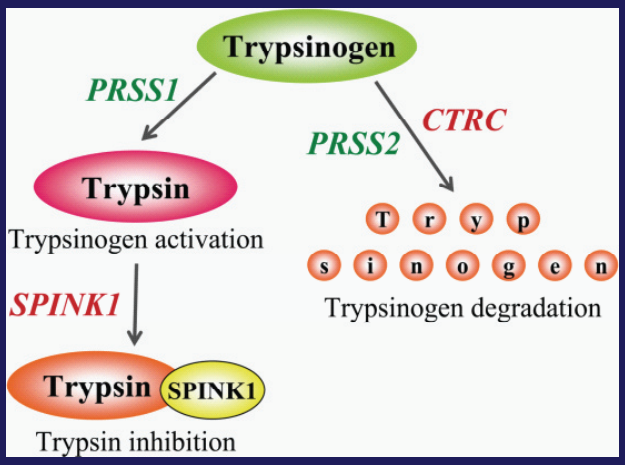
Overexpression of DPAGT1 is a feature of a subset of human Oral Squamous Cells Carcinoma (OSCC)



Adhesion in oral cancer

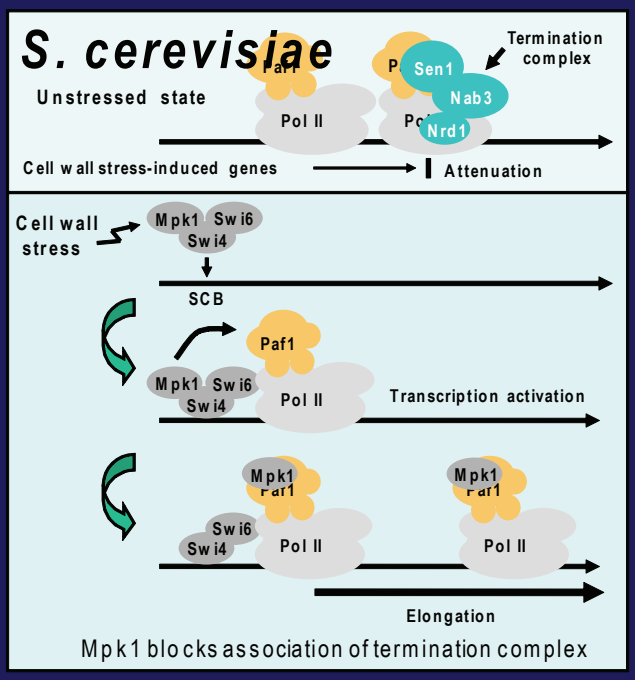


Miklos Sahin-Toth, MD, PhD



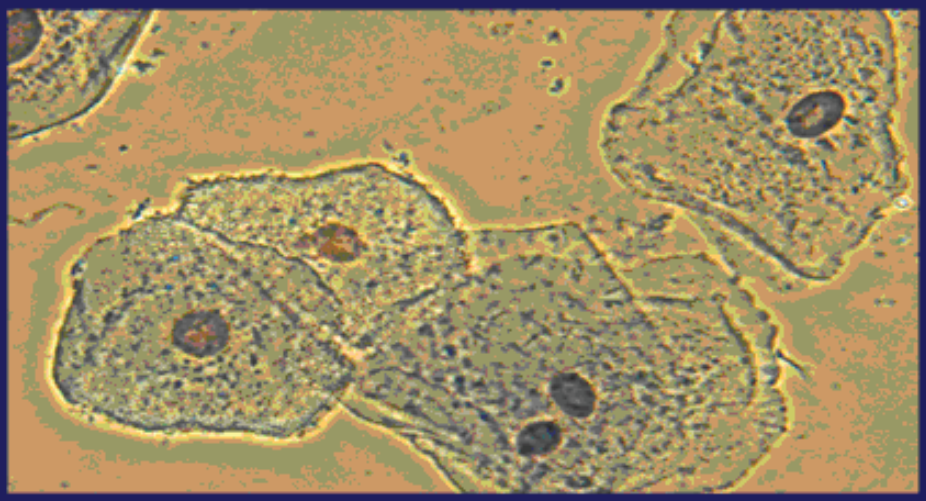
The trypsin-dependent pathological pathway in chronic pancreatitis associated with genetic mutations. Activation of trypsinogen to active trypsin is mitigated by trypsinogen degradation and active trypsin is inhibited by pancreatic secretory trypsin inhibitor (SPINK1). Mutations in *PRSS1* stimulate autoactivation of cationic trypsinogen. Loss-of-function mutations in *SPINK1* reduce inhibitor expression and compromise trypsin inhibition. The p.G191R variant in *PRSS2* stimulates trypsin-mediated degradation of anionic trypsinogen and thereby protects against chronic pancreatitis. Loss-of function mutations in *CTRC* reduce secretion or activity of chymotrypsin C and thus impair protective trypsinogen degradation.

David Levin, PhD



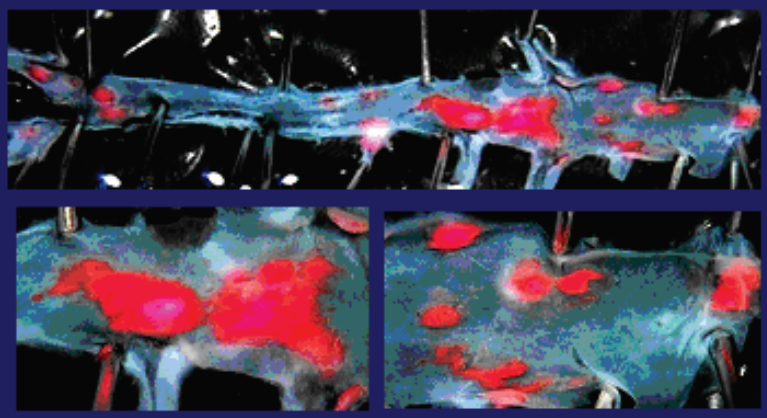
Use of the yeast *Saccharomyces cerevisiae* as a model system for understanding stress signaling in eukaryotic cells.

Frank Oppenheim DMD, PhD



Desquamated oral buccal epithelial cells with attached streptococci

Salomon Amar DMD, PhD



Aortic tree of mice infected with Porphyromonas gingivalis, a major pathogen in periodontal disease; red area denotes lipid-rich atherosclerotic plaque

Russell Giordano DMD, DMSc

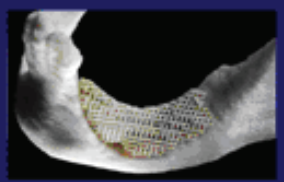
- **Materials for CAD/CAM**
  Developing new materials for computer aided fabrication of restorations; test a variety of existing ceramic materials under accelerated aging.
- **Ceramic Materials via 3D Printing**
  Collaborating with Sandia National Labs to develop a system to print ceramics for tissue matrix devices for bone defects and dental restorations.
- **Any dental material including orthodontic and endodontic materials.**

CAD/CAM Design

Milled Crown

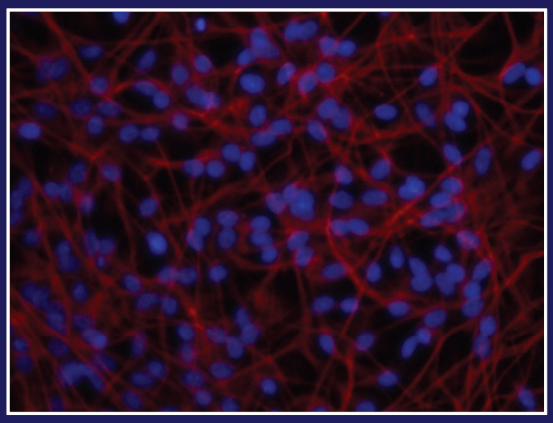
Ceramic "Printer"

Ceramic Matrix



George Huang, DDS, MSD, DSc

Stem Cell Biology/ Tissue Regeneration



Spontaneous neurogenic differentiation of dental stem cells reprogrammed into induced pluripotent stem cells. Cells were stained with a neurogenic marker Tuj1 (red). The cell nucleus was stained with a dye DAPI (blue).

Predoctoral Research Program (PRP)

The GSDM developed a highly successful Predoctoral Research Program for DMD students. The mission of the Program is: 1) to shape the future of dental medicine and dental education through research; 2) to educate students from diverse backgrounds about the importance of research in dental medicine; and 3) to mentor students to make informed decisions about research career opportunities.

The PRP at the GSDM benefits individual students and the field of dental medicine. Through participation in research students enhance their analytical thinking abilities, become trained in the design and execution of scientific studies, gain a better understanding of innovative dental techniques, materials and tools, improve their eligibility for postgraduate specialty training programs and academic appointments, become more informed dental clinicians, and contribute to the dental literature by publishing their research findings.

The GSDM provides state-of-the-art research training resources. Students choose faculty mentors from 36 research scientists involved in more than 100 research projects that span broad areas of basic and applied biomedical sciences, as well as clinical and public health research. In addition, to direct mentor-student interactions, student trainees are expected to become important contributors to research teams and to participate in the full range of research-related activities, including laboratory/ team meetings and journal clubs. At the completion of research training, students

are expected to showcase their accomplishments at the School's Science Day and at the University's Science and Engineering Day. In addition, students are encouraged to participate in national and international scientific meetings in the areas of their research training. Information about the PRP and the Student Research Group (SRG) can be obtained at http://dentalschool.bu.edu/research/predoctoral/index.html. Information on the GSDM Science Day abstracts and awardees is available at http://dentalschool.bu.edu/research/predoctoral/events.htm.

Program Structure

Because of its unique curriculum, the GSDM offers formal research training for credit to students. Students who maintain a 3.0 GPA or higher in their didactic and clinical courses are considered for research training. Students selected by Committee can participate in the Program. The first-year training takes place following the completion of the DMD didactic courses during the Apex rotation from May to July. The rotation is based on a five-day week as follows:

a. students dedicate two days for research training and three days for the Apex clinical assignment;

b. students dedicate three days for research training (30 hours per week) and two days for the Apex clinical assignment under the Intensive Research Elective Course (IREC). Students are considered for the IREC 1 if they have participated in research during the second semester of their dental education on a voluntary basis or if they have prior research experience;

c. students can do research on a voluntary basis and are expected to spend no less than 10 hours per week in research training. Advanced Standing students can start research during the second semester of their dental education.

Prior to engaging in research training, the Predoctoral Research Office meets with the applicants to advise them of their assignments and to inform them of the prerequisites to research training including NIH training in the Protection of Human Subjects in Research and other regulatory requirements. The students are given a copy of the Research Handbook that contains a detailed description of the program. During research rotations, student trainees are expected to attend meetings with the Office of the Predoctoral Research that include presentations on scientific writing skills and approaches to better presentations. Trainees are also expected to attend seminars relevant to their research organized by the GSDM, the School of Medicine and other research institutions in the greater Boston area. In addition, students are required to participate in research competitions. Students have the option to do research rotations outside of Boston University that require the execution of an affiliation agreement that governs the relationship between Boston University and the outside institution.

Student research training is overseen by the Predoctoral Research Committee, chaired by the Director of the PRP and composed of members of the GSDM biomedical science and clinical faculty, the Associate Dean for Academic Affairs, the APEX Program Administrator, a student representative and the Assistant Director of

Predoctoral Research. The mission of this committee is to guide and monitor research activities among DMD students, evaluate the effectiveness of the PRP and make recommendations for program improvements.

The Intensive Research Elective Course (IREC)
The goal of the IREC is to provide intensive and structured research experience throughout the dental school curriculum for students who are interested in careers in oral health research.

The IREC objectives are: 1) to carry out well-defined research projects under the guidance of research mentors; 2) to enhance critical thinking skills; 3) to participate in the full range of research-related activities, including scientific meetings and journal clubs. Scientific meetings will provide platforms for discussions of research findings, for troubleshooting research strategies and methodologies and for critiquing results and their interpretation; 4) to train in the design and execution of scientific studies, gain better understanding of innovative dental techniques, materials and tools, develop analytical thinking abilities, contribute to the dental literature by publishing results, showcase accomplishments at local, national and international scientific meetings, become more informed dental clinicians and improve eligibility for academic appointments; and 5) to contribute to the discovery of new knowledge.

The IREC components include mentored research and a completed project. Students need to complete the mentored project for the section and report the results at Science Day and at other scientific events. The project could be ongoing throughout the IREC training.

There will be three options to IREC:
IREC 1 - Intensive Research DMD year 1 (3 credits);
IREC 2 - Intensive Research DMD year 2 (2 credits);
IREC 3 - Intensive Research DMD year 3 (2 credits).

Research Training Eligibility
Students who maintain a 3.0 GPA or higher in their didactic and clinical courses are considered for research training. Students selected by Committee can participate in the IREC.

1- The IREC 1 course takes place during the first-year following the completion of the DMD didactic courses from May to July. The rotation is based on a five-day week as follows:

a. Students dedicate three days for research training (30 hours per week) and two days for the Apex clinical assignment under the Intensive Research Elective Course (IREC). Students are considered for the IREC 1 if they have participated in research during the second semester of their dental education on a voluntary basis or if they have prior research experience.

b. IREC 1 trainees will be graded by the end of the Apex rotation.

2- The IREC 2 course takes place during DMD year 2. Students who completed IREC 1 training or those with prior research experience can apply.

a. The expected number of hours is 100 contact hours minimum in the laboratory or in the clinical setting. The activities outlined below need to be accomplished outside the contact hours.

b. Students need to complete the mentored project and present it at Science Day and at other scientific events. The project could be an ongoing product carried from IREC 1.

c. IREC 2 trainees will be graded by the end of DMD year 2.

3- The IREC 3 course takes place during DMD year 3. Students who completed IREC 1 and/or IREC 2 training or those with prior research experience can apply.

a. The expected number of hours is 100 contact hours minimum in the laboratory or in the clinical setting. The activities outlined below need to be accomplished outside the contact hours.

b. Students need to complete the mentored project and report the results at Science Day and at other scientific events. The project could be an ongoing product throughout the IREC training.

c. IREC 3 trainees will be graded by the end of DMD year 3.

Activities during the IREC

Project Development

IRP trainees will work together with their mentors on the preparation of research proposals through literature reviews, analyses of preliminary data and pilot studies. Project description will include concept definition, formulation of specific hypotheses, aims and timelines, as well as expected outcomes. Mentors assigned to train IREC students assume the responsibility for supporting the students through the selection, design and execution of a project. Once the project is completed, students are expected to present at local, national and international meetings.

Seminar Series

The Predoctoral Research Program (PRP) office will organize a seminar series through which IREC trainees will learn about different scientific methodologies and approaches. These seminars will enrich the trainees' research experience by exposing them to the latest scientific findings and will facilitate development of personal relationships among peers.

Journal Club

Each trainee will be required to attend a journal club directed at developing skills in the critical evaluation of literature by critiquing research papers.

Scientific Writing and Presentation Skills
The PRP Office will assist the IRP trainees in the presentation of the research accomplishments at scientific meetings. An emphasis will be made on improving oral presentation and writing skills.

Scientific Events
The PRP Office will support the IRP trainees to present their research projects at the IADR/AADR meetings, the Hinman Symposium, the Yankee Dental Symposium, the annual GSDM Science Day and Boston University Science and Engineering Day.

Instructions in the Responsible Conduct of Research (RCR)
Prior to Apex training, The PRP Office will inform trainees of their responsibilities that include a session on the NIH training in the Protection of Human Subjects in Research. During the IREC training, the PRP office will facilitate training attendance in RCR. The activities include discussion of standards of good practice and policies for handling misconduct allegations. The training program on RCR will consist of a series of lectures, seminars and workshops on several major issues that include Human Subjects, Research Notebooks, Authorship Responsibility, Institutional Policies on Scientific Misconduct, Proper Application of Statistical Analysis and Conflict of Interest.

Training and Assessment
Each research mentor will be expected to provide guidance and supervision to the trainee. Each mentor will formally meet with the IREC trainee on a regular basis to review progress. In addition, mentors will also be expected to interact informally with the IREC trainees on a regular basis during the elective course in years two and three. The IREC trainee's progress will be determined by an evaluation questionnaire completed by the research mentor to provide an assessment of the trainee's degree of research progress and knowledge of the specific subject area. In addition, the IREC trainee's research experience will be evaluated in relation to subsequent research activities and his/her future career plans. A final grade will be issued and an assessment summary upon completion of training will provide the trainee with a comprehensive overview of his/her performance.

Program Evaluation
Assessment of the educational outcome will be used by measuring the initial baseline through a pre-program questionnaire. A post-program questionnaire will be used to quantify changes in knowledge, skills and career choices. Feedback gathered through evaluation will be documented and used to improve the quality of the Program. The student's self-assessment will be triangulated with the actual assessment by the mentor. The evaluation will help in the adjustment of goals and objectives of the research training to improve the Program outcome.

Benefits while in the PRP

- AADR membership;
- IADR/AADR annual meeting;
- Poster/Oral Presentations at Science Day, Science and Engineering Research Symposium, Hinnman symposium, Yankee Dental Congress, etc;
- Publishing opportunity;
- Sigma Xi membership;
- Regulatory and ethical conduct of research training;
- Fogarty International Clinical Research Scholars Program opportunity;
- Howard Hughes Medical Institute Research Training Programs opportunity.

Expectations during first-year research rotation

Meeting I (during the second week of rotation)

- Students are advised on principles in conducting research. Expectations are emphasized regarding presentation of their work at scientific meetings and in particular GSDM Science Day and BU Science and Engineering Research Symposium Day (during March of every year). Students are expected to report on their project and research experience. Information on end of rotation presentations, American Association for Dental Research (AADR) memberships and AADR meeting attendance are discussed. Information on mentor end of rotation assessment is given.

Meeting II (during the second month of rotation)

- Students attend a seminar on "Scientific Writing Skills." Information on optional seminars on "Approached to Better Presentations" is discussed.

Meeting III (end of rotation)

- present orally a summary of their research to their colleagues (first-year rotation) and present orally or as a poster during GSDM Science Day;
- complete a program evaluation and a detailed report of the research experience (disk or email).

Evaluation criteria:

- Mentor evaluation: 50% (mentor)
- Other assignments: 30% (mentor)
- Presentations: 10% (PRP office)
- Meeting attendance: 10% (PRP office)
  Mentor evaluation criteria include research science aptitudes, report writing, research skills, and interpersonal/communication skills.

Rotation prerequisites

- Research Rotation Approval Form;
- Research outline;
- NIH tutorial on the protection of human subjects in research certificate;
  You can go online http://cme.nci.nih.gov to take a course and quiz. Once the two-hour tutorial is completed, a completion certificate can be downloaded. Onsite training sessions are available and information can be obtained at http://www.bumc.bu.edu/ocr;

- Laboratory safety training for lab settings. Information on upcoming sessions at: http://www.bu.edu/research/compliance/files/LabSafeTrainingSchedule08.pdf;
- Institutional Animal Care and Use Committee (IACUC) training, Laboratory Animal Science Center (LASC) New Researcher Orientation, Research Occupational Health Program (ROHP) assessment may be needed if animals are used during the research training. Course training schedules can be found at: http://www.bumc.bu.edu/IACUC; Mentor needs to add the trainee to the protocol.
- Students who will be working in direct contact with the subjects and/or identifiable data must be listed in INSPIR, Section A4 of the IRB protocol;
- Students who will be handling Human-Derived samples (including cell lines) or recombinant DNA, PI needs to file an amendment to add the student's name to the form found at: http://www.bumc.bu.edu/Dept/Home.aspx?DepartmentID=357
- Additional requirements as per individual mentor.

The Student Research Group (SRG)

Interested students are encouraged to participate in the School's SRG, a local chapter of the American Association for Dental Research (AADR) Student Research Group. The national SRG was established in 1980 as a means by which the AADR could foster a major source of future researchers from the ranks of dental students. The SRG at GSDM was established in 1992 and is a component of the AADR in the Boston/Connecticut Region. This region includes: Boston University, Tufts University, Harvard School of Dental Medicine, and the University of Connecticut. The AADR strongly urges schools within a region to work together to promote student research activity, and to share experiences inclusive of: competitions, conferences and interaction with research faculty. Intercampus events have been established. All students are invited to attend.

SRG Officer's Duties

Motivated students involved with the SRG are encouraged to run for officer's positions. Democratic elections are held annually.

President

- runs SRG meetings and officers' meetings;
- conducts elections;
- assures that other officers' duties are carried out;
- organizes required tasks of the SRG, including official school recognition;
- directs SRG-dental school relations and visibility;
- writes the welcome letter to incoming freshman students before school
- starts; and acts as AADR contact with help from the faculty advisor.

Vice President

- organizes big-brother/sister assignment for new student researchers;
- assists the president when necessary;

- assists the secretary in completing membership applications; and
- assists the treasurer when necessary.

Secretary

- records minutes from all officers' meetings and distributes them; and
- ensures that all students have completed AADR membership applications.

Treasurer

- authorizes and records all monetary transactions of the SRG; and
- ensures that SRG budget is balanced and appropriately distributed.

Timeline of events

January 2, 2012	Deadline for APEX I rotation applications
January 28, 2012	Yankee Dental Congress (YDC37) Student Table Clinics
February 1, 2012	Deadline for IREC1
March 15, 2012	GSDM Science Day
March 21, 2012	BU Science and Engineering Day
March 21 - 24, 2012	American Association for Dental Research (AADR) Meeting, Tampa, FL
April 2012	Orientation DMDI and ASI (11 a.m. - 12 p.m.)
April 1, 2012	Orientation to IREC1
May 2012	ADA Dental Student Conference on Research
May 21 – July 13, 2012	APEX Rotation
August 2012	Rotation oral presentations
October 2012	ADA/Dentsply Student Clinician Program
October 2012	Hinman Symposium

Information about research

- http://www.bu.edu/dental-research
- http:// blackboard.bu.edu/

Sample Outline

Goal:

To determine the role of monocyte chemoattractant protein-1(MCP-1) in the formation of lesions of endodontic origin.

Rationale:

Inflammation resulting from tissue injury or exposure to pathogenic stimuli are known to cause release of inflammatory mediators. The release of one of these mediators, IL-1, subsequently stimulates the osteoblasts to express the monocyte chemoattractant protein-1(MCP-1) gene. In several studies, MCP-1 has been documented to attract monocytes, memory T-lymphocytes, and natural killer cells. In models of inflammation, MCP-1 deficient mice were unable to recruit monocytes, suggesting that MCP-1 plays an integral and unique role in attracting monocytes to the sites of inflammation. The expression of this gene has been documented in

several disorders characterized by mononuclear infiltrates, and has been shown to contribute to the inflammatory component of these diseases. In this study, we will determine the functional significance of the expression of MCP-1 as related to the lesions of endodontic origin.

Specific Aims:

In this study, we will examine the effect of MCP-1 deletion in transgenic mice on endodontic lesion as measured by the following factors:

1) The size of the lesion
2) The recruitment of monocytes

Sample Abstract

Role of E-cadherin Junctions in Sjogren's Disease

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1Boston University, Boston, MA, USA, 2Harvard University, Charlestown, MA, USA

Objectives: Sjogren disease is an autoimmune systemic inflammatory disorder that affects a number of organs including salivary glands. Current understanding is that altered cell-cell adhesion of autoimmune target organs occurs prior to the establishment of lymphocytic infiltrates. The goal of this study was to gain insights into the cell biology of the developing submandibular glands (SMG) from a NOD mouse, a model for diabetes and Sjogren-like disease. Our hypothesis is that dysfunctional cell-cell adhesion in the developing SMG renders it a target for lymphocytic infiltration. Here, we investigated E-cadherin, the major salivary cell-cell adhesion receptor, in the embryonic staged SMGs from the NOD mouse to assess their functional status and effect on branching morphogenesis and cytodifferentiation. **Methods:** Submandibular gland rudiments were dissected from E13.5 and E18 NOD mice and cultured. Isolated glands were fixed, permeabilized and blocked overnight. The glands were then stained for E-cadherin and actin cytoskeleton using the indirect immunofluorescence staining method. Primary antibody to E-cadherin was obtained from BD Transduction and the secondary antibody, AfiiniPure Fab fragment goat anti-mouse IgG from Jackson ImmunoReseach Laboratories. Phallodin, a stain for filamentous actin (F-actin), was purchased from Molecular Probes. The slides were analyzed using confocal microscopy. **Results:** E13.5 SMGs from NOD mice displayed altered morphology. Indirect immunofluorescence staining of E-cadherin showed mislocalized distribution of E-cadherin junctional complexes with a pronounced lack of targeting to the apical lateral cell-cell borders. Phalloidin staining for F-actin revealed disorganization of the actin cytoskeleton and this correlated with the loss of salivary cell polarity. Similarly, a population of SMGs at E18 displayed discohesive morphology, altered acinar structures and an apparent collapse of ductal structures. **Conclusion:** Our studies show that impaired cell-cell adhesion in the embryonically developing SMG may explain the susceptibility of this tissue to autoimmunity.
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Sample Research Report

Functional Significance of FoxO1A in Osteoblastic Differentiation
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INTRODUCTION

The FOXO, or Forkhead Box-containing protein, family (FoxO1/FKHR, FoxO3a/FKHRL1, FoxO4/AFX, and FoxO6) is a structurally related group of winged helix transcriptional factors. These factors have been shown to regulate a variety of critically important cellular processes including apoptosis, metabolism, DNA repair, cell cycle arrest, differentiation, and defense against oxidative stress. For these events to occur FOXO requires dephosphorylation and translocation to the nucleus, where it acts by binding to specific DNA sequence regions, thereby functioning as a transcriptional activator. FOXO is deactivated via phosphorylation by the Akt family of proteins in response to insulin, by this means being retained in the cytosol unable to act on its target.
While FOXO's mechanism and method is emerging in recent literature, it is not yet clear the role it plays in assorted cell lines. This fact, teamed with FOXO's apparent encompassing and dynamic regulatory mechanism, was the foundation of this project. The aim was to determine FOXO's significance in mesenchymal cells, specifically in the differentiation of osteoblasts. A clonal murine calvarial-derived cell line (MC3T3-E1) was used which performs a developmental progression similar to osteoblasts.
It exhibits early proliferation without differentiation followed by osteoblastic differentiation with the expression of alkaline phosphatase, formation of extra cellular matrix and the production of osteocalcin.

METHODS

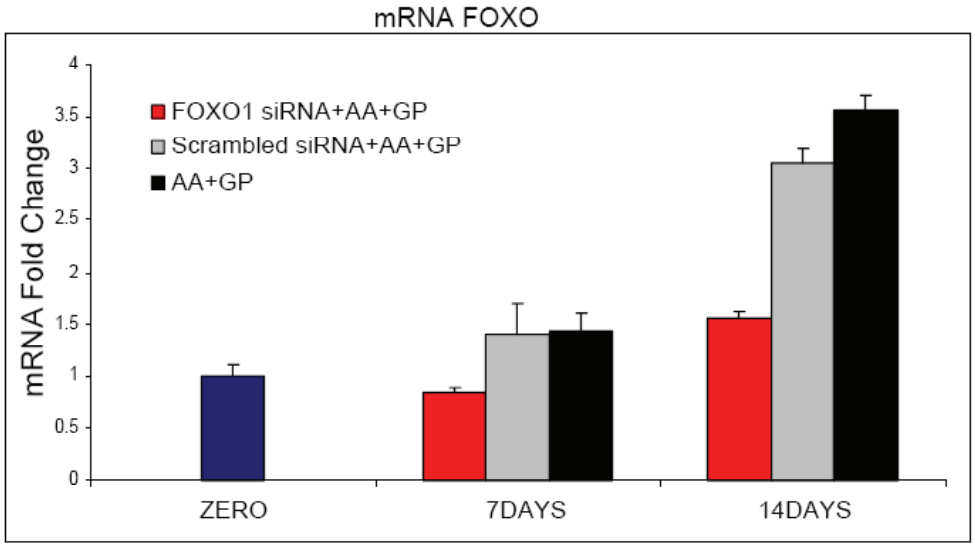
To determine the role of FOXO1 in the differentiation of osteoblasts, MC3T3 cells were cultured and treated with ascorbic acid and beta-glycerolphosphate (as detailed below) for 14 days to trigger osteoblastic differentiation. 72 hours into this treatment, small interrupting RNA (siRNA) was introduced as either FOXO1 or scrambled. RNA was collected at 7 and 14 days and Real Time PCR of FOXO1 and other differentiation related factors was performed.
Cell Culture. Pre-osteoblast (MC3T3) mouse cells were cultured using Alpha MEM media with 10% filtered Fetal Bovine Serum (FBS), 1% penicillin/streptomycin and 1% non-essential amino acids in a 37oC humidified atmosphere with 5% CO2. Passages were performed at or below 80% confluence.
Transfection. 72 hours prior to transfection 20L of 0.03M Ascorbic acid was introduced to cultures. Transfection was performed utilizing Hiperfect (12 μL), silencing RNA (1μL of each 881 & 746) or scrambled RNA (2L) in 400μL of 0.5% FBS. Transfection was continued for 24 hours. Following transfection, 100μL/10mL

of 1.08g/5mL beta-glycerolphosphate along with the ascorbic acid in 10% FBS was continued until collection. Collection points were Day 7 and Day 14 following transfection.

RNA extraction. Media was removed at collection time and cultures were rapidly frozen utilizing liquid nitrogen. RNA extraction was performed utilizing the Qiagen miRNeasy kit and protocol.

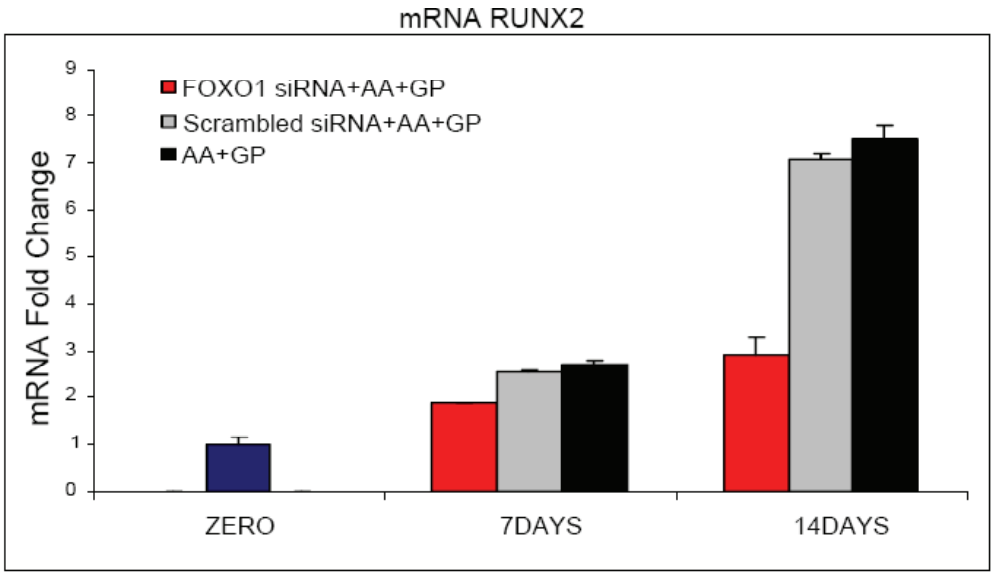
Reverse Transcription. 2µg of RNA was combined with 6.61µL MgCl2, 6µL dNTPs, 3µL RT buffer 1.5µL random hexomers, 1.89µL reverse transcriptase and 0.6µL RNAse inhibitor.

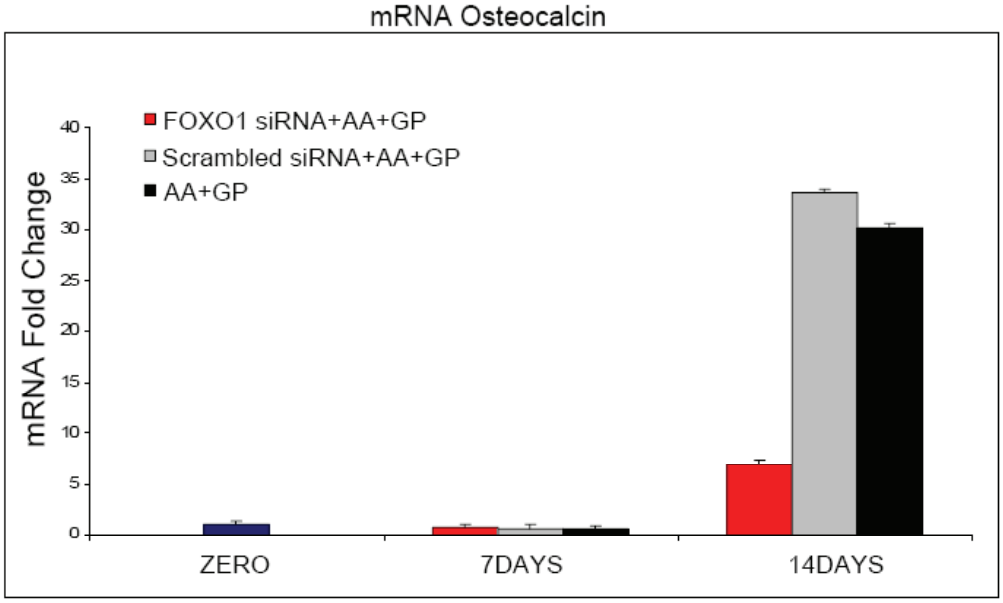
RT-PCR. 4µL of cDNA was diluted in 196µL DNAse-free water. 9µL of the dilute was combined with 10µL of PCR MasterMix (concentrated solution of DNA Polymerase, dNTPs and all the components needed for PCR except primers) and 1µL of TaqMan FOXO1 probe.



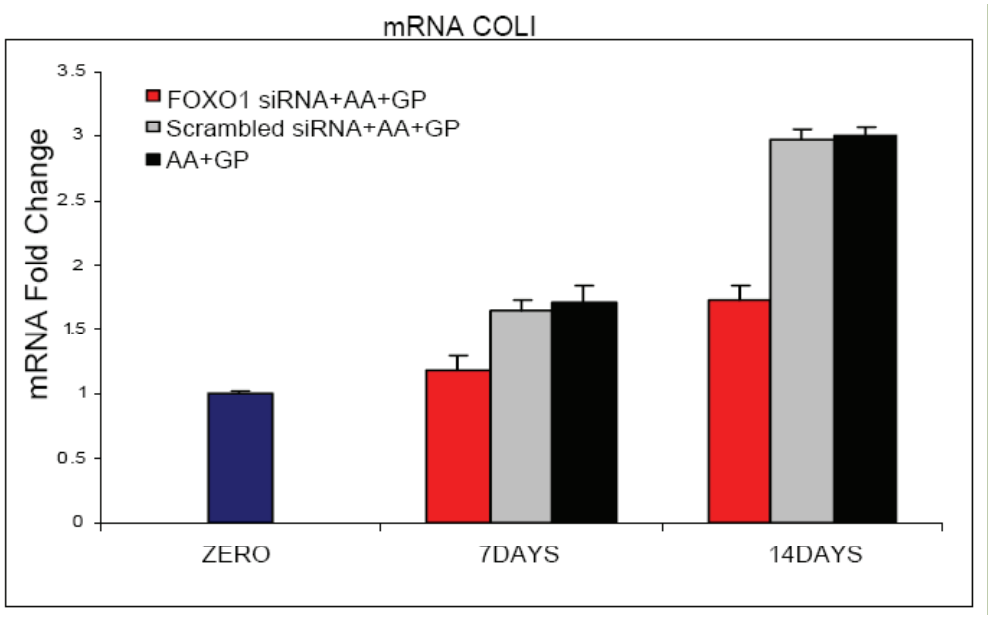
RESULTS

The RT-PCR of FOXO1 mRNA shows that the small interfering RNA decreased gene expression of FOXO1 when compared to scrambled siRNA or the differentiated osteoblasts, though only by a significant level after 14 days. After 7 days of treatment, results show little change in FOXO expression in any of the groups. There is approximately a 4-fold increase in FOXO expression comparing the scrambled with the zero day control while a 2 fold decrease comparing the FOXO siRNA to scrambled.

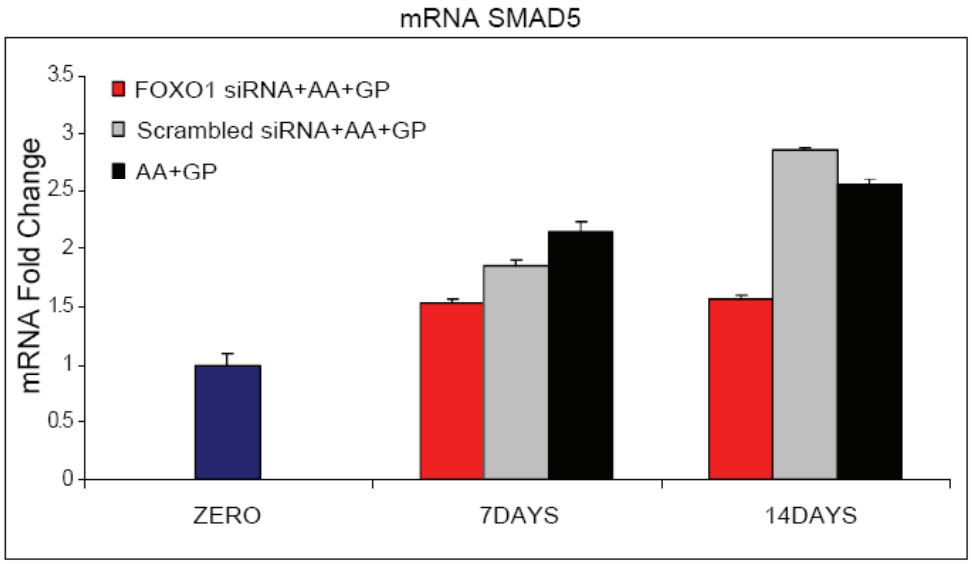


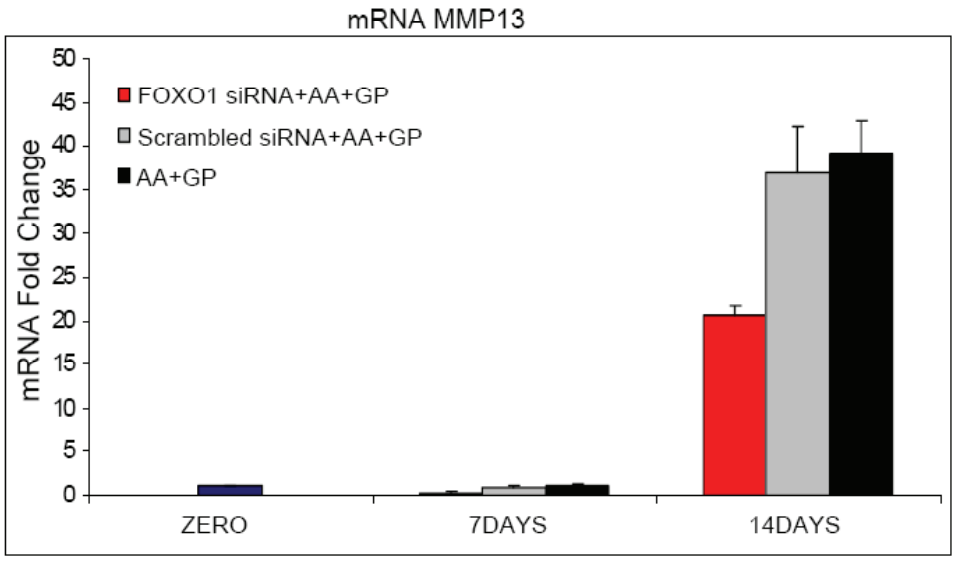


The second gene examined by RT-PCR was Runt-Related Transcription Factor 2 (RUNX2) which is a factor known to be essential for osteoblast differentiation. The 14 day scrambled and control groups show a 9-fold increase in RUNX2 mRNA expression, confirming differentiation, while the FOXO1 siRNA shows a 3-fold decrease in RUNX2 expression when compared to the scrambled and control.



Another indicator for differentiation was tested. Osteocalcin, a noncollagenous protein manufactured by osteoblasts and found in bone, is a common biochemical marker for bone formation. This was also tested by RT-PCR showing a 35-fold increase in the scrambled and controlled groups when compared to the zero point with a 7-fold decrease when the FOXO siRNA was compared to the scrambled.





Type-1 Collagen represents an organic component of bone matrix. The gene Col1 (A1&A2) was also tested by RT-PCR. There was a 3-fold increase in the scrambled and control groups when compared to the zero time point, while a 2-fold decrease when FOXO1 siRNA was compared with scrambled.

SMAD5, a member of the TGFβ family of modulators for bone morphogenetic proteins (BMPs), was also examined. The scrambled and control showed a 3-fold increase compared with the zero point, while the FOXO siRNA displayed a 2-fold decrease compared with the scrambled.

Lastly Matrix metalloproteinase 13 (Collaginase 3), a secreted peptide known to degrade collagen in normal processes like remodeling, was also examined. The 14 day scrambled and control showed a 40-fold increase when compared with the zero day point while the FOXO siRNA showed a 2-fold decrease when compared with the scrambled.

DISCUSSION

The aim of this project was to determine the significance of FOXO1 in the differentiation of osteoblasts. To achieve this, three successive goals were required. The first was to demonstrate the successful ability to adequately silence FOXO's translation by the introduction of siRNA to near confluent pre-osteoblasts at the onset of differentiation. This was shown with the up regulation of FOXO mRNA in 14 day scrambled and control groups with a 2-fold decrease in the intended silence group. The second objective was to confirm MC3T3 differentiation to osteoblasts. This was achieved by showing up regulation in several common osteoblast genes

(RUNX2, Osteocalcin, Col1, SMAD5, MMP13) from pre-treatment to 14 days post differentiation treatment. Lastly, by achieving the previous two, it was shown that FOXO1 holds a proportional relationship to several known osteoblast differentiation factors by showing that impairing FOXO1 translation resulted in a down regulation of the aforementioned genes. With that, it has become evident that FOXO plays an important regulatory role in the differentiation of osteoblasts.

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