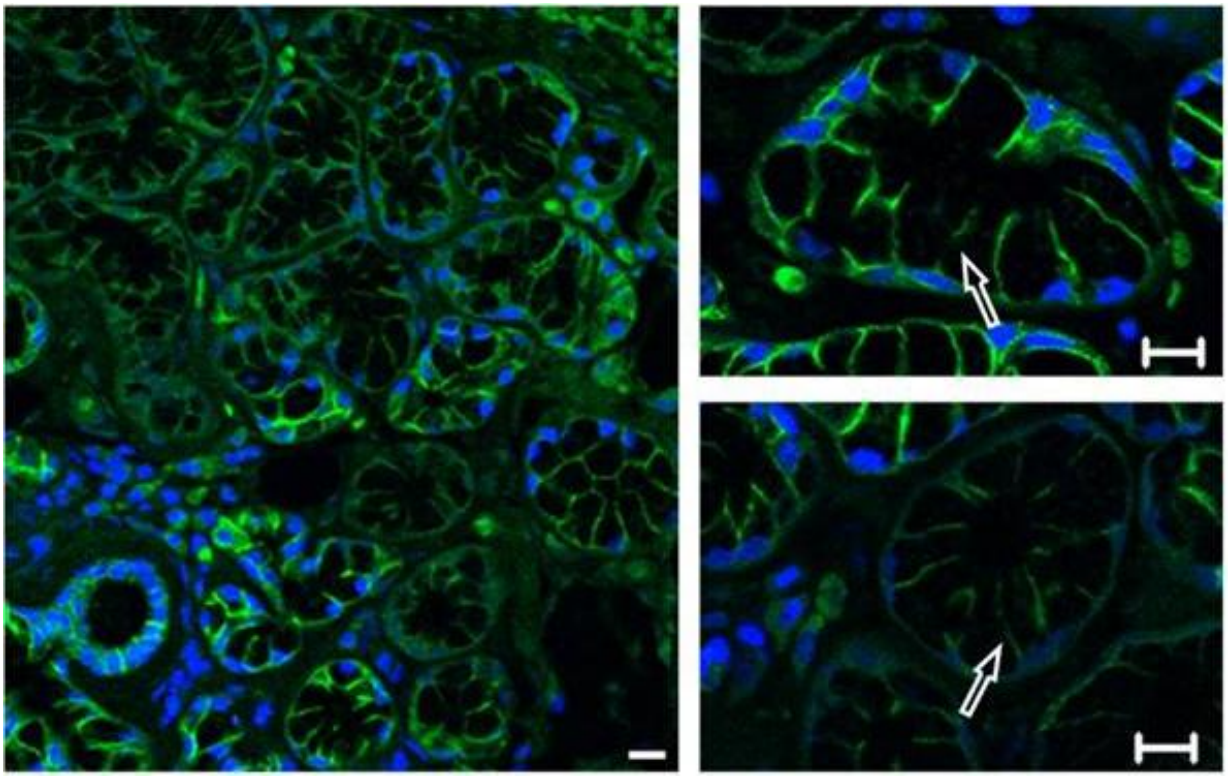
RESEARCH HANDBOOK 2014 - 2015



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 5, 2015
IREC1: Feb 2, 2015
IREC2: ongoing during second-year
IREC3: 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.
- Department of Periodontology, CABR Building, 700 Albany Street
- Center for Clinical Research, 100 East Newton Street
- Center for Anti-inflammatory Therapeutics, 650 Albany Street
- Departments of Endodontics, General Dentistry, Oral and Maxillofacial Surgery, Orthodontics and Dentofacial Orthopedics, Pediatric Dentistry, 100 East Newton Street
- Department of Health Policy and Health Services Research, 560 Harrison Avenue
- Department of Restorative Sciences/Biomaterials, 72 East Concord St. R 520 and 650 Albany Street

Other research sites:

- Boston University School of Medicine (BUSM)
- Any other research facility approved by the Pre-doctoral 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

- Paula Friedman, DDS, MSD, MPH, Professor and Director of the Geriatric Dentistry Fellowship Program. Research Area: Geriatric dentistry
- Anita Gohel, BDS, CAGS, PhD, Associate Professor and Director of Oral Diagnosis & Radiology. Research Area: Radiology imaging and interpretation
- Judith Jones, DDS, MPH, Professor and Asst Dean for Faculty Development and Director of the Center for Clinical Research. Research Area: Health outcomes research, oral-systemic relationships

HEALTH POLICY & HEALTH SERVICES RESEARCH

- Raul Garcia, DMD, MMS, Professor and Chair. Research Area: Epidemiology
- Michelle Henshaw, DDS, MPH, 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, DrPH, Associate Professor, Director of Advanced Specialty Education Program in Dental Public Health. Research Area: Cardiology and oral health disparities

MEDICINE/INFECTIOUS DISEASES

- Frank Gibson, PhD, Associate Professor. Research Area: Microbiology

MOLECULAR & CELL BIOLOGY

- Ruslan Afasizhev,PhD, Professor. Research Area: Molecular mechanisms of RNA processing in trypanosomes
- Salomon Amar, DMD, PhD, Professor and Director of Center of Anti-inflammatory Therapeutics. Research Area: Cell biology
- Eva Helmerhorst, MS, PhD, Associate Professor. Research Area: Biochemistry
- Maria Kukuruzinska, PhD, Professor and Associate Dean for Research. Research Area: Molecular and cell biology/development
- Cataldo Leone, DMD, DMSc, Professor and Associate Dean for Academic Affairs and Advanced Education & International Programs. Research areas: biochemistry/periodontology
- David Levin, PhD, Professor and Chair. Research Area: Biochemistry/molecular biology
- Yoshiyuki Mochida, DDS, PhD, Associate Professor. Research Area: Molecular biology
- Frank Oppenheim, DMD, PhD, Professor. Research Area: Biochemistry
- Phillips Robbins, PhD, Professor. Research Area: Molecular and cell biology
- Miklos Sahin-Toth, MD, PhD, Professor. Research Area: Biochemistry
- Erdjan Salih, PhD, Associate Professor. Research Area: Biomedical Sciences/ Biochemistry/Bone Biology and Bone Cancer Interaction/Mass Spectrometry
- John Samuelson, MD, PhD, professor. Research Ares: Microbiology

ORAL & MAXILLOFACIAL SURGERY

- Radhika Chigurupati, DMD, MS, Associate Professor and Director of Research. Research Area: Global health, Early diagnosis of oral cancer, clinical informatics
- Richard D'Innocenzo, DMD, MD, Associate Clinical Professor. Research Area: Trauma, fracture, maxillofacial management and anesthesia
- Pushkar Mehra, BDS, DMD, Associate Professor and Chair. Research Area: Trauma, fracture, maxillofacial management and orthognathic surgery
- Vicki Noonan, DMD, DMSc, Associate Professor and Director of the Clinical Oral & Maxillofacial Pathology Practice. Research area: pathology/oral biology
- 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

ORTHODONTICS & DENTOFACIAL ORTHOPEDICS

- Leslie Will, DMD, CAGS, MSD, Professor and Chair. Research Area: Normal and abnormal growth, treatment outcomes and diagnostic tools

ORTHOPEDIC SURGERY

- Louis Gerstenfeld, PhD, Professor. Research Area: Cell biology/bone

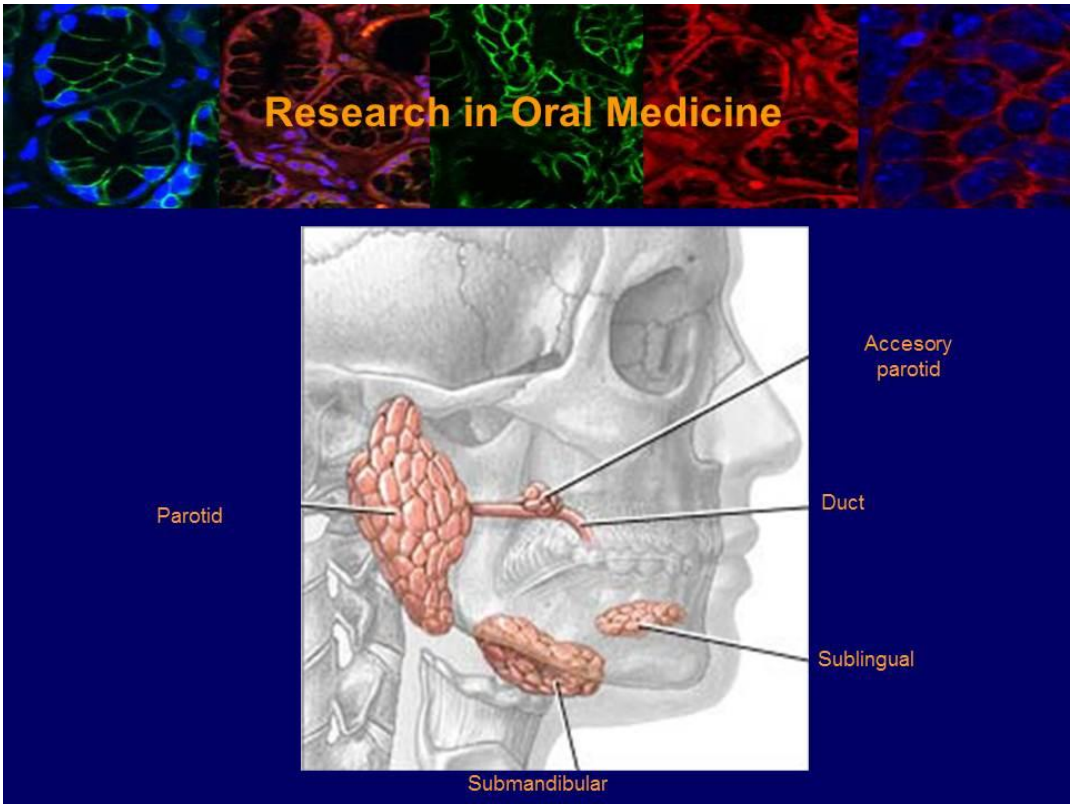
PERIODONTOLOGY

- Serge Dibart, DDS, DMD, Professor and Program Director. Research Area: Gingival epithelial cells

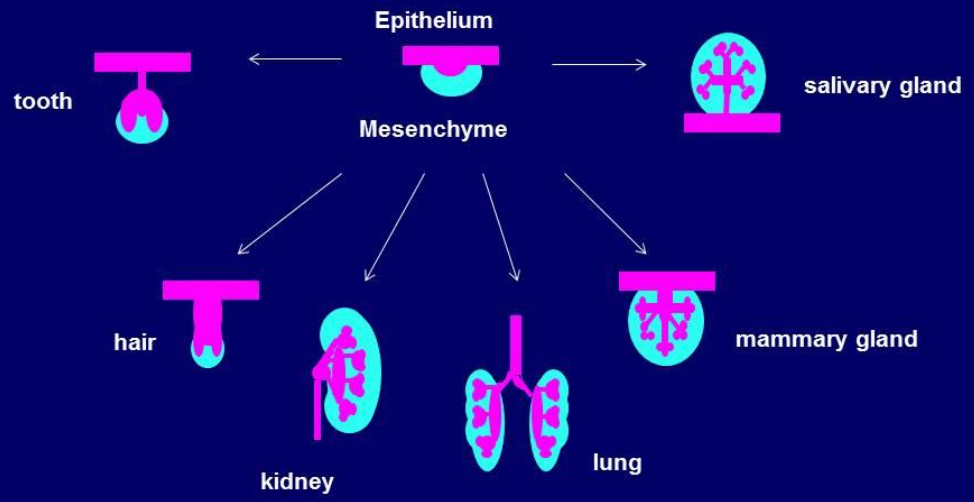
RESTORATIVE SCIENCES/BIOMATERIALS

- Laisheng Chou, DMD, PhD, Professor. Research Area: Cell biology/oral medicine
- Russell Giordano, DMD, DMSc, Associate Professor. Research Area: Biomaterials
- Dan Nathanson, DMD, MSD, Professor and Chair. Research Area: Biomaterials

The following images represent selected research by GSDM faculty.



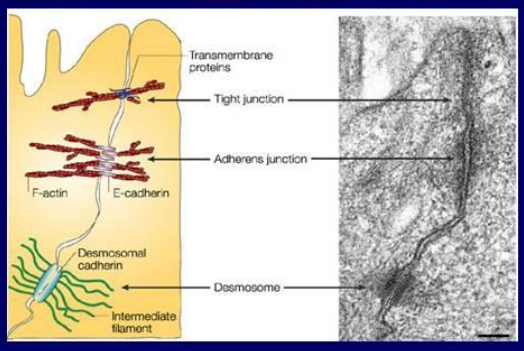
Salivary Glands and Tooth Development Through Branching Morphogenesis



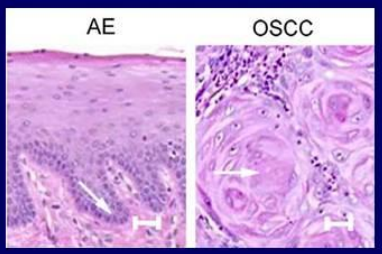
Maria Kukuruzinska, PhD



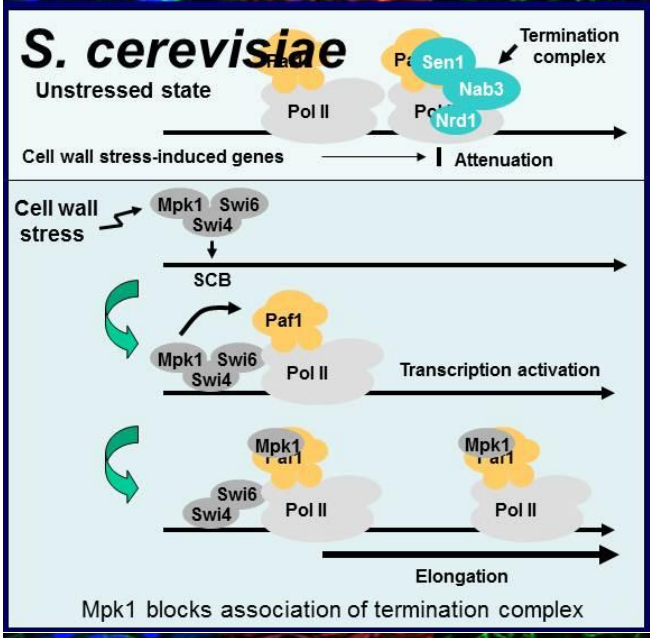
Overexpression of DPAGT1 is a feature of a subset of human Oral Squamous Cells Carcinoma (OSCC)



Adhesion in oral cancer



David Levin, PhD



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

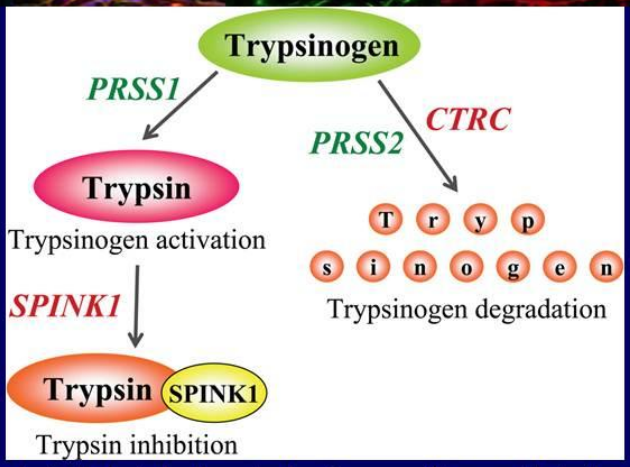
Ruslan Afasizhev, PhD



Trypanosomes in the human blood

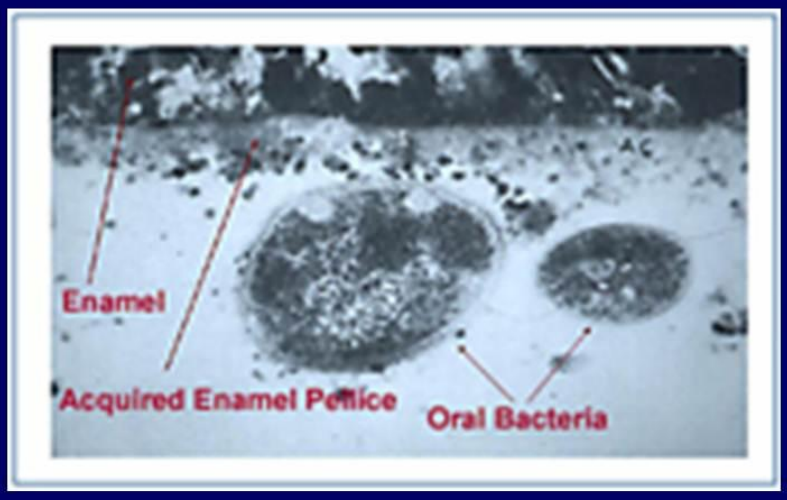
Colored scanning electron micrograph of Trypanosoma brucei among red blood cells. Insect-transmitted trypanosomatid parasites cause chronic and ultimately fatal infections in humans and livestock, for which few safe therapies exist

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

Frank Oppenheim, DMD, PhD



Acquired enamel pellicle (EP) is a protein film resulting from the selective adsorption of proteins present in the oral cavity onto tooth surface



Pre-doctoral Research Program (PRP)

The GSDM developed a highly successful Pre-doctoral 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 www.bu.edu/dental/research/predoctoral. Information on the GSDM Science Day abstracts and awardees is available at www.bu.edu/dental/research/predoctoral/scienceday.

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 Pre-doctoral 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 Pre-doctoral 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 Pre-doctoral Research Committee (PRC), a sub-set of the Research Committee, chaired by the Associate Dean for Research and Director of the PRP. The PRC is 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 Pre-doctoral Research. The mission of the PRC 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 are three options to IREC:
IREC1 - Intensive Research DMD year 1 under Apex;
IREC2 - Intensive Research DMD year 2 (2 credits);
IREC3 - 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 IREC1 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 IREC. Students are considered for the IREC1 if they have participated in research during the second semester on a voluntary basis or if they have prior research experience.
    b. IREC1 trainees are graded by the end of the Apex rotation.

2) The IREC2 takes place during DMD year 2. Students who completed IREC1 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 IREC1.
    c. IREC 2 trainees are graded by the end of Year 2.

3) The IREC3 takes place during DMD year 3. Students who completed IREC1 and/or IRE2 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. IREC3 trainees are graded by the end of DMD year 3.

Activities during the IREC

Project Development
IREC trainees work together with their mentors on the preparation of research proposals through literature reviews, analyses of preliminary data and pilot studies. Project description includes 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 PRP office organizes a seminar series through which IREC trainees learn about different scientific methodologies and approaches. These seminars enrich the trainees' research experience by exposing them to the latest scientific findings and facilitate development of personal relationships among peers.

Journal Club
Each trainee is required to attend a least one journal club directed at developing skills in the critical evaluation of literature by critiquing research papers.

Scientific Writing and Presentation Skills
The PRP Office assists the IREC trainees in the presentation of the research accomplishments at scientific meetings. An emphasis is made on improving oral presentation and writing skills.

Scientific Events
The PRP Office supports the IREC trainees to present their research projects at the IADR/AADR meetings, the Hinman Research Symposium, the Yankee Dental Symposium, the annual GSDM Science Day and Boston University Scholars' Day.

Instructions in the Responsible Conduct of Research (RCR)
Prior to Apex training, The PRP Office informs 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 facilitates training attendance in RCR. The activities include discussion of standards of good practice and policies for handling misconduct allegations. The training program on RCR consists 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 expects to provide guidance and supervision to the trainee. Each mentor formally meets with the IREC trainee on a regular basis to review progress. In addition, mentors are 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 is 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 is evaluated in relation to subsequent research activities and his/her future career plans. A final grade is issued and an assessment summary upon completion of training is provided to the trainee with a comprehensive overview of his/her performance.

Program Evaluation

Assessment of the educational outcome is used by measuring the initial baseline through a pre-program questionnaire. A post-program questionnaire is used to quantify changes in knowledge, skills and career choices. Feedback gathered through evaluation is documented and used to improve the quality of the Program. The student's self-assessment is triangulated with the actual assessment by the mentor. The evaluation helps 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, Scholars Day, Hinnman Symposium, Yankee Dental Congress, etc.
- Publishing opportunity
- AAAS/Science membership
- Regulatory and ethical conduct of research training
- Medical Research Scholars Program
- NIDCR Summer Dental Student Award

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 the GSDM Science Day and BU Scholar's Day. 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.

Timeline of events

August 15, 2014	Apex Rotation Oral Presentations
October 31 - Nov 2, 2014	Hinman Symposium
October 9-12, 2014	ADA/Dentsply Student Clinician Program, New Orleans
January 5, 2015	Deadline for APEX I rotation applications
January 31, 2015	Yankee Dental Congress (YDC39) Student Table Clinics
February 2, 2015	Deadline for IREC1
March 19, 2015	GSDM Science Day
March 11-14, 2015	American Association for Dental Research (AADR), Boston
April 7, 2015	Orientation DMDI and ASI (11 a.m.– 12 p.m.)
April 3, 2015	Orientation to IREC1
April, 2015	BU Scholar's Day
April, 2015	ADA Dental Student Conference on Research, Bethesda, MD
May 18 – July 10, 2015	APEX Rotation

Rotation prerequisites

- Research Rotation Approval Form
- Research outline
- CITI Courses for the protection of human subjects in research and the HIPAA training at http://www.bumc.bu.edu/ocr/instructions-for-taking-bumc-citi-courses/#one.
  sign up as BU first timer and click on biomedical researchers and then on HIPAA. A completion certificate can be downloaded at the end of each course
- Laboratory safety training for lab settings. Information on upcoming sessions at: www.bu.edu/orctraining/ehs/research-safety-training/lab-safety-training-schedule/
- Animal training for students working with animals. Mentor needs to add the trainee to the protocol.
  Course training schedules at www.bumc.bu.edu/IACUC. The animal requirements are:
  - Institutional Animal Care and Use Committee (IACUC) Orientation
  - Laboratory Animal Science Center (LASC) New Researcher Orientation
  - Medical Surveillance Clearance by OEM Research Occupational Health Program.

  To schedule an appointment (ROHP) 617-414-7647 or rohp@bu.edu
- Students who will be working in direct contact with the subjects and/or identifiable data must be added to 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: www.bumc.bu.edu/Dept/Home.aspx?DepartmentID=357

Additional requirements as per individual mentor.

Information about research

- www.bu.edu/dental-research
- http://learn.bu.edu (Blackboard)
- www.bu.edu/dental/research

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.

Sample Outline

Goal:
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

D.M. AFSHAR1, S. KHALIL1, L. BAN2, D. FAUSTMAN2, and M. KUKURUZINSKA1, 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. Supported by grants PHS RO1 DE10183 and RO1 DE14437.

Sample Research Report

Identification of Vimentin-Enriched Cells in Embryonic Submandibular Glands After Wounding

Erin Breen, Meghan Bouchie and Maria Kukuruzinska
Department of Molecular and Cell Biology
Boston University Henry M. Goldman School of Dental Medicine

ABSTRACT

The ability to grow embryonic submandibular glands in organ culture has proved to be beneficial to study various developmental and physiological processes. An important process is the epithelial to mesenchymal transition. EMT is characterized by the loss of epithelial adhesion and gain of mesenchymal features and crucial to embryonic development and the healing process after injury to tissues. Vimentin, a type III intermediate filament, has been found to be involved with EMT and wound healing. By using an ex vivo wound healing model and introducing an injury to the developing embryonic submandibular gland we observed the level of vimentin expression at the site of injury after wounding. We found an increase in vimentin positive cells at and around the site of injury.

INTRODUCTION

The submandibular gland belongs to a group of epithelial tissues, including the lung, kidney, and mammary gland, which develops through a series of morphogenetic changes referred to as branching morphogenesis. Central to this developmental process is the inductive interactions between mesenchymal and epithelial cells. Epithelial-mesenchymal transition is characterized by the loss of epithelial adhesion and gain of mesenchymal features, an important mechanism needed during embryonic development. The submandibular gland initiates at approximately 11 days post-coitum (E11) when an ectoderm derived oral epithelium interacts with neural crest derived mesenchyme. By E12, the epithelium invaginates into the mesenchyme and an end bud enlarges. At E13, clefts form on the enlarged end bud and branching morphogenesis begins. As morphogenesis progresses, regions of the branching epithelium undergo cyto-differentiation, eventually giving rise to a structure consisting of differentiated terminal secretory units, the acini, and an array of secondary ducts that empty into the principal excretory duct and ultimately into the oral cavity. It should also be noted that during epithelial morphogenesis, primitive stem/progenitor cells also undergo a series of cell fate decisions which give rise to more differentiated cell types while simultaneously maintaining a reservoir of stem/progenitor cells.

In addition to its role in early development of the submandibular gland, the epithelial-mesenchymal transition has been shown to play a role in tissue repair in various tissues. Studies have shown that human pancreatic Beta cells undergo EMT before re-differentiating into insulin producing cells. A specific EMT marker is the type III intermediate filament vimentin. Vimentin is normally expressed in cells of mesenchymal origin such as fibroblasts. Vimentin expression has been seen in epithelial cells involved with wound healing. Impaired wound healing in embryonic and adult mice lacking vimentin has been reported and shown to be due to retarded fibroblast invasion and subsequent contraction of wounds, suggesting that vimentin is important for cell motility. In addition, it has been seen that the expression of vimentin decreases after the cells become stationary after wound closure.

To date, however, all the data sited in the literature has been obtained using mostly cell lines. An in vitro wound-healing model by introducing an injury to the intact submandibular gland after it has been extracted from the embryo

has not yet been developed. In this study, we attempt to design a wound-healing model using the submandibular gland from E13.5 mice and subsequently look at the expression of vimentin in wounded and non-wounded glands using immunofluorescence over a 48 hour time period.

METHODS AND MATERIALS

Submandibular Gland Organ Cultures

TSubmandibular glands were dissected from pregnant E13.5 mice. An injury was made using the edge of a pair of surgical forceps. The glands were cultured on Whatman Nuclepore Trach-etch filters at 37° C and humidified 5% CO2 atmosphere. The filters containing either eight wounded or non-wounded glands were floated in 200 ul of DMEM/F-12 supplemented with 100 ug/ml penicillin, 100 ug/ml streptomycin, 150 ug/ml vitamin C, and 50 ug/ml transferrin in 50 mm glass-bottom microwell dishes.

Fixation

Glands were fixed at time 0, 18, 24, and 48 hours. Prior to being fixed, pictures were taken of all the glands in order to course changes in morphology. Glands were fixed using 3.7% PFM solution. 200 ul was added underneath the filter and the filter was put on the shaker for 45 minutes at room temperature. The PFM solution was removed and the filters were rinsed 4 times with 1x PBS followed by one large volume wash (3-4 ml) and placed on the shaker for 10 minutes. The glands were then stored at 4° C in the large volume wash.

Blocking

Prior to blocking, the filters were removed from the 4°C and the large volume wash was removed. 200 ul of 0.1% Triton-X PBS was added over the filter and left on the shaker for 20 minutes at room temperature. The solution was removed and 3 large volumes washes were done using PBS 0.1% tween. Each wash was left on for 10 minutes while the filters were on the shaker. The glands were blocked overnight at 4°C using bovine serum albumin (BSA) with 10% goat serum in 1x PBS 0.1% tween. The PBS 0.1% tween solution was made using 500 ml PBS and 500 ul tween 20. The 10% BSA solution was made by adding 1 gram BSA to the final volume of 10 ml using 1x PBS 0.1% tween. This solution was passed through a filter. The final blocking solution was made using 1 ml 100% goat serum, 1 ml 10% BSA, and 8 ml 1x PBS 0.1% tween.

Staining

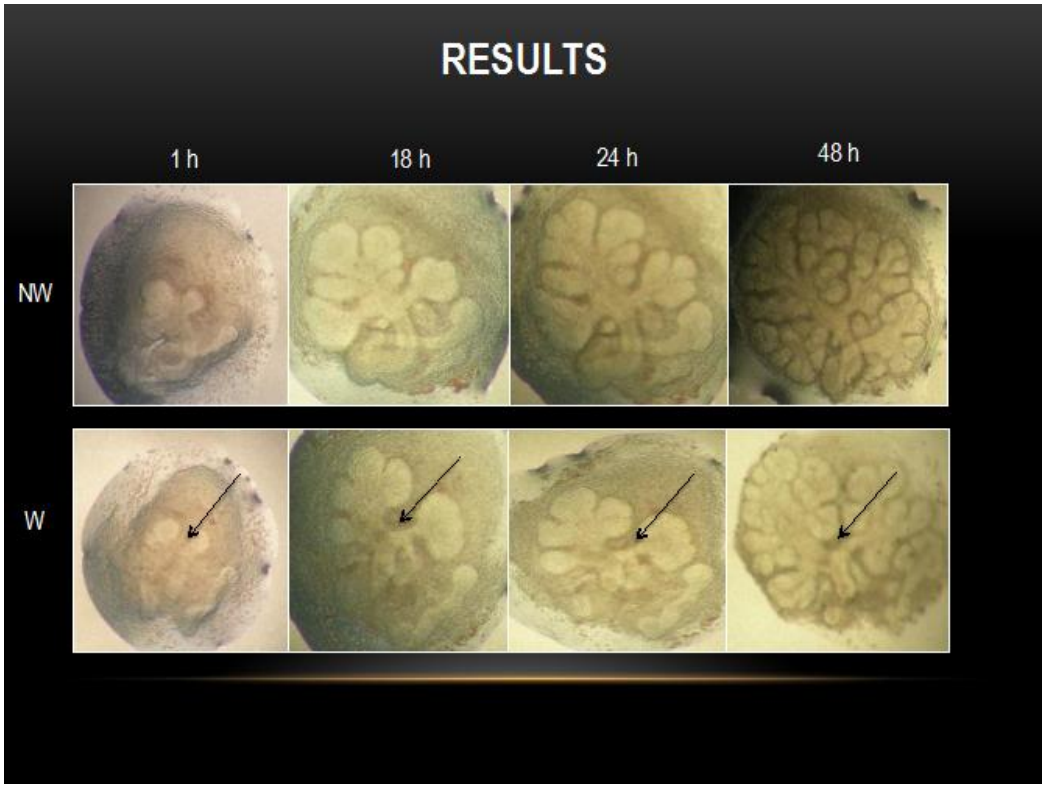
The primary antibody was diluted 1:10. To each filter 200 ul of solution consisting of 160 ul 1x PBS 0.1% tween, 20 ul 10% BSA, and 20 ul of primary antibody was added. The primary antibody was left on the filters for 3 hours on the shaker at room temperature. After the filters were removed from the shaker, 4 quick washes of 200 ul using PBS 0.1% tween were performed. One large volume was then done and the filters were put on the shaker for 10 minutes. The secondary antibody was diluted 1:100 (198 ul of PBS 0.1% tween and 2 ul goat anti-mouse secondary antibody). 200 ul was added to each filter and put on the shaker for 1.5 hours after which the solution was removed. Finally, an F-actin nuclear stain was applied for 30 minutes. After this solution was removed, the glands were kept in PBS 0.1% tween until mounting.

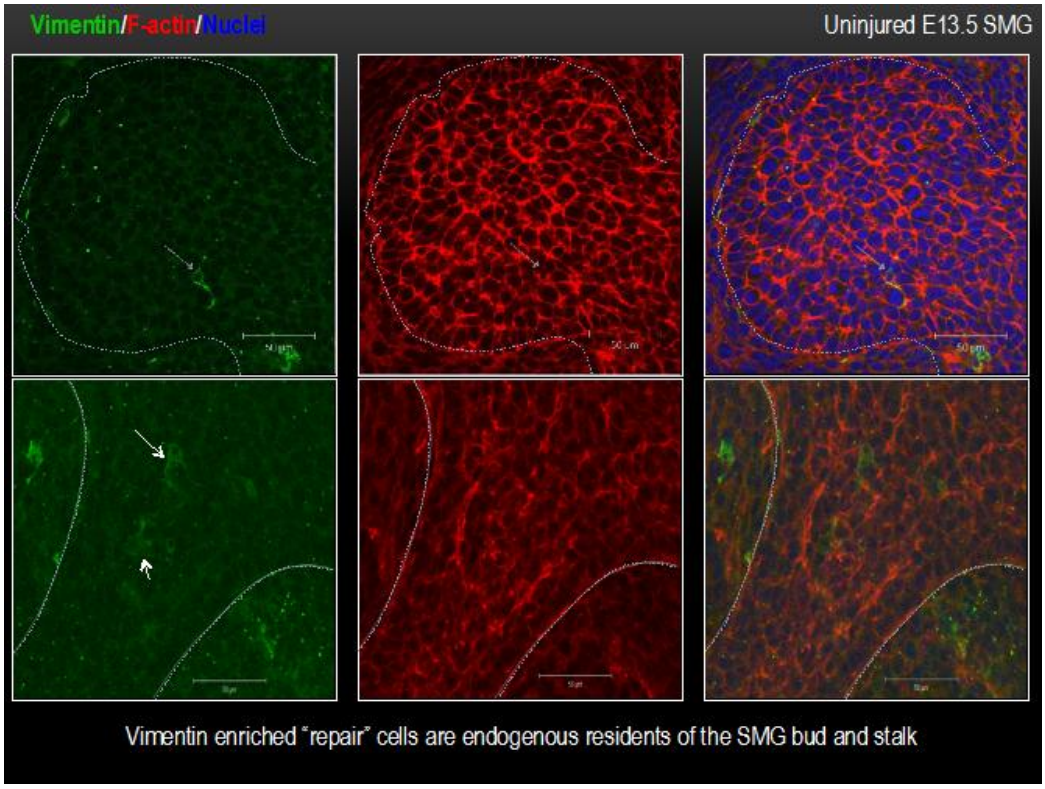
Mounting

Slides were cleaned with ethanol and labeled with the date of dissection and primary antibody. A custom chamber was placed on each slide and 15 ul of vector shield was applied to the center of the slide and spread. The filters were submerged in PBS and the glands were removed from the filters using forceps. The glands were placed on the

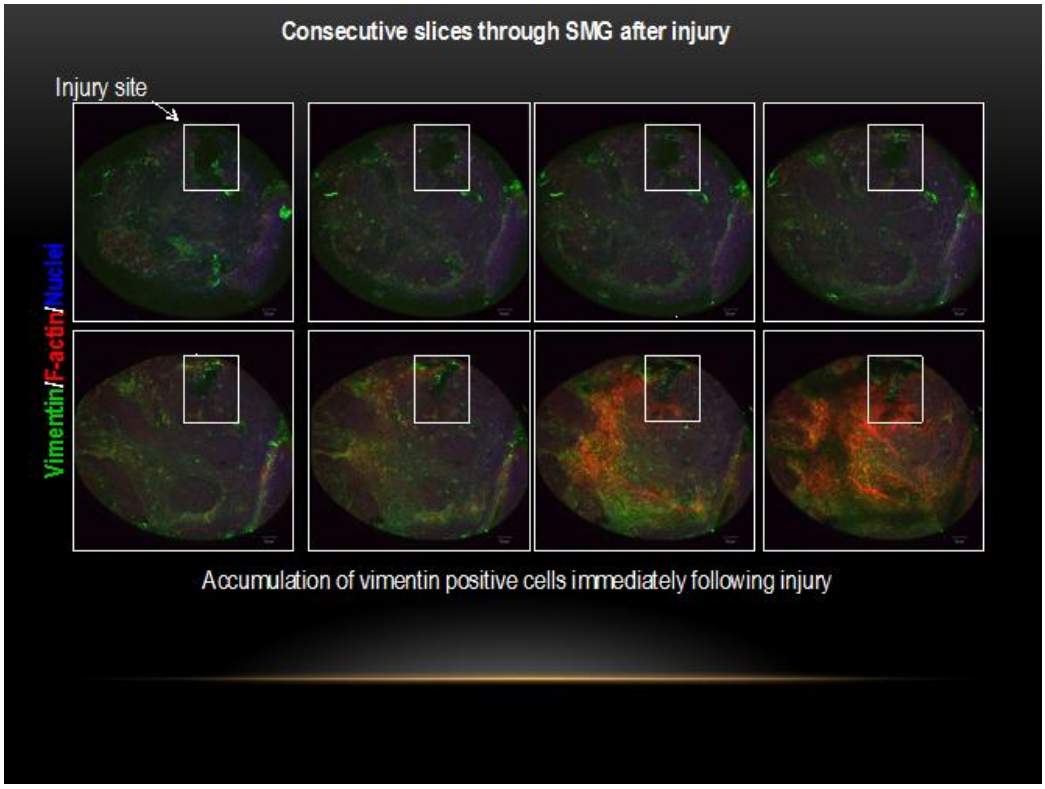
slide in the vector shield and a coverslip was added on top. Slides were wrapped in aluminum foil and kept at 4°C. Episcope images were taken to confirm there was staining, but confocal images were taken to localize the staining.

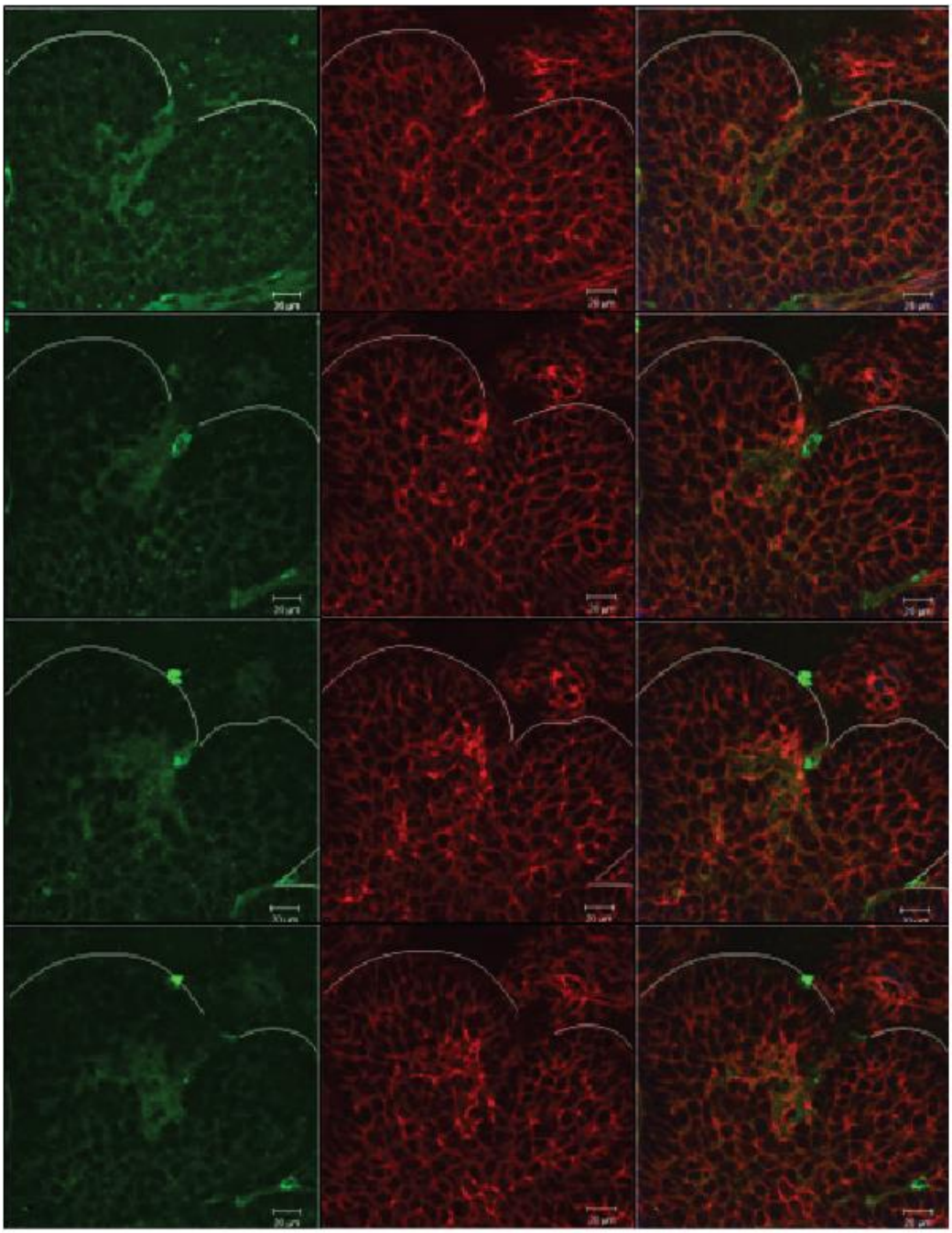
RESULTS





Vimentin positive cells accumulate around the injury site





DISCUSSION

After the initial studies we can conclude increased expression of vimentin was detected around the site of injury. In addition, vimentin enriched repair cells are endogenous residents of the SMG bud and stalk. Previous reports suggest that vimentin positive cells involved in wound healing represent stem cell niches. It is possible that the cells recruited to the site of injury of the wounded SMG were recruited from this niche of cells. Finally, it appears that the SMG is likely to serve as a wound healing model based. Further studies will increase the understanding of the mechanisms of regeneration and repair of the SMG.

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Loan repayment program: www.lrp.nih.gov

Dental research organizations:

www.dentalresearch.org

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