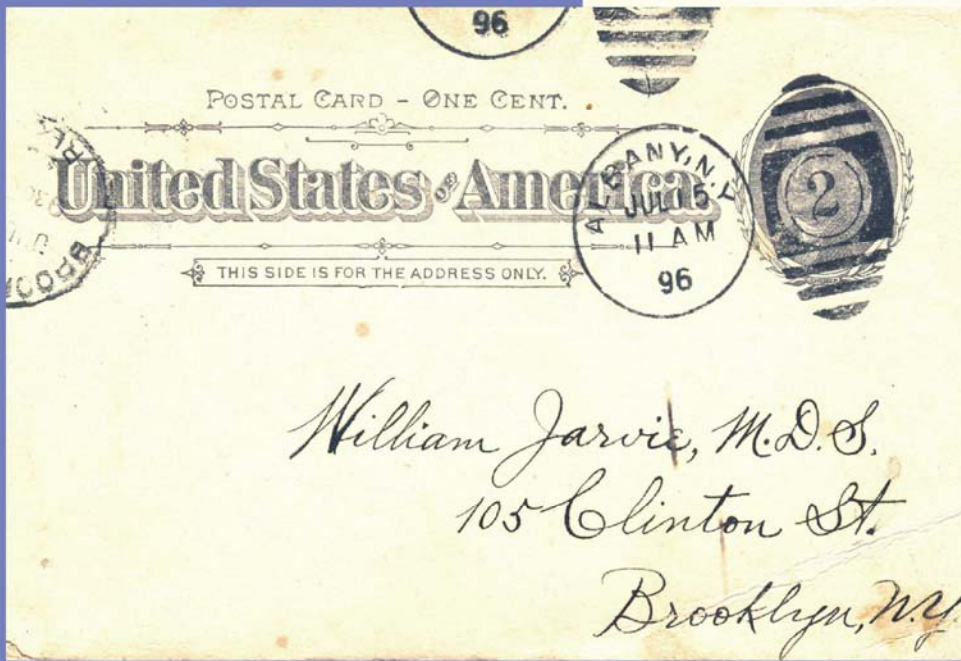
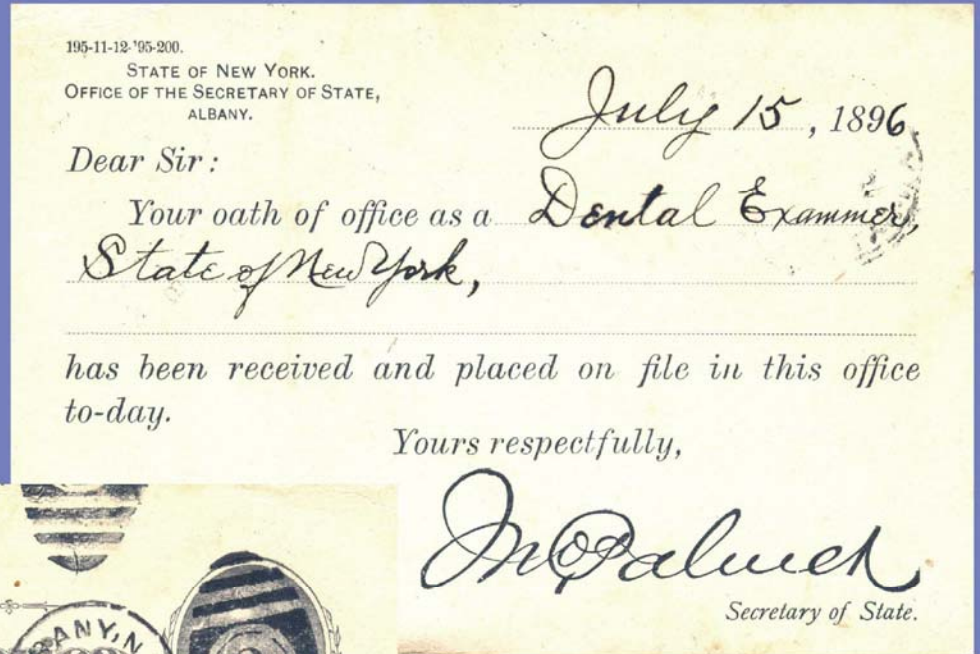




Jarvie

Journal of the William Jarvie Society

Volume 51, Spring 2008



Published by the William Jarvie Society
College of Dental Medicine
Columbia University





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Volume 51, Spring 2008

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College of Dental Medicine
Columbia University
City of New York
www.dental.columbia.edu/jarvie**

**Birnberg Research Program
April 9-10, 2008**

Editor-in-Chief

Tina Vani '08

Associate Editors

Phil Mann '09 Peter Ok '08 Deborah Weng '09

College of Dental Medicine, Columbia University, 630 W. 168th Street, New York, NY 10032

“When apparently we have reached the limits of possibility, new avenues of progress and advancement are opened to our view and advances which shall make our knowledge of today seem in the light of the future to be but the densest ignorance.”

William Jarvie 1905

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A Message from the Editor

For the 51st year, we present *Jarvie*, the *Journal of the William Jarvie Society*, as an official tribute to Columbia University College of Dental Medicine's commitment to research for over half a century. Our new cover displays a historic document from 1896 that belonged to William Jarvie, himself, and takes us back to the origin of the Jarvie Society.

Vision, determination, and persistence have led to the longevity of this journal. Curiosity, however, is often what initiates such intellectual investigations. As Zora Neale Hurston (1891-1960), graduate of Barnard College, American folklorist, and author during the time of the Harlem Renaissance said, "Research is formalized curiosity. It is poking and prying with a purpose. It is a seeking that he who wishes may know the cosmic secrets of the world and that they dwell therein."

The abstracts here reflect the curiosity and hard work of students and mentors across the scientific spectrum. We thank them for sharing their ideas and findings with the Columbia community.

We would like to thank our devoted mentors for their guidance throughout the year and in making this journal: Dr. Richard Abbott, Director of the Office of Research Administration; Dr. Jeremy Mao, our new faculty advisor; Dean Ira Lamster; Dean Letty Moss-Salentijn; and Dean Martin Davis.

Thank you to the executive board of the Jarvie Society and its members for their efforts this year. It is an honor to be a part of this distinguished group. We are also very grateful to Dentsply for sponsoring the Student Clinician Award.

Tina Vani
Class of 2008



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March 25, 2008

Members of the Jarvie Society:

Last year marked the 50th anniversary of the Jarvie Journal, and over the past few years we have seen a notable increase in the number of abstracts presented by predoctoral and postdoctoral students at our Student Research event, known as Birnberg Day.

Birnberg Day and the publication of the Jarvie Journal is one highlight of our academic calendar. On a personal level, I greatly enjoy listening to the presentations, as each participant demonstrates great enthusiasm for their work. Our presenters are invariably well prepared, and the faculty is always impressed by the sophistication of the research.

The Columbia University Medical Center (CUMC) is justifiably proud of its research program. The publication of the Jarvie Journal and the presentations on Birnberg Day are an important part of the College of Dental Medicine's contributions to the CUMC culture of scholarship and discovery.

This is an incredibly exciting time to be part of biomedical research. Our understanding of the essential elements of life is coming into focus, we have a new appreciation for the etiology of many diseases, and stem cell research offers exciting new possibilities for improving the quality of life. Your involvement in research is important from many perspectives, and emphasizes dental medicine as an integral part of the health sciences.

Enjoy the experience!

Sincerely,

Ira B. Lamster, DDS, MMSc
Dean

Columbia University Medical Center



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March 21, 2008

Dear Members of the William Jarvie Society,

It is clear that you are all aware of the importance of research in the health care professions since you followed the advice that was given to you when you matriculated in the College of Dental Medicine, to take advantage of the opportunities that the Health Sciences environment of this great research university offers you. Importantly, you have been fortunate enough to find excellent research mentors and your work that is presented here on our annual student research day, is the result both of the guidance of your mentors and of your own sustained efforts.

Research is the lifeblood of our profession and in truth, when research stagnates the profession cannot hope to stay alive. Some of you may think of your research efforts as a single episode in your training, useful to enhance your chances to get into a coveted postdoctoral residency. Although I am a realist, I would be disappointed if that were the case. I hope that most of you will remember the excitement of engaging in original research, that spark that lit the “fire in your belly”, so that you will have the desire to continue your explorations in order to advance your profession’s knowledge base.

Each year I believed that the presentations could not possibly get any better and each year I have been proven wrong. Your work is once again outstanding. We all look forward to your presentations and hope to engage in some interesting dialogues.

Congratulations on a job well done. I wish you all a successful student research day.

Sincerely,

Letty Moss-Salentijn, DDS, PhD
Robinson Professor of Dental Medicine
(in Anatomy and Cell Biology)
Senior Associate Dean for Academic Affairs

Columbia University Medical Center

History of the William Jarvie Society*

The William Jarvie Society for Dental Research was organized on December 16, 1920. At the invitation of Dr. William J. Gies, all the undergraduate students of dentistry at Columbia University conferred with him for the purpose of considering the desirability of organizing a society of students, teachers, and benefactors for the promotion of the spirit of research in the School of Dentistry.

After general discussion, it was unanimously voted to proceed with the proposed organization and Joseph Schroff, M.D.**, was elected temporary chairman. Because of the important relation, which Dr. William Jarvie bore to the establishment of the School of Dentistry, and because of high interest in the promotion of dental research, it was unanimously voted that the society be named the William Jarvie Society for Dental Research, and that Dr. William Jarvie be elected an honorary member.

Dr. Schroff served ably as president during 1922. Dr. Monasch officiated during 1923, and in 1924, because of the amalgamation of the College of Dental and Oral Surgery with the School of Dentistry of Columbia University, interest in the organization diminished and the society ceased its activities in 1925. On February 7, 1929, the society resumed activity and elected officers. Interest revived and the organization was again brought into prominent place in the extracurricular life of the school.

During 1932-33, several members of the faculty who had contributed greatly to research in dentistry and allied fields addressed the members of the society and their guests. Dr. Charles C. Bodecker, Professor of Oral Histology and Embryology, spoke on "Dental Caries and Allied Subjects" and illustrated his talk with a liberal number of lantern slides. Dr. Bodecker spoke of the various theories and the classification of dental caries, and also explained the caries index for recording the extent of caries. He also briefly outlined the work done by various investigators in this field.

Dr. Byron Stookey, Associate Professor of Neurological Surgery, addressed the next open meeting, which was held as a feature of the alumni day activities. His topic was, "The Interpretation and Treatment of Painful Affections of the Trigeminal Nerve." In a most interesting and instructive lecture, Dr. Stookey showed the relationship of diseases of this nerve to dental diagnosis. He explained the past work done in this field and the newer methods of surgical treatment, illustrating his talk with many lantern slides. He also presented several patients to demonstrate the effectiveness of his surgical treatment of this disease.

The Jarvie Society recorded another year of activity and accomplishment. Student interest in the organization was never greater, and a long and vigorous future for the society seems assured. The future of dentistry lies in its research into the problems that beset it and the Jarvie Society has done its share in stimulating interest in this long-neglected phase of our work.

*An excerpt from the *Dental Columbian*, 1933.

** Editor's Note: Dr. Joseph Schroff, M.D., one of the first two students admitted to the dental school through the Columbia admissions process, became the first student to receive the Columbia DDS degree in 1922. Dr. Schroff subsequently joined the SDOS faculty, teaching Oral Surgery to generations of students until his retirement as head of Oral and Maxillofacial Surgery in the early 1950s.

The Birnberg Research Award

The Birnberg Research Award was established by the Alumni Association of the Columbia University School of Dental and Oral Surgery in the early 1950s to encourage dental research of excellence and to help stimulate public interest in support of dental research. The award is named in honor of Dr. Frederick Birnberg (1893-1968), class of 1915, who helped to establish a research fund.

The College of Dental Medicine faculty research committee, in conjunction with the school's Alumni Association, considers individuals who have made important contributions to dentistry through both research and mentoring for selection as Birnberg Lecturer and recipient of the Birnberg Award. Fifty-one outstanding scientists and teachers have been honored as the Birnberg Lecturer since the first Birnberg Award was presented in 1954.

Birnberg Lecturers and Award Recipients

1954--- Dr. Charles F. Bodecker	1976--- Dr. Jersome Schweitzer	1994--- Dr. Ze'ev Davidovitch
1955--- Dr. Joseph Appleton	1977--- Dr. George Green	1995--- Dr. Ivar Mjor
1956--- Dr. Isaac Schour	1978--- Dr. David Scott	1996--- Dr. Lorne M. Golub
1957--- Dr. Ralph Phillips	1979--- Dr. Berge Hampar	1997--- Dr. Bruce J. Baum
1958--- Dr. Reider F. Soqnaes	1980--- Dr. Barnet Levy	1998--- Dr. Kenneth Anusavice
1959--- Dr. John Knuston	1981--- Dr. Ronald Dubner	1999--- Dr. James D. Bader
1960--- Dr. Maxwell Karshan	1982--- Dr. Martin A. Taubman	2000--- Dr. Lars Hammerström
1961--- Dr. George Paffenbarger	1983--- Dr. Louis T. Grossman	2001--- Dr. David T. W. Wong
1962--- Dr. Eli Goldsmith	1984--- Dr. Solon A. Ellison	2002--- Dr. Henning Birkedal-Hansen
1963--- Dr. Edward V. Zegarelli	1985--- Dr. Norton S. Taichman	2003--- Dr. Barbara Dale-Boyan
1964--- Dr. Francis A. Arnold	1986--- Dr. Ronald J. Gibbons	2004--- Dr. Paul B. Robertson
1965--- Dr. Seymour Kreshover	1987--- Dr. Robert J. Gorlin	2005--- Dr. Bruce L. Pihlstrom
1966--- Dr. Paul Goldhaber	1988--- Dr. Enid A. Neidle	2006--- Dr. Jeffrey D. Hillman
1968--- Dr. Sholom Peariman	1989--- Dr. David H. Pashley	2007--- Dr. Ralph V. Katz
1970--- Dr. Melvin Moss	1990--- Dr. William H. Bowen	2008--- Dr. Robert J. Genco
1971--- Dr. Irwin Mandel	1991--- Dr. Harold C. Slavkin	
1973--- Dr. Lester Chan	1992--- Dr. George R. Martin	
1975--- Dr. Russell Ross	1993--- Dr. Richard Skalak	

2008 Birnberg Lecturer

Robert J. Genco, DDS, PhD

Dr. Genco received his DDS from the State University of New York at Buffalo School of Dentistry in 1963. Following dental school, Dr. Genco studied at the University of Pennsylvania where he earned the Certificate in Periodontics from the School of Dental Medicine and the PhD degree from the Graduate School (Department of Microbiology and Immunology) in 1967. Dr. Genco holds appointments at the State University of New York at Buffalo as SUNY Distinguished Professor in the Department of Oral Biology (School of Dental Medicine), Department of Microbiology (School of Medicine and Biomedical Sciences) and the Department of Immunology (Roswell Park Cancer Institute). Presently, Dr. Genco is at the Joslin Diabetes Center, midway through a two and one half year appointment as Visiting Professor of Medicine at the Harvard Medical School.

Dr. Genco has received numerous honors and awards, including the William J. Gies Foundation Award (American Academy of Periodontology, 1983), the Seymour J. Kreshover Lecture (Sponsored by the National Institutes of Health, 1985), the Gold Medal for Excellence in Dental Research (American Dental Association, 1991), and the William J. Gies Award (American Dental Association, 2002). Throughout his career, Dr. Genco has been selected to deliver special lectures including the Kreshover Lecture at the National Institute of Dental and Craniofacial Research (National Institutes of Health, 1985) and the Moskow Lecture at the College of Dental Medicine (Columbia University, 2002). Dr. Genco holds memberships in the National Academy of Science (Institute of Medicine), the American Association for Dental Research (served as president: 1985), and the International Association for Dental Research (served as president: 1991) among other scientific and professional organizations.

Through most of his career, Dr. Genco's research has focused on oral infections, with an emphasis on the systemic consequences of oral infection, as well as the effect of systemic conditions on the progression of oral disease, particularly in the patients with diabetes mellitus, cardiovascular and cerebrovascular diseases. In 1999, Dr. Genco was one of the co-chairs, along with Dr. Ann Marie Schmidt (Columbia University) of an NIDCR sponsored workshop on "Oral Diseases and Diabetes." Two other areas of interest have been the effects on oral bones of osteoporosis and the reduced estrogen in postmenopausal women, and the use of tissue engineering for the regeneration of the periodontium and the bones around implants.

Birnberg Research Program

WEDNESDAY, April 9, 2008, 2:00-5:00 P.M.

THURSDAY, April 10, 2008, 12:00-2:00 P.M.

WEDNESDAY, April 9, 2008

2:00-5:00 P.M. Table Clinic Presentations
Hammer Health Science Center
Riverview Lounge
HHSC-Fourth Floor

THURSDAY, April 10, 2008

12:00-1:00 P.M. Birnberg Research Lecture
Robert J. Genco, DDS, PhD
SUNY Distinguished Professor (Department of Oral Biology,
School of Dental Medicine; School of Medicine and
Biomedical Sciences; Roswell Park Cancer Institute)
Director, Periodontal Disease Clinical Research Center,
School of Dental Medicine;
Vice Provost of Science, Technology Transfer, and Economic
Outreach (STOR); State University of New York at Buffalo

HHSC-301

“Oral and Systemic Disease: Crossing the Barriers of Interdisciplinary Research in Academic Health Centers”

1:00-2:00 P.M. Dentsply Award Presentation
Buffet Luncheon
**Special event: Dedication of the Irwin Mandel
Student Research Program at CU-CDM**
Riverview Lounge
HHSC-Fourth Floor

A Message from the Jarvie President

This year, the Jarvie Society celebrated our most renowned researchers within the College of Dental Medicine in an effort to encourage students to realize the impact Columbia University has within dental research. A mission of the organization is to create an appreciation for research. Not only do we want students to pursue research as dental students, but we also want them to consider research as a career option. Though a majority of students do end up in clinical fields, our university, with the guidance of Jarvie Society, would like to develop clinicians who are aware of and incorporate the newest innovations and findings within research into their clinical practice.

As we present the 51st edition of the *Journal of the William Jarvie Society*, we celebrate the achievements of students, residents and their mentors alike. Without the continued support of the mentors, the achievements of the authors of the journal would not be possible.

In our celebration of the “Greatness of Columbia” we have several individuals to extend gratitude towards. In our introduction to research for the incoming first year class, Dr. Richard Abbott, Dr. Heera Chang and Dr. Shantanu Lal provided their personal reflections of how research made an impact on their personal, professional and clinical lives. Dr. Jeremy Mao jump started our “lunch and learn” sessions with his well-published work on stem cells. Dr. Ira Lamster, Dean of the College, provided options for students within research and academics. As a final presenter of our “lunch and learn” session, Dr. John Grbic presented his work on bisphosphonate-related osteonecrosis of the jaw recently published in the *Journal of the American Dental Association*. This year, our members have truly heard from some of Columbia’s best in research.

The Jarvie Board has worked tirelessly to make access to and awareness of research projects much easier for its members. Our second annual Jarvie Student Research Panel was again a great success. Second and third-year students discussed experiences in research and externships abroad, including participants in NIDCR, Amsterdam, Australia, Turkey, Japan, CUMC, and other national meetings. We brought forward a new social event for our members to provide a more informal setting to discuss research opportunities at the College of Dental Medicine.

Our organization has made a transition in its leadership. Dr. Jeremy Mao has graciously accepted a role as a faculty advisor and in his first year has brought forth major contributions to our organization. The Jarvie Research Board would like to express special gratitude to Dr. Heera Chang in her role as faculty advisor and past president of Jarvie Society as she moves along in her career. The successes of this organization would not have been possible without the continued support of Dr. Richard Abbott, Dr. Ira Lamster, Dr. Letty Moss-Salentijn and Dr. Martin Davis.

At the end of my term as president of the Jarvie Society, I would like to thank Kristina Rodriguez, Hannah Ahn, Ken Zi Wang, and Ryan Turner for the unequivocal dedication they have displayed as members of the executive board. Our editors, Tina Vani, Debbie Weng, Phil Mann and Peter Ok have spent countless hours producing this year’s Jarvie Journal and their work has been remarkable. Lastly, thank you to our members, for without them, this organization, this journal and student research would not have been such a success at Columbia University.

Neeraj Panchal
Class of 2009

2008 William Jarvie Society Membership

Officers:

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President:	Neeraj Panchal '09
Vice President:	Kristina Rodriguez '09
Secretary:	Hannah Ahn '08
Treasurer:	Ken Zi Wang '09
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Webmaster:	Phil Mann '09
Associate Editors:	Phil Mann '09 Peter Ok '08 Deborah Weng '09

Advisors:

Dr. Richard Abbott
Dr. Jeremy Mao

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Kavita Bhalala	Gloria Lee	Pooria Shahin
Walter Chen	Stephenie Lee	David Simhaee
Ray Cheng	Bernard Lam	Scott Solow
Jung Inn Choi	Ben Liu	Laura Sotomayor
Toby Cohen	Sathya Mahendrarajah	Shahnaz Tendulkar
Janish Desai	Phil Mann	Tran To
Hai Do	Petro Matsyshyn	Ryan Turner
Amanda Eidelson	Steven Nadler	Tina Vani
Idar Hsin	Jeffrey Nichelini	Violetta Vayner
Lisa Hsu	Mariel Nortick	Ken Zi Wang
Michael Huang	Aisling O'Connor	Deborah Weng
Betty Huang	Peter Ok	Ben Yagoubian
Raymond Jone	Neeraj Panchal	Charles Yau
Yasemin Kilical	Derek Park	
Junghyun Kim	Tasneem Rangwala	

Visit us at www.dental.columbia.edu/jarvie

Pre-Doctoral Student Abstracts

Nicotine Nasal Spray for Pain and Inflammation After Third Molar Surgery

Payam Afzali¹, Benjamin Yagoubian¹, Joseph Akkara¹, David Alfi²,
Jessamyn Conell-Price³, Janet Yeh³, Pamela Flood^{3,*}

¹College of Dental Medicine, Columbia University, New York, NY; ²Division of Oral and Maxillofacial Surgery, College of Dental Medicine, Columbia University, New York, NY; ³Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, New York, NY; *Faculty Mentor

Background: Third molar extraction is a common paradigm for the study of post surgical pain with a major inflammatory component. Clinical trials have suggested that nicotinic receptor activation with nicotine nasal spray (or patch) provides analgesia and opioid sparing in a general surgery population. Clinical trials have also suggested that nicotine nasal spray dosage is not limited to the adverse side effects common in analgesics prescribed for postoperative pain. Common analgesics prescribed are combinations of acetylsalicylic acid, acetaminophen with codeine and the non-steroidal anti-inflammatory analgesics. The effect of the analgesics is more effective early on if given before the effect of local anesthesia subsides and may require a less potent analgesic. The post surgical pain begins as the effects of local anesthesia diminishes and reaches its maximum intensity within the first 12 hours post operatively. The most important determination of the amount of pain is the surgical time. A nicotine nasal spray may be beneficial as adjunctive treatment in control of postoperative pain.

Objective: The objective of this study is to determine whether treatment with nicotine nasal spray (3mg) reduces pain and or inflammation post third molar extraction.

Materials and Methods: This is a prospective, double blind randomized crossover trial. Subjects will be treated with nicotine (spray) prior to ipsilateral removal of maxillary and mandibular third molars and a placebo prior to removal of molars on the contralateral side at a subsequent visit. The primary outcome variable is a numerical analog pain score (NRS 0-10). Secondary variables are NRS scores for inflammation and Vicodin use. Planned enrollment is for 26 non-smoking subjects.

Results: Eleven subjects have been enrolled. Data from 8 subjects has been analyzed. The average NRS in the first hour was 1.3 ± 2.0 (Mean \pm SD) during the nicotine session and 2.3 ± 2.7 during the placebo session ($P < 0.35$). The strongest analgesic effect occurred the first three days after surgery. Data on inflammation and nausea has not yet been analyzed.

Conclusion: With data from only 8 of 26 subjects analyzed it is premature to have conclusions. However, there is some suggestion that the subjects have less pain after the session in which they were treated with nicotine.

Payam Afzali was supported by a College of Dental Medicine Research Assistantship

The Use of MTA Second Ferrule Post Space Barriers to Decrease the Incidence of Complications from Microleakage and Root Fracture Following Post and Core Preparation

Cory Bailey, Luba Borukhova, Rush Davidson, Phillip Josephs, Sung-Woo Kang, Eunice Kim, Jaepil Kim, Thao Le, Reyna Nguyen, BJ Park, Tina Vani, Jack Levi*
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**Faculty Mentor*

Introduction: Apical migration of microorganisms and their toxins through inadequate coronal, and post and core restorations, may lead to persistent periapical inflammation. The use of posts in endodontically treated teeth has led to fracture susceptibility of the tooth, post, and root. The placement of additional material between the gutta percha and the terminus of the post will allow the post to be incorporated into the material, forming a collar for greater stabilization and inhibition of microleakage of oral microorganisms. Portland cement, (PC), and MTA are similar and can anchor the post at its apical end, as well as preventing the penetration of dye. PC is less expensive and more available. (PC) and MTA are biocompatible, antimicrobial, and have calcium hydroxide releasing properties.

Aim: To investigate if placement of a post space, second ferrule on a root canal treated tooth with a post and core preparation will lessen microleakage, post dislodgement and possible root fracture.

Methods: Twenty extracted human molar teeth were accessed, instrumented with hand and Sequence NiTi rotary files. The root canals were filled with gutta percha and Columbia root canal sealer. Post and core preparations were made, using $\frac{1}{2}$ the root length plus 2mm for the post preparation length. Parallel sided, passive; Para posts were selected according to root width diameter. Using a Centrix syringe, with a 19 gauge needle, 2 mm of (PC) was placed over the gutta percha to prepare the 2nd ferrule post space barrier. The ends of the Para posts were angled 30 degrees and flattened to enable the post to be incorporated into the PC barrier material. The controls had posts fitted to the gutta percha without a PC barrier. Posts for the experimental and the controls were cemented with Panavia cement, a self curing resin bonding cement. Ti Core with Titanium cement was used to form the core. Crown preparations with a ferrule were made on all teeth. A # 6 round bur was used to drill a hole below the ferrule to simulate caries. Methylene blue dye enhanced with barium sulfate for radiopaque contrast, was injected for one hour into the holes of the control and experimental teeth. The apices of these teeth had 1mm cut off and inserted into a high vacuum suction tip lined with gauze. Observations and comparisons of dye penetration were made. Both experimental and control teeth were subjected to two hours total of lateral and horizontal striking forces using a gold foil electric plugger. X rays were taken to compare displacement or root fracture between the experimental and control. Dye penetration was again performed through the drilled holes, and evaluated for one hour of high speed suction.

Results and Conclusions: The combination of Panavia Cement and Ti-Core Titanium in cementing Para posts and core build up, played a very positive role in preventing root, and post fracture, as well as acting as a good barrier for microleakage. In all twenty teeth, there was only one case of a root fracture to the experimental, one core fracture to the control and one post and core displacement. The remaining 17 teeth experienced no incidents to fracture of the post, displacement of the post, fracture of the root, or fracture of the core, all after impaction.. The results were unable to single out strong differences with microleakage and post failure, between the controls, and experimental containing the MTA ferrule barriers. One control experienced microleakage before impaction. Four experimental and four control teeth experienced microleakage after impaction. This study was unable to evaluate micro-displacement of materials in the root canal, micro movement of the post or micro fracture of the root. We can only infer that. the second ferrule, post space barrier will prevent post failures and protect the tooth from microleakage associated with infection.

Predominant Expression of Chemokine GCP-2 (CXCL6) in Periodontal Disease

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Background: The chemokine granulocyte chemoattractant protein 2 (GCP-2/CXCL6) is involved in neutrophil recruitment and migration. Previous studies showed that GCP-2 is upregulated in mucosal inflammation, e.g., in inflammatory bowel disease, similarly to the functionally and structurally related chemokine interleukin 8 (IL-8). Nevertheless, unlike IL-8, a role of GCP-2 in gingival inflammation has not yet been demonstrated.

Objectives: We aimed to evaluate the expression of GCP-2 in clinically healthy versus diseased gingival tissues and to explore possible correlations with clinical, microbiological and immunological factors.

Materials and Methods: Gene expression in 184 diseased and 63 healthy gingival tissue specimens of 90 periodontitis patients was analyzed using Affymetrix U133Plus2.0 arrays. GCP-2 expression was further confirmed by real-time RT-PCR, western blotting, and ELISA while the localization of GCP-2 expression in the gingival tissues was analyzed by immunohistochemistry. Plaque samples from adjacent periodontal pockets were collected and evaluated for 19 periodontal species using checkerboard DNA-DNA hybridizations.

Results: Among all inflammatory cytokines, GCP-2 mRNA was the most highly expressed (3.8 fold, $p < 1.1 \times 10^{-16}$) in diseased versus healthy tissue, as compared to a 2.6 fold increased expression of IL-8 mRNA ($p < 1.2 \times 10^{-15}$). Increased expression of GCP-2 correlated with higher levels of red and orange complex pathogens and with increased probing depth, but not with attachment loss. GCP-2 was found to be expressed in gingival vascular endothelium and pocket epithelium.

Conclusion: GCP-2 correlates with the severity of periodontitis and appears to act as a hitherto unrecognized functional adjunct to IL-8 in diseased gingival tissues.

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Role of the ETS-1 in Skin Tumorigenesis

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Introduction: Ets-1 is a proto-oncogene and a member of the Ets family of transcription factors that share a unique DNA binding domain, the Ets domain. Ets transcription factors play an important role in a variety of physiological and pathological processes, including embryogenesis, wound healing, and tumor progression. Ets-1 binding sites have been described on the promoters or enhancers of many of metastasis and invasion-associated genes, including matrix metallo- proteinases (MMPs) and integrin $\beta 6$. ETS-1 is normally expressed in basal layer of the epidermis. Interestingly mis-expressing ETS-1 in the suprabasal layers can generate epithelial tumors. We analyzed the expression of integrins and basement membrane (BM) proteins in these ETS-1 generated tumors in order to study the correlation between integrin expression and loss of BM integrity.

Objective: The purpose of this study is to characterize tumors generated by mis-expression of the ETS-1 transcription factor in the supra-basal layers of epithelium of experimental mice. ETS-1 is a member of the ETS family of transcription factors and has been identified as having a role in formation and progression of cancerous skin lesions. Normally ETS-1 is found in layers of the epithelium near the basement membrane. However, by introducing ETS-1 into epithelial layers further from the basement membrane, tumors can be generated in experimental mice. Analyzing these tumors and characterizing them based on various cellular and extracellular markers will increase our understanding of the factors that are important in cancer progression.

Materials and Methods: Skin and Oral tissue samples from mice in which ETS-1 had been mis-expressed in the suprabasal epithelium were divided into two groups: affected and unaffected. The affected group represented mice in which the tumor phenotype was strongly expressed whereas the unaffected group consisted of mice in which the tumor phenotype was mildly expressed or not expressed. The tumors were analyzed using antibodies against basement membrane markers, integrins, e-cadherin etc and visualized under a fluorescence microscope. Wild type mice were used as the control for the study.

Results: We found a strong correlation between the loss of the BM integrity and changes the integrin expression in the tumors that we analyzed.

Discussion: The Ets family of transcription factors may play an important role in the progression of cancer by virtue of its ability to regulate the expression of ECM remodeling enzymes the MMPs as well as integrins (which are involved in maintaining an intact ECM).

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The Use of Super-EBA 2nd Ferrule Post Space Barriers, to Decrease the Incidence of Microleakage, Post/Tooth Complications Following Root Canal Therapy

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Introduction & Objective: The use of an embracing marginal collar preparation of 1.5-2mm, called a ferrule, assists in preventing tooth fractures. The ferrule transfers horizontal forces onto the root, and decreases the forces transferred by the post cervically. The objective of our pilot study was to show that the placement Super EBA (super ethoxy benzoic acid) as a post space barrier, into the root canal, can serve as a second ferrule when the post terminus has been embedded 1.5 mm into this material. It is anticipated that it will prevent post displacement, fractures as well as inhibit the penetration of microleakage from the coronal portion of the tooth. Super EBA is zinc oxide eugenol cement reinforced with ethoxybenzoic acid to increase the strength of the mixture. This material has better dentinal bonding properties than gutta percha, will inhibit microleakage and serve as a component to align the post and assist the tooth from root and post micro fracture. A Para post was cemented using Rely X, and Ti-Core cement for the core build up. Rely X cement is dual cure composite cement, moisture tolerant and self adherent, Ti- Core with Titanium is a hybrid autopolymerizing composite – resin cement which is reinforced with titanium. The coronal core build ups were subjected to one hour impaction, using gold foil condensers, for the occlusal and one hour for the lateral side of the tooth (to simulate masticatory forces). The experimental and controls were compared for microleakage, root displacement and fracture.

Methods: Sixteen extracted human, anterior and bicuspid teeth were used in this study. Both experimental (n=8), and controls (n=8) were treated endodontically with hand and then rotary NiTi sequence file instrumentation. All root canals were filled with gutta percha and Columbia Cement using the lateral condensation technique. Post space preparation of ½ the root length, plus 2mm were made and radiographed. Eight (n=8) experimental teeth had Super EBA injected into the post preparation space to a thickness of 2mm (using a Centrix syringe with special 19 gauge needle). A 30 degree bevel was made at the terminus of the Para post, to facilitate cementation into the Super EBA ferrule and barrier preparation. All the prepared root canals were treated with Rely X, and Para posts were cemented into place. The controls (n=8) contained the cemented Para posts without the Super EBA barrier-ferrule. After the core preparation using Ti-Core with Titanium, a #6 round bur hole was drilled below the coronal ferrule of all teeth. Methylene blue dye was injected into this preparation. The root apices of each prepared canal were cut and placed in high speed evacuation unit lined with gauze to determine dye penetration to the apex. All teeth were subjected to one hour of lateral and one hour of vertical force condensation, using automatic gold foil condensers on the coronal core preparation (used to simulate masticatory vector forces). The experimental and controls were compared for: dye leakage before and after impaction. X-rays were taken to assess fracture of the core, root, or post.

Results: The combination of Rely X and Ti-Core Titanium in cementing Para posts and core build ups played a very positive role in preventing root, and post fracture, as well as acting as a good barrier for microleakage. There were only two cases out 16 teeth that experienced a core fracture. Only three controls and one experimental experienced dye leakage after impaction, six experimental experienced no leakage before and after impaction. The results were unable to single out strong differences between the controls, containing no Super EBA ferrule barriers, with those that provided the intended extra protection for the post at its terminus. This study was unable to evaluate micro-displacement of materials in the root canal, micro movement of the post or micro fracture of the root. Gross evaluation showed no differences between the controls and experimental in root fracture, post breakage or post displacement. We can only infer that the second ferrule and post space barrier will reduce apical microleakage and post/tooth complication.

Mutational Frequency of the *BRAF*, *KRAS*, and *PIK3CA* Genes in Oral Squamous Cell Carcinoma

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Introduction: Oral squamous cell carcinoma (OSCC) is a disease process that can be quite debilitating when left untreated and requires an intervention as early as possible. OSCC involves multiple oncogenes and tumor suppressor genes that function in a complex process; many of which have yet to be identified. Genes that regulate cell growth and apoptosis are of vital concern when dealing with OSCC.

Objective: This study investigates the *BRAF*, *KRAS*, and *PIK3CA* genes in the tumorigenesis of OSCC. *BRAF* and *KRAS* are key participants in the *RAS-RAF-MEK-ERK-MAP* kinase pathway. Deregulation of the *BRAF* and *KRAS* genes can affect cell growth and result in cell transformation. *PIK3CA* functions in numerous cell pathways, including inhibition of cell apoptosis.

Methods: To study possible roles of *BRAF*, *KRAS*, and *PIK3CA* genes in OSCC tumorigenesis, direct genomic sequencing was used to identify mutations in clinical samples of OSCC.

Results: As was evidenced by sampling 42 OSCC specimens, we found a mutational frequency of 2% (1/42) for both *BRAF* and *KRAS* genes. The mutational frequency of the *PIK3CA* gene was 3% (1/35) among 35 OSCC samples.

Conclusion: Our study supports the hypothesis that *BRAF*, *KRAS*, and *PIK3CA* are important for the tumorigenesis of OSCC and can be valuable drug targets for treatments.

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Dental Enamel Defects in Celiac Disease: A Case Control Study

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Introduction: Celiac disease (CD) is an immune-mediated enteropathy triggered by exposure to dietary gluten in genetically susceptible individuals. It occurs in approximately 1% of the population in the US and throughout the world. Patients with CD typically experience gastrointestinal symptoms such as diarrhea and abdominal pain. However, affected patients are also at increased risk for developing a variety of autoimmune diseases as well as malignancies. Diagnosis of CD requires clinical suspicion of CD and/or a positive serology screen for CD antibodies, in conjunction with a confirmatory small intestine biopsy. Unfortunately, the variable clinical presentation of CD makes diagnosis difficult at times and an estimated 97% of affected individuals remain undiagnosed. Early detection is desirable to facilitate monitoring for other systemic manifestations, including autoimmune conditions and malignancies.

Objective: Dental enamel defects reflect disease processes that occur during the period of enamel formation. Several European studies have demonstrated that patients with CD have a higher incidence of enamel defects, proposing the utility of such defects as a screening tool for CD. We conducted a case control study in adults and children to assess the association between enamel defects and CD in the US.

Methods: Biopsy-proven CD patients and age-matched controls will be recruited from the Columbia University Celiac Disease Center, CD support groups, and the dental clinic. The cohort will be divided into two groups: adults (≥ 16 years) and children (≤ 16 years). A total of 100 patients will be accrued for each group (adult CD, adult control, child CD, and child control). Patients will complete a questionnaire and undergo a full oral exam. The oral exam will include a dental exam with notation of any enamel defects as well as decayed, missing, and filled teeth (DMFT). Significant Caries Index (SCI), signifying prevalence of caries, will also be calculated. The dental exam will be documented with 5 photographs (right buccal, left buccal, facial, maxillary occlusal, mandibular occlusal). Enamel defects will be graded as 0 (absent) through 4, according to Aine's classification. The oral mucosa will be examined for other manifestations associated with CD, including aphthous ulcers, candidiasis, and lichenoid lesions. Chi-square tests will be used to compare dichotomous variables. Linear regression will be used for DMFT correlation.

Results: Accrual of patients is ongoing. Variables to be investigated include: 1) enamel defects in CD patients compared to controls, 2) the correlation between enamel defects and DMFT, 3) oral mucosal lesions in CD patients compared to controls, and 4) the correlation between enamel defects and oral mucosal lesions.

Conclusion: Previous studies have shown an increased prevalence of enamel defects in both adults and children with CD compared to controls, although only those in children reached statistical significance. This is likely related to fewer restorations in this younger population. Continued study of the oral manifestations of CD is important as these findings may represent the first objective sign of disease in previously undiagnosed individuals. As such, the dental practitioner may play a critical role in early detection of CD.

Analysis of the Oral Epithelium and Keratinocytes of Conditional $\beta 1$ Integrin Knockout Mice

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Introduction: In the stratified epithelia, the basal cell layer utilizes integrins to attach to the underlying basement membrane. Integrins are a large family of heterodimeric trans-membrane receptors, comprising of $\alpha\beta 1$ sub-units that link the extracellular matrix (ECM) to the cellular cytoskeleton. Integrins signal across the cell membrane in a bi-directional manner. These signaling events regulate cellular processes such as cell adhesion, migration, proliferation, differentiation and apoptosis. While several gene knockout studies have addressed the function of integrins in the epidermis, less is known about the consequences of the loss of these integrin subunits to the oral epithelia.

Objective: The objective of this study is to investigate the consequences of the Knock Out (KO) of integrin $\beta 1$ expression in the oral epithelium. We are particularly interested in following the phenotypes of the KOs in the tongue, tooth buds and gingival epithelia, to ask how structural and functional integrity of oral epithelia including changes in expression of proliferation and apoptosis are affected by loss of $\beta 1$ integrins. We isolated WT and KO oral keratinocytes and performed cell biological experiments.

Materials and Methods: Histological Analysis: 10um frozen sagittal and coronal sections of heads of WT and $\beta 1$ conditional KO mice were collected and processed for histological staining with H&E, and indirect immunofluorescence staining with antibodies and markers including Keratin 14 and $\alpha 6$. Ki67 antibody was used to assess for cell proliferation. TdT-mediated dUTP nick-end (TUNEL) assay was used to assess for apoptosis. All staining sections were viewed using a Zeiss Axiophot microscope and photographed.

Oral Keratinocyte Cell Culture: We isolated oral keratinocytes from the tongue of E17.5 WT and $\beta 1$ KO embryos. The tongue was isolated and digested with 1 unit/ml dispase at 4°C to separate the mucosal epithelium. The epithelium was incubated at 37°C in trypsin-EDTA for 5 minutes and warm keratinocyte growth media (with serum) was added to terminate trypsin activity. The keratinocytes were dissociated by pipetting vigorously and strained using a 40um cell strainer and centrifuged. The cell pellet was resuspended in high calcium (1.2mM) keratinocyte growth media. The cells were plated on 3T3 fibroblast feeders that have been treated with mitomycin C and grown at 32°C and 5% CO₂. The cells were passaged after 7 days, and maintained on fibroblast feeders.

Results: The loss of $\beta 1$ integrin in the oral epithelium results in the loss of basement membrane integrity predominantly in the tongue and around the tooth buds as indicated previously and loss of orientation in the developing lower incisors. We will continue to gather data and establish oral keratinocyte cell cultures to perform analysis of cell adhesion, proliferation and migration of these cells.

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Temporal Release of Platelet Rich Plasma (PRP)-Derived Factors from a Hydrogel Carrier with Application in Dental Pulp Repair

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Introduction: Platelet-Rich Plasma (PRP) is derived from blood plasma and serves as an autologous source of growth factors such as Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth factor (VEGF), Transforming Growth Factor beta-1 (TGF- β 1), and Insulin-like Growth Factor (IGF). These factors are important for both vascularization and regeneration of dental and craniofacial tissues(1). Clinical efficacy of PRP is however largely dependent on the temporal bioavailability of the PRP-derived factors(2). Previously, we designed an alginate hydrogel-based carrier for controlling growth factor release(3) and our long term goal is to evaluate the potential of this carrier combined with PRP in promoting vascularization and dental pulp repair.

Objective: The objective of this study is to determine the temporal release of VEGF from alginate beads with PRP. In addition, we will test the feasibility of encapsulating dental pulp cells in alginate beads and evaluate the effects of PRP on the viability of these cells. It is hypothesized that VEGF release from the PRP will gradually increase with time, and cell viability will be maintained in the alginate carrier with PRP.

Methods: PRP Alginate Beads: PRP was prepared following Landesberg *et al.*(4), and alginate carriers were fabricated by mixing 2% alginate (Sigma) with PRP and then dispensed into a 6% CaCl₂ solution(3). The wet weight (n=6) and dimensions (n=6) of the as-fabricated PRP alginate beads as well as the PRP/pulp cell-containing alginate beads were measured at each time point. The amount of VEGF released (n=6) as well as remaining in alginate beads (n=6) were measured by ELISA (R&D System) over three weeks.

Pulp cells/PRP Alginate beads: Human dental pulp cells were cultured for three weeks in F/S DMEM (Invitrogen). The PRP/pulp cell-embedded alginate beads were fabricated by first mixing the pulp cells with PRP, followed by combining the mixture with the alginate solution. The suspension was then dispensed into a 6% CaCl₂ solution. The resulting seeding density was 70,000 pulp cells/bead, and cell viability (n=6) was determined over two weeks via the live-dead assay (Molecular Probes).

Results and Discussion: Characterization of PRP Hydrogel Carriers: No significant change in alginate wet weight and dimension were observed over time. The intense red coloration of the bead due to the presence of PRP observed on Day 0 diminished over time, corresponding to the diffusion of growth factors out of the alginate beads.

Temporal Release of VEGF from PRP in alginate beads: It was found that the percent release of VEGF from beads increased gradually and reached a maximum at 21 days. The total amount of VEGF (released from plus remaining in carrier) measured at each time point did not change significantly over time when compared to Day 0, suggesting that there is little interaction between the VEGF and the hydrogel matrix.

Effects of PRP on viability of pulp cells in alginate beads: Live/Dead staining of the cell-laden alginate beads revealed that the pulps cells remained viable in the PRP+alginate matrix over the two-week culturing period.

Conclusion: The results of this study demonstrate the efficacy of the alginate carrier in supporting the temporal release of VEGF from PRP. Moreover, dental pulp cells embedded with PRP in alginate beads remained viable. Future studies will focus on evaluating the effects of PRP-derived factors such as VEGF and PDGF on the vascularization potential of dental pulp cells grown in the hydrogel carriers.

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Imaging *nfm-1* Mutants in *C. elegans* Using Confocal Microscopy and Spinning Disk Confocal Microscopy

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Introduction: Dr. Gobel's laboratory has previously characterized cytoskeletal linker proteins of the FERM (protein 4.1-ezrin-radixin-moesin) family in *C. elegans* and showed that ERM-1, an ancestral member of this family, is required for apical membrane modeling during epithelial morphogenesis, particularly tubulogenesis. Separate studies have suggested that two vertebrate homologs of ERM-1, ezrin and radixin, are also implicated in the formation of the mouse enamel organ in the region where preameloblasts come into contact with the stratum intermedium. During tubulogenesis as well as enamel organ formation, these proteins have a role in the cytoskeletal organization at cell boundaries contributing to organ morphogenesis and they appear to be involved in cortical membrane rearrangement. This project is part of the characterization of *nfm-1* (the *C. elegans* Neurofibromatosis 2 homolog), which is another member of the FERM family with a putative role in epithelial morphogenesis.

Objective: We are testing the hypothesis that loss-of-function and null mutations in *nfm-1* cause defects in the morphogenesis of tubular epithelia, namely the intestine. One specific question is if NFM-1, which is located at the basolateral intestinal membrane, may contribute to the generation of apico-lateral junctions and the apical membrane itself, as previously demonstrated for other basolateral membrane molecules.

Materials and Methods: Specimens used for the experiments included one null mutation and one loss-of-function mutation in *nfm-1*. A confocal microscope was used to analyze the intestinal morphology of the two mutant strains. To see the effects of *nfm-1* mutations on membrane formation, *nfm-1* mutant embryos were stained with antibodies binding to apical junction and apical membrane proteins. The spinning disc confocal microscopy was piloted on an *erm-1-GFP* labeled strain to gain experience in visualizing the intestinal development in real-time.

Results and Conclusions: Our confocal analysis suggests that in *nfm-1* loss-of-function and null mutants, apical cytoskeletal and junctional markers are correctly placed at their respective locations. However, structural irregularities of the apical membrane that reach into the junctional area are more pronounced in the null mutant (complete absence of *nfm-1*), possibly indicating a requirement of *nfm-1* in membrane microdomains. We currently favor the hypothesis that *nfm-1*'s function lies directly at the basolateral membrane (experiments in progress). The spinning disc confocal microscopy experiment presented itself with challenges involving detrimental levels of photobleaching and therefore no conclusive result has been obtained for this experiment.

Discussion: It is expected that highly conserved and ancestral cytoskeletal molecules such as *erm-1* and *nfm-1* will govern basic biological mechanisms that will be relevant to different tissues and to a wide variety of multicellular organisms, including humans. Our confocal microscopy results contribute to the functional characterization of *nfm-1* in epithelial morphogenesis. Further investigation, including the use of other advanced imaging systems such as transmission electron microscopy, will be used to identify the effect of loss of *nfm-1* on membrane microdomains. The exact stage in intestinal tubulogenesis where NFM-1 plays a morphological role awaits the establishment of spinning confocal analysis, which is in progress.

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Regeneration of the Dental Pulp by the Release of Biologically Active Cues

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Introduction: Root canal therapy is the conventional dental and endodontic treatment for infected or injured dental pulp tissue. Although tooth function may be successfully restored, current canal therapy leads to non-vital teeth. Clinical observation reveals that a number of root canal-treated teeth undergo discoloration, increased brittleness and incidence of fractures. Some teeth need secondary treatment due to undetected bacterial colonies in lateral root canals or voids in the filling of root canal with gutta percha. In this study, we hypothesized that the vitality of dental pulp can be restored by the delivery of bioactive cues. Growth factor delivery is a technology that has been developed from the field of biomaterials and entails the encapsulation of bioactive cues, such as peptides, proteins and gene fragments, in micro- or nano-scaled biomaterials¹, and has the potential to recruit endogenous stem cells, odontoblasts and other pulp cells into the dental pulp².

Objective: To regenerate the vasculature into the root canal and pulp chamber of endodontically treated human incisors.

Materials and Methods: Eight extracted human incisor teeth were treated with root canal therapy, following conventional endodontic procedures³. Extracted teeth were disinfected in 10% NaOCl for 1 week. An opening was made through the crown of the tooth into the pulp chamber. Pulpal tissue was removed and the root canal was cleaned and shaped with hand files and rotary instruments. The root canal was enlarged to create 1mm of opening at the apex. Following autoclave to further remove any biological and organic components, collagen matrix was placed into the pulp canals and they were inoculated with one of the following: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), VEGF + bFGF, or empty (control). VEGF and bFGF are known to exert chemoattractant, mitogenic, and angiogenic effects *in vivo*^{1,2,4,5}. The endodontically treated and growth factor-loaded teeth were then implanted subcutaneously into the dorsum of 12-wk old mice and harvested 2 weeks post-op. Harvested teeth were decalcified and embedded in paraffin for histological analysis. Sections were stained with hematoxylin and eosin (H&E) and observed under a light microscope.

Results: Upon *in vivo* harvest, gross examination revealed red pigmentation in both the opening of the pulp chamber coronally and the apical foramen in bFGF alone and bFGF + VEGF groups. In contrast, there was a lack of red pigmentation in the control group (growth factor-free collagen group) and VEGF alone group. Granular-like tissue infiltrated the pulp chamber and root canal of teeth treated with bFGF and bFGF+VEGF groups. Given that no cells were seeded in the collagen sponges that were inserted into the root canal in any groups, the infiltrating soft tissue is expected to be host derived. VEGF alone group induced small to moderate host tissue infiltration into the pulp chamber and root canal. In contrast, the control group did not induce host tissue infiltration.

Conclusion: The present findings indicate the feasibility to use biologically active cues that are known to participate in native angiogenesis process towards the regeneration of dental pulp. We are in the process of analyzing the characteristics of the infiltrated host tissue and cells. Our ongoing work on pulp derived stem cells suggests that they readily respond to various growth factors and regulatory cues. The dental pulp represents a heterogeneous environment consisting of multiple cell types. The regeneration of dental pulp is a challenging but attainable goal, given the discoveries in related disciplines of stem cell biology, biomaterials and tissue engineering^{1,2}. The delivery of angiogenic growth factors is the first step towards the induction of host-derived vascular tissue migration into treated teeth. The present tissue engineering approach using biocompatible scaffolds and angiogenic growth factors may be useful during root canal treatment. There is potential that the replacement of synthetic fillers of current root canal therapy with biological cues may restore or maintain the vitality of the dental pulp.

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A Literature Review: Oral Health and Hygiene in the Sub-Saharan African Countries Visited by the Millennium Villages Projects.

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Introduction: The Millennium Villages Project (MVP) is a multidisciplinary plan dedicated to advancing health, food production, education, access to clean water and essential infrastructure in African rural communities. The Project's specific goals focus on significantly reducing poverty, hunger, disease, child mortality, and improving primary education, gender equality, maternal health, environmental sustainability and global involvement by 2015. The health care initiative is focused on chronic illnesses. In reference to oral health, the rapid urbanization in Africa has resulted in the introduction of a diet richer in refined carbohydrates and fats, which may adversely affect oral health. In 2006, an oral health and hygiene component was added to the MVP. To date, this project has visited 4 villages to determine the prevalence of oral disease and provide emergency services. With a paucity of available resources, the MVP oral health project seeks to develop a self-sustaining preventative program.

Objective: The objective of this literature review is to compile an archive of oral and dental health data in the countries in which MVP has visited since 2006: Ethiopia, Rwanda, Senegal and Tanzania. The compilation of this information will allow a comparison of data collected on the current trips to the existing literature.

Materials and Methods: Information was obtained from journal articles in the English language found in the PubMed and Medline databases.

Results: Of the 14 studies identified, 11 contained original data and 3 were review articles. Among the four African countries visited by the oral health team, the largest number of reports was for Tanzania followed by Senegal, Ethiopia, and Rwanda. In general, DMFT scores were most commonly used to report dental status and as expected, prevalence of disease increased with age. There was a trend of more disease in urban environments than in rural environments, but those living in rural environments had greater difficulty accessing care. One important consideration is the naturally occurring levels of fluoride in the drinking water. In the 14 articles reviewed, the number of patients examined ranged from 196 to 5,532. However, the majority of the articles had a sample size of greater than 1,000 patients. Data from the MVP trips is preliminary, yet indicates a common finding of oral pain. The percentage of individuals who presented with oral pain ranged from 51% to 86%. There was an obvious lack of availability of oral health care services in the four villages.

Conclusion: One shortcoming of this review is that only studies written in English were identified and summarized. Additional information on oral health and hygiene in these underdeveloped countries needs to be gathered. Our goal is to gather publish findings and to compare the information to data gathered by the oral health teams to assess trends over time. This effort will be used to help address the current oral health problems in the villages. The intent is to significantly improve the quality of life of the people living in these sub-Sahara African countries by addressing their oral health problems.

Hematopoietic Stem Cell Derived Endothelial Progenitors to Promote Vascularization of Tissue Engineered Bone Grafts

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Background and Objective: Hard tissue defects resulting from trauma, congenital anomalies, or cancer resections require bone implants for reconstruction and re-establishment of function. Current procedures including allografts and autografts suffer from donor site scarring, immune rejection, inappropriate regeneration and repair, or lack of a feasible donor site. Stem cell based tissue engineering provides tools for the regeneration of autologous bone that may circumvent these hurdles, yet still demand improved design considerations, such as adequate vascularization. Metabolic demands in current engineered bone grafts are not met due to suboptimal ingrowth of blood vessels, resulting in subsequent hypoxic cell death and implant failure. Engineering an appropriate vascular supply in the regenerated bone is imperative for its long-term survival. Two distinct stem cell populations from bone marrow, mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs), have been demonstrated to be suitable progenitors for bone forming osteoblasts and vascular endothelial cells, respectively. The objective of the present study is to differentiate MSCs and HSCs into osteoblasts and endothelial cells capable of forming tissue analogous to bone with high potential for vascularization.

Methods: Human HSCs and MSCs were isolated from commercially available human bone marrow samples of multiple donors under IRB exemption. MSCs were expanded and differentiated into osteoblast-like cells. HSCs (CD34+) were differentiated into endothelial-like cells. The phenotypes of differentiated osteoblasts and endothelial cells were confirmed using immunohistochemistry and ELISA for vascular endothelial growth factor receptor (VEGFR) expression and von Willebrand factor (vWF) (for endothelial cells) and mineral matrix deposition (for osteoblasts). Continuing studies will determine the osteogenic and vasculogenic potential of the present approach in an in vivo model for vascularized bone engineering.

Results: Human bone marrow derived HSCs were cultured and differentiated into endothelial-like cells as shown by increased VEGFR and vWF expression in differentiated cells by immunolocalization using fluorescent staining and ELISA respectively. Human bone marrow derived MSCs differentiated into osteoblast-like cells as observed by increased alkaline phosphatase (ALP) activity and mineral matrix deposition (von Kossa stain). Calcium phosphate scaffolds were seeded with either MSC-derived osteoblasts and HSC-derived endothelial cells or MSC-derived osteoblasts alone (control). Significantly more blood vessels were present in bone grafts seeded with both HSC-derived endothelial cells and MSC-derived osteoblasts than MSCs alone.

Conclusion: Vascularized bone grafts can become an integral part of surgery. hHSC are a suitable source for endothelial cells and may increase vascularization of bone grafts. The current techniques for endothelial cell and osteoblast differentiation from a single bone marrow sample may improve the long-term survival of engineered bone grafts by inducing vascularization and nutrient supply. Integration of endothelial cells and osteoblasts may be a useful strategy in promoting the survival of engineered bone as well as achieving successful bone grafts.

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Exploring the Use of a PLGA Microsphere Delivery System in the Treatment of Craniosynostosis

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Introduction: Craniosynostosis is a pathological condition defined as the premature fusion of the sutures of the skull, relative to the cessation of brain growth and normal suture fusion timing. Premature suture fusion is associated with secondary deformities in the cranial vault, cranial base, and midface. Such skeletal deformities often result in significantly elevated intracranial pressure, altered intracranial volume, and dilation of the subarachnoid spaces. The resulting craniofacial, ocular, and neural abnormalities can lead to extensive clinical and surgical management problems. The manipulation of various cell mediators, including connective tissue growth factor (CTGF) and growth and differentiation factor 5 (GDF-5), is suspected to regulate the ossification of fibrous tissue in cranial sutures. Examination of the prolonged release profiles of these cell mediators via microencapsulation using poly(lactic-co-glycolic acid) (PLGA) microspheres (MS) is necessary to evaluate their plausibility as treatments. PLGA microspheres can be readily fabricated using the double-emulsion solvent-extraction technique. This method allows the control of sphere diameter and degradation kinetics, while maintaining the stability and bioactivity of the encapsulated growth factors.

Objectives: The purpose of this project is to examine the efficiency of microencapsulating cell mediators, specifically CTGF and GDF-5, using PLGA microspheres. If proven as an effective *in vitro* delivery system, PLGA microspheres can then be tested in an *in vivo* system, ultimately focusing on curbing the effects of craniosynostosis.

Methods: Microspheres of poly (d-l-lactic-co-glycolic acid) of 50:50 and 75:25 PLA/PGA ratios were prepared using double-emulsion technique ([water-in-oil]-in-water). A total of 250 mg PLGA was dissolved into 1 mL dichloromethane, and 2.5 µg of recombinant human growth factor (CTGF or GDF-5) was diluted in 50 µL of reconstituting solution per manufacturer protocol and added to the PLGA solution, forming a mixture (primary emulsion) that was emulsified for 1 minute (water-in-oil). The primary emulsion was then added to 2 mL of 1% polyvinyl alcohol (PVA, 30,000– 70,000 MW), followed by 1 minute of mixing ([water-in-oil]-in-water). Upon adding 100 mL PVA solution, the mixture was stirred for 1 minute. A total of 100 mL of 2% isopropanol was added to the final emulsion and continuously stirred for 2 hours under a chemical hood to remove the solvent. Control microspheres (empty and without growth factor) were fabricated using the same procedures, with the exception of using 50 µL distilled water instead of the growth factor solution. PLGA microspheres containing one of the growth factors or distilled water were isolated using filtration and washed with distilled water. Microspheres were frozen in liquid nitrogen for 30 minutes and lyophilized for 48 hours. Freeze-dried PLGA microspheres were stored at 20°C prior to use. The efficiency of cell mediator encapsulation was tested using a Fluorophore-Linked Immunosorbent Assay kit (FLISA). This method mirrors an Enzyme-Linked Immunosorbent Assay (ELISA) kit system, but substitutes the HRP/AP-streptavidin with a fluorescent IRDye-labeled streptavidin.

Results and Conclusion: Using the FLISA analysis technique, it was found that the encapsulation yields of CTGF and GDF-5 within a PLGA microsphere delivery system were 87.83ng/10mg MS and 10.25ng/10mg MS, respectively. When packaged in PLGA microspheres, both CTGF and GDF-5 were found to follow predictable *in vitro* sustained release profiles throughout a 6-week time span.

Discussion: Since the conclusion of this adjunctive study, these applications have already been applied *in vivo*. An animal study, conducted by Lee *et al.*, demonstrated that the delivery of CTGF encapsulated PLGA MS into an artificially created defect on rat calvaria induced clear fibrous suture-like tissue between calvaria bones after 4 weeks. Furthermore, sustained release of CTGF lasted up to 6 weeks, showing the effectiveness of PLGA MS as controlled release vehicles. The *in vitro* encapsulation yield of CTGF was significantly higher than that of GDF-5. This may have been caused by the differences in molecular weight between CTGF (38 kDa) and GDF-5 (21 kDa). Overall, this project has shown that PLGA MS are excellent controlled release carriers for the CTGF cell mediator, advancing research toward finding a successful treatment for craniosynostosis.

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Expression Levels of pChk2, Hint1, and pSmad2 in Oral Precancerous Lesions and Squamous Cell Carcinomas

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Introduction: Approximately 34,000 people are newly diagnosed with oral squamous cell carcinoma (scc) annually in US. If discovered early before the metastasis occurs, the prognosis is excellent with 80 to 90% survival. Because of its asymptomatic nature, most are diagnosed at a later stage and the survival rate is less than 50%. Early detection hence yields improved survival. The molecules implicated in oral carcinogenesis signaling pathways, such as pChk2, Hint1 and pSmad2, may serve as useful markers for identification of the oral precancerous and cancerous lesions. Phosphorylated Chk2 (pChk2) is a DNA damage response molecule activated during the initiation period of carcinogenesis. Hint1 and pSmad2 are tumor suppressor genes, involved in cell cycle control. There is evidence that the decreased expressions of these tumor suppressors are critical events in malignant transformation.

Objectives: This preliminary study aims to observe the expression levels of two putative tumor suppressors, Hint1 and pSmad2, and a DNA damage response molecule, pChk2, in normal, precancerous lesions and sccs. Our ultimate goal is to devise a panel of biomarkers with the highest diagnostic power for the precancerous lesions and carcinomas.

Materials and Methods: Expression levels of pChk2, Hint1, and pSmad2 were assessed on twenty cases with known clinical outcome, using immunohistochemistry (IHC). Five 'normal' (control) cases consisted of those diagnosed as hyperkeratosis or epithelial hyperplasia, which did not progress to cancer within 6 year period. Ten cases were of precancerous lesions that later transformed into carcinomas. The last five cases consisted of scc. Immunoassays were performed on the paraffin-embedded tissue blocks of the selected cases. For each case, the intensity of the epithelial nuclear staining, ranging from no staining (0) to strong staining (+3), the staining pattern (focal versus diffused) and the staining distribution (basal one third, basal two thirds or involving the full thickness) were examined.

Result/Conclusion: pChk2 was selectively expressed in precancerous and cancerous lesions ($p < 0.0001$) but not in controls ('normal'). Progressive decrease in Hint1 expression, from 'normal' to scc, was observed. Statistically significant decrease in Hint1 expression was detected in carcinomas compared to that of the 'normal' ($p = 0.002$). In contrast, decreased expression of pSmad2 was observed only after the malignant transformation had occurred ($p = 0.004$). Hence a lesion with positive pChk2, and significantly decreased expression of Hint1 and pSmad2 may be indicative of squamous cell carcinoma. These promising results warrant further studies.

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The Role of Axin in Craniofacial development

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Introduction: Axin, a scaffold protein in the axin/GSK-3/APC complex, serves as a negative regulator of the Wnt/ β -catenin signaling pathway by promoting the phosphorylation of β -catenin, thereby leading to subsequent β -catenin degradation. Axin has also been implicated as a positive regulator of the TGF- β signaling pathway, which promotes the ubiquitination and degradation of the inhibitory Smad, Smad7 proteins. Loss of *Axin* results in early embryonic lethality at E9.5, apparently due to excessive β -catenin accumulation and signaling. Dr. Costantini's laboratory made a mutant allele of the Axin gene, Axin- Δ C6, which lacks the last 6 amino acids of the C-terminus. The C6 motif has been reported to be one of the domains required for JNK activation and a main site for the SUMOylation of Axin. Axin- Δ C6 homozygous embryos are indistinguishable with Axin null embryos and also die at E9.5-10.5. When the Axin- Δ C6 mutant was crossed to a β -catenin knockout mouse, which reduced the amount of β -catenin by 2-fold, the compound mutant embryos survived to a much later stage. However, these embryos developed severe craniofacial abnormalities including cleft lip and cleft palate.

Objectives: We propose to study the role of Axin in craniofacial development of mice with 129S1/Sv1 background. The experiments proposed are to 1) conduct a thorough histological analysis of the embryos 2) perform *in situ* hybridization and immunohistochemistry on these mutant embryos to examine the expression of different genes/proteins in the TGF-beta and Wnt signaling pathways.

Materials/Methods: In this study, *Axin* ^{Δ C6/+}; β -cat^{+/-} male mice of the C57BL/6J background were crossed with *Axin* ^{Δ C6/+}; β -cat^{+/+} females of the 129/S1/Sv1 background for 3 generations. Female mice from N2 and N3 with 129/S1/Sv1 background were sacrificed at E10.5, E11.5, E12.5 and E13.5 according to institutional guidelines and mutant embryos were collected in phosphate buffered saline solution. Embryos were fixed in 4% paraformaldehyde and embedded in paraffin. Coronal sections (10um) were prepared and processed H&E staining.

Results: Of the 95 N2 embryos dissected, seven were genotyped as "rescued" embryos. Of the seven embryos, only two showed some degree of a "rescued" phenotype. The rescued embryo at E13.5 had a well-developed body with craniofacial defects the rescued embryo at E10.5 was a mass of tissue that did not have any distinct or distinguishable facial or body features. The rest were dissolved. Of the 64 N3 embryos dissected, three were genotyped as "rescued". Two of the rescued embryos were an indistinct mass of tissue and one was dissolved.

We compared our results to those of the initial experiment performed by Ian Vui En Chia and the results of our 2006 summer research project. Preliminary experiments performed by Dr. Chia utilized mice with a mixture of background that included a 129/S1/Sv1 strain and our summer project utilized mice of the C57BL/6J strain. In Dr. Chia's experiment, the "rescued" embryos have a well developed body with one or more distinct facial abnormalities, including a cleft lip, brain protruding out of the oral cavity, impaired fusion of the medial nasal prominences, and a protruding tongue. In the summer experiment, the "rescued" embryos had abnormal facial features but lacked an advanced body. Unlike the phenotype of the compound mutant embryos seen in mice of Dr Chia's experiments, mutant mice of the pure C57BL/6J background and varying degrees of the 29/S1/Sv1 background exhibited less degree of "rescue" with the removal of one allele of β -catenin.

Conclusion: Due to the fragility of the embryos in this experiment we were unable to fix the embryos and perform *in situ* hybridization and immunohistochemistry. Mice with loss of Axin can be "rescued" from embryonic lethality with the removal of one allele of β -catenin. The phenotype observed in *AX* ^{Δ C6/+}; β C^{del/+} compound mutant embryos seem to be dependent on the genetic background of the mice.

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Differentiation of Bone Marrow Derived Cells into Keratinocytes: A Critical Step Towards Understanding Their Interactions in Epithelial Cancer

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Introduction: While cancer of the mouth and pharynx only accounts for 2 to 4 percent of all cancers diagnosed in the United States, it has a low survival rate that gives it a poor prognosis. In addition, the prognosis has not changed significantly for over the last 40 years. Despite the knowledge has been gained through research and clinical studies, the mortality demonstrates there is still more information that needs to be discovered.

Objective: Several converging lines of evidence have suggested the existence of a cancer stem cell because cancer cells and normal stem cells share many similar characteristics. There are three candidate origins of cancer stem cells: tissue specific stem cells, transient amplifying progenitor cells, and differentiated cells, able to give rise tumor formation. Moreover, previous studies have shown that bone marrow derived cells (BMDCs) have a role as a tissue specific stem cell in skin injury. However, a recent gastric cancer study has demonstrated that BMDCs are involved in gastric cancer formation as a malignant precursor (1, 2). This study strongly suggests that the BMDCs may have a role as a cancer stem cell. My study in Morris' lab focuses on demonstrating the possible interaction of BMDCs and keratinocytes as a first step to determining their interactions in cancer and transformation into cancer stem cells.

Materials and Methods: I harvested keratinocytes from 8 week female mice and bone marrow cells from 8 week male mice. I seeded BMDCs on the bottom of a 6-well plate and seeded keratinocytes on the chamber inserts. One week after cell harvest, I put the inserts into the 6-well plate and set three different time periods, 1 week, 2 week, and 3 week, of co-culture. After co-culture, I checked for bone marrow cell differentiation into keratinocyte by detecting keratin expression with a pan-keratin antibody and immunohistochemistry.

Results and Conclusions: The co-cultured BMDCs were examined after 1 week, 2 week, and 3 week intervals. We were able to detect keratin expression in a large number of BMDCs after co-culture but not without co-culture. These results support our hypothesis that BMDCs can differentiate into keratin immunoreactive cells when co-cultured with keratinocytes.

Discussion: Stem cells and cancer cells share similar characteristics, such as immortality and differentiation, an ability that has led to the hypothesis that stem cells can become cancerous cells (3). In addition, many studies have verified that BMDCs can transform into other cell types, such as epithelial cells, skeletal muscle, and hepatocytes. A problem can arise when BMDCs migrate to areas of inflammation and injury as they can differentiate either into healthy tissues or neoplastic ones. Our experiment demonstrates that keratinocytes are able to induce BMDC differentiation to keratinocytes. Therefore, we suggest that migrated BMDCs to epithelium during inflammation can differentiate into keratinocytes and these cells might be transformed to cancer stem cells in chronic wounds. This has implications in cancer development and growth and could be a new model for oral cancer. Further studies are necessary, but if this is a model for cancer growth and development, it could have important implications for early cancer detection, cancer treatment and care.

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Differentiation of Tooth Derived Stem Cells into Osteogenic, Chondrogenic and Adipogenic Cells

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Introduction: Although previous work has shown that exfoliated deciduous teeth can be an alternative source for cells with several properties of progenitor cells, the understanding of these tooth derived progenitor cells is sketchy. In the present study, we demonstrated that cells isolated from the dental pulp of exfoliated deciduous teeth differentiate into multiple cell lineages. As compared to stem cells from the other somatic stem cell sources such as bone marrow and adipose tissue, this unique postnatal stem cell may be capable of extensive proliferation and multi-potential differentiation.

Objective: This study was designed to explore the protocol of TSC differentiation into several previously unexplored cell lineages.

Materials & Methods: TSCs were isolated from exfoliating deciduous incisors of 5-7 year-old donors after IRB approval. The dental pulp samples were separated from the remnant crowns and then digested in 3 mg/ml collagenase type I and 4 mg/ml dispase for 1 hr at 37 °C. The cells were isolated using a tissue filter and centrifugation at 500-g for 8 min and then culture-expanded in monolayer. The isolated, mononucleated cells from the dental pulp were differentiated into osteogenic, chondrogenic, and adipogenic cells. Following 2-4 wks of differentiation, TSC-derived cells showed a number of positive markers of osteogenesis, chondrogenesis, and adipogenesis including alizarin Red (AR), Safranin-O (Saf-O), and Oil-Red O (ORO).

Results & Conclusion: The isolated TSCs were spindle-shaped like bone marrow or adipose tissue derived stem cells. Alizarin red-positive nodules were formed in the TSCs cultures after 4 weeks of osteogenic induction, indicating calcium accumulation in vitro. After 4 weeks of culture with CIS, TSCs formed a pellet detached from the culture dish forming a cartilage-like tissue. Unexpectedly, TSCs cultured in regular growth media for 4 weeks also showed positive Saf-O staining. These findings indicate that TSCs are capable of spontaneous differentiation into chondrocytes. However, TSCs cultured in AIS failed to show adipogenic differentiation as indicated by ORO staining.

Discussion: The present findings suggest several new differentiation pathways of tooth derived stem cells. These data indicated that TSCs possessed the ability to differentiate into functional osteoblasts and chondrocytes in vitro. Ongoing studies are designed to further explore the molecular and genetic pathways leading to chondrogenesis, osteogenesis and adipogenesis. In summary, TSCs from more readily accessible tissue sources – deciduous teeth - may have potential applications in tissue engineering and future treatment of various tissue injuries or degenerative diseases. Funded by NIH/NIDCR grants (DE13964 and DE15391 to J.J.M.).

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Copolymeric Scaffolds for Bone Tissue Engineering: *in vitro* 3D Culture of Human Mesenchymal Stem Cell-Derived Osteoblasts

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Introduction: Effort in bone tissue engineering has been motivated by bony defects seen in the clinical care of patients, such as maxillary and mandibular fractures, craniofacial tumor resection and congenital anomalies. As a departure from the conventional approaches such as autologous bone grafts or prosthetic devices, bone tissue engineering relies on novel biomaterials frequently in combination with osteoprogenitor cells and/or osteoinductive growth factors (Mao *et al.*, 2006). A porous polymeric biomaterial provides the surfaces and internal structures for the attachment, growth and differentiation of stem cells and/or osteoprogenitor cells. A wide range of resorbable scaffolds have been investigated in experimental and clinical studies. We hypothesized that a composite of poly(ethylene glycol)diacrylate (PEG) and hydroxyapatite (HA) may take advantage of complementary properties of the two materials and provide the ease for cell seeding in bone tissue engineering applications. In this study, hydrogel cylinders were constructed with a copolymer of PEG and varying concentrations of HA, and seeded with human mesenchymal stem cells (hMSCs) to induce osteoblasts in the osteogenic medium.

Objectives: (1) To determine the viability of encapsulated hMSC-derived osteoblasts in 3D copolymer of PEG and HA at various concentrations; and, (2) to determine whether HA/PEG copolymer accommodates the osteogenesis of hMSC-derived osteoblasts.

Materials and Method: The hMSCs were isolated from commercially available bone marrow donors under IRB exemption. After 2-4 weeks of culture expansion, the mononuclear and adherent hMSCs were trypsinized and encapsulated in hydrogel cylinders (3mm height by 5mm diameter) that were fabricated with PEG and varying concentrations of HA powder. The PEG/HA hydrogel cylinders were made at concentrations of 0.001, 0.01 and 0.1 % (weight/volume) HA in PEG. The hydrogel cylinders that were prepared with PEG alone (i.e., no HA) were used as controls. The hMSCs were incorporated into the hydrogel cylinders by mixing hMSCs with the PEG/HA solutions at a cell density of 10×10^6 cells/mL, prior to photopolymerization under a long-wavelength UV light (365 nm). The cell-hydrogel cylinders were incubated up to 6 weeks in an osteogenic medium. The cylinders were harvested after 5 weeks and cells were determined to be alive by live/dead test. The cell constructs were evaluated by: (1) histology staining for bone tissue using H & E, Alzarin Red and ALP; and, (2) RNA isolation and RT-PCR analysis of gene expression for collagen I and osteocalcin.

Results and Conclusion: The results of histological staining showed that after 6 weeks in the osteogenic medium, the 3D PEG hydrogel with HA showed the formation of bone tissue at all HA concentrations (0.001 to 0.1 %HA), while no bone formation was observed in the control (PEG only). The greatest amount of bone formation was observed in the 0.1% HA sample. The gene expression of collagen 1 and osteocalcin showed statistically higher values for the 0.1% HA sample when compared to the control or 0.001 % HA sample. Therefore, it is concluded that the 3D PEG/HA copolymeric showed enhanced osteogenic differentiation of the hMSC derived osteoblasts at a seeding density of 10×10^6 cells/mL. Ongoing experiments include an *in vivo* study where PEG-HA copolymer seeded with hMSC derived osteoblasts will be implanted in mice and harvested after 6 weeks to be tested for osteogenic activity and biomechanical properties. The present findings demonstrate the proof of concept for using two biocompatible materials, PEG and HA, for bone tissue engineering applications for craniofacial trauma, congenital anomalies and tumor resection.

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Comparison of the Supra- and Subgingival RPS Flora of a Single Individual

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Introduction: Streptococcal receptor polysaccharides (RPS) are recognition molecules for oral biofilm formation. These polysaccharides function as receptors for the lectin-like adhesins present on other oral species such as *Actinomyces. naeslundii*. Six structural types of RPS have been identified from strains of *Streptococcus sanguinus*, *S. gordonii*, *S. oralis*, and *S.mitis* that coaggregate with *A. naeslundii*. Each RPS contains a recognition motif, either GalNAβ1-3Gal (Gn) or Galβ1-3GalNAc (G), within its hexa- or heptasaccharide repeating unit.

Objective: To characterize and compare the RPS-producing streptococcal clones from supra and subgingival dental plaque of the same individual.

Materials and Methods: Swabbed tooth and plated onto THB. After overnight incubation, random colonies were chosen. We performed initial screening by colony immunoblotting with a cocktail of RPS-specific antibodies. RPS-positive colonies were selected and antigenic-typing was performed by dot immunoblotting with different RPS-specific antibodies. Additionally, with the same colonies, we performed receptor-typing through coaggregation with other types of oral bacteria. Finally, RPS producing clones were identified by Repetitive Extragenic Panadromic (REP)-PCR

Results and Discussion: Of the 140 randomly selected supragingival colonies and 70 randomly selected subgingival colonies from an 8h plaque sample plated on THB, 19 supragingival and 43 subgingival were positive for cell surface RPS. Of 60 RPS-Positive isolates, 52 produced serotype of 1 RPS, whereas 8 produced a serotype of 4/5 RPS. Furthermore, all of these isolates produced receptor type Gn. Finally, 42 type 1Gn RPS isolates exhibited seven different REP-PCR patterns and 6 type 4/5 Gn exhibited the same REP-PCR pattern.

Conclusion: The supra- and subgingival RPS-bearing flora of one individual appear to be similar. This flora consists of at least eight RPS-producing clones, which together make two types of RPS (i.e. 1Gn and 4/5 Gn). The Gn-bearing streptococci predominate in early supragingival plaque. However, the primary niche of G-bearing streptococci remains to be identified.

Hand Hygiene: Use of Soap and Alcohol Rubs among New York State General Dentists

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Introduction: The hands of healthcare providers have been recognized for more than a century as a major reservoir of potential pathogens causing clinical infections. Traditionally, washing with soap and water has been the primary method of hand cleansing, but in 2002 the Centers for Disease Control and Prevention (CDC) published the “Guideline for Hand Hygiene in Health-Care settings,” recommending that handwashing be replaced by an alcohol-based hand sanitizer for patient contacts, except if hands were visibly soiled. This was further documented in dentistry through the CDC Guidelines for Infection Control in Dental Health-Care Settings-2003.

Objective: (1) To describe the frequency with which general practice dentists (GPDs) utilize soap and alcohol rubs for hand hygiene (HH), while noting the rate of adherence to the CDC Guidelines for hand hygiene. (2) To identify the most influential variables predicting appropriate HH practices and predicting frequency of alcohol rub use.

Materials and Methods: A random sample (n=360) of actively practicing GPDs in New York State was drawn from a list supplied by the New York State Dental Association. From the initial total sample, a net sample of 234 GPDs participated [response rate = 66 percent]. Using a four-page structured mail questionnaire, the subjects’ responses were measured for acceptable HH behavior and frequency of alcohol rub use. GPDs were said to have acceptable HH behavior if they reported using soap often/almost always/always (with at least 15 seconds washing time) and/or an alcohol rub in three situations [before beginning to provide care, between patients, and after removing their gloves]. Frequency of alcohol rub use was an average of usage in four different situations [before beginning to provide care, between patients, after removal of gloves, and re-gloving/resuming care after interruption], where response values ranged from 5 [always] to 1 [never]. We included in each predictive model all variables correlated with the respective dependent variables at ≤ 0.1 , variables remaining in the final stepwise regression model into which all candidate variables were entered, and variables with $p \leq 0.5$ in the final model.

Results and Conclusion: About 65% of GPDs reported acceptable HH behavior for all three of the following situations: before beginning to provide care, between patients, and after removing their gloves, while 19% did not meet the acceptable standard of care for any of the three situations. In regards to alcohol rub use, about 2% reported they “always” disinfect their hands with rubs in all four situations, while half the participants reported “never” using alcohol rubs in all four situations. The first predictive model regressed appropriate HH on eleven variables identified in the process described above [$R^2=.268$]. The three most influential predictors were knowledge of CDC Guidelines (B= .323, $p=.005$), access to necessary supplies (B= -.229; $p=.009$), and knowledge of the association between HH and infection control (B= -.222, $p=.024$). The second predictive model regressed frequency of alcohol rub use on eight predictor variables identified in a similar manner [$R^2=.239$]. The three most significant predictors were how often the GPDs washed their hands after removal of gloves (B= -.212, $p=.017$), how often hands were washed between patients (B= -.191, $p=.032$), and level of agreement with the notion that HH prevents infection (B= -.200, $p=.008$). Study findings suggest approaches to increasing dentists’ compliance with HH standards of care and for increasing use of alcohol rubs.

Discussion: According to the first predictive model, more specific knowledge of the guidelines, rather than more general, theoretical knowledge of the scientific guidelines facilitates appropriate HH. Furthermore, access to necessary HH supplies facilitates compliance. The second predictive model suggests that dentists’ hand washing habits need to be changed (decreased) in order to facilitate more frequent use of alcohol rubs. Also, increasing GPDs’ belief that appropriate HH can prevent infection is predictive of more alcohol rub use and seemingly has the potential to assist in the process of increasing the use of alcohol rubs in dental practice settings. Such findings are an important first step in the development of a targeted strategy aimed at improving HH compliance, accompanied by increased alcohol rub usage, among dental clinicians.

Correlates of Dental Coverage, 2001-2005

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Purpose: Having dental coverage is consequential to dental care and oral health status, but only two-thirds of American adults have this coverage. American adults that typically experience the highest rates of dental disease are often those with the least adequate dental coverage. Not having dental insurance varies significantly by age, level of education, income, race, and work status. Therefore, the objectives of this study are to examine correlates of and temporal trends in dental coverage.

Methods: Data from The Commonwealth Fund's 2001, 2003, and 2005 nationally representative telephone survey of US adults over age 18 were analyzed for frequency and correlates of the question, "Please tell me whether you have any insurance to cover all or part of the following health care needs. Do you have insurance for your dental care?" This *Biennial Health Insurance Survey* sampled 3508 individuals in 2001, 4052 in 2003, and 4350 in 2005. Using SPSS software, independent variables assessed for correlation with dental insurance were (1) age: 19-64 versus 65+, (2) race: Caucasian, African-American, Hispanic, and other, (3) income: greater or less than \$35,000 in 2001, 2003 or \$40,000 in 2005, (4) education: greater or less than high school, and (5) medical insurance source: employer sponsored insurance (ESI), private coverage, Medicare, Medicaid, combinations, and dual Medicare/Medicaid coverage. In addition, t-tests and logistic regression modeling were performed to determine the significance and contribution of the five independent variables to dental insurance. Adjusted odds ratios (AOR) were calculated to determine the independent contribution of each predictor.

Results: In 2005, the percentage of adults older than 65 years (74.2%) with dental coverage was greater than two times that of adults 19-64 years (32.6%). African-Americans had more dental coverage than Hispanics, who had slightly more than Caucasians (73.6%, 65.5%, and 64.0% respectively). More adults with higher incomes (76.2%) and higher education (67.6) had dental insurance than lower incomes (54.6%) and less education (48.0%). 82.8% of adults with ESI had dental coverage, 58.0% with Medicaid, 49.7% with dual, 38.5% with combination, 25.6% with private, and 19.9% with Medicare coverage. Retirement-age adults (>65) are half as likely to have dental insurance as those of working age (AOR .449). African-Americans have much higher and Hispanics slightly higher odds of having dental coverage (AOR 1.86, 1.06 respectively). Higher income and higher educated Americans are 1.6 and 1.1 times more likely to have dental insurance. Type of medical insurance source relates to having dental coverage with few who are not employer insured having dental coverage (AOR are .37, .36, .29, .12, and .07 respectively for Medicaid, dual, combination, Medicare, and self insured). All differences were statistically significant at <.05% level.

From 2001 to 2005, the percentage with dental coverage increased overall from 62.4% to 65.5%. Similarly, the percentage with dental coverage increased overall between 3.1% and 12.8% for each correlate of age, race, income, education, and medical insurance source. The two exceptions were declines in dental coverage for Hispanics (66.0% to 65.5%) and privately insured (33.7% to 25.6%). The odds of having dental coverage increased among African-Americans and decreased among Hispanics over time compared to Caucasians. In 2005, those individuals with higher incomes were 1.66 times more likely to have dental insurance than those with lower incomes, a decline from 1.83 times in 2001. All types of medical insurance, except combination coverage, became less likely to predict dental coverage from 2001 to 2005.

Conclusions: Although dental coverage among American adults is increasing slightly, disparities still exist. This study demonstrates the uneven distribution of dental coverage by age, education, income, race and source of medical insurance, as well as worsening trends in dental coverage, especially among Hispanics and the privately insured. Finally, having medical insurance and the type of health coverage significantly impacts dental coverage.

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Coping with Dental Symptoms: Self-Care Strategies Used by African Americans Living in Central Harlem

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Introduction: Although oral health status in U.S. has improved over the years, the burden of oral disease is still disproportionately endured by people of color and the socio-economically disadvantaged. These disparities have been attributed to, among others things, limited access to dental services, fear of intervention, and a deep-rooted belief regarding the inevitability of dental problems. Thus, it is possible that members of this population, when faced with oral problems, may forego professional dental treatment and use alternative or self-care strategies to ameliorate their oral problems. To explore this, a study was conducted to investigate the strategies used to manage oral symptoms among adults living in Central Harlem.

Objective: (1) To describe the principal coping strategies used to manage toothache and sensitivity to cold by adults in Central Harlem; (2) To identify which coping strategies are most frequently used for each symptom, (3) To contrast the types and prevalence of coping strategies used for each of these two common symptoms.

Materials and Methods: Study participants were recruited in locations throughout Central Harlem, using a street intercept approach to screen for eligibility. Individuals who met the study's eligibility criteria were invited to participate in an in-depth interview about their attitudes, beliefs, and strategies regarding management of an oral symptom. A sample of N=118 African American men and women agreed to participate in in-depth interviews with a trained interviewer, lasting from 90 to 120 minutes, conducted from September 2004 to June 2005. The interviews were tape recorded and transcribed. Interview transcripts were then analyzed using the qualitative software, Atlas.ti, followed by more detailed thematic coding/analysis by members of the study team. Due to the small sample size, statistical comparisons were computed using Fisher's exact test.

Results and Conclusions: Study data indicated that a toothache (38%) and sensitivity to cold (48%) were the dental symptoms most often experienced by the respondents during the past 6 months. A number of significant differences were identified in the coping strategies used to manage these two symptoms. Participants with toothache pain were significantly more likely than those with teeth sensitive to cold to use systemic oral pain medication (e.g., Tylenol; 78% vs 48%, $p = .04$), to use topical analgesics (e.g., Orajel; 56% vs. 22%, $p = .02$), and tended to be more likely to do things to calm themselves down (e.g., sleep, lay down; 26% vs. 4%, $p = .06$). In contrast, those with a sensitivity to cold were significantly more likely to manage the symptom using special toothpastes (e.g., Sensodyne, 22% vs. 0%, $p = .02$), by changing their eating habits (e.g., avoiding certain foods, chewing on one side; 57% vs. 4%, $p = .001$), and tended to do things to distract themselves (e.g., slapped myself, made a fist; 39% vs. 15%, $p = .06$). There were no significant differences in the use of oral rinse or gargling (67% vs. 43%, $p = .15$) or in the seeking of professional dental care (30% vs. 26%, $p = 1.0$) between those with toothache or sensitivity to cold. Findings provided evidence that the African American adults of Central Harlem applied diverse coping strategies in response to their dental symptoms and that the strategies used varied by the type of symptom. Use of professional care was less than 30% for both symptoms and was often the last alternative used.

Discussion: Study findings suggest that many African Americans in Harlem may be unable or unwilling to seek professional dental care, and that they may use self-care strategies in an effort to postpone or prevent the need to seek professional dental care. Thus, further research is needed to identify the barriers (both financial and attitudinal) to obtaining dental care. This research also suggests the need to provide information about the importance of professional dental treatment, the risks of treatment delay, and how to access low-cost emergency dental care.

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Screening Ameloblasts for Apoptosis

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Background: The initial deposition of dentin matrix is followed by the differentiation of pre-ameloblasts into secretory ameloblasts (SA) that deposit enamel matrix, which acts as a protein scaffold for provisional mineralization. Later, maturation stage ameloblasts (MA) fully mineralize the enamel while simultaneously they remove most of the enamel matrix using proteases such as KLK4 and MMP-20. Currently, there is no complete model for the mechanism of enamel mineralization, but the presence of Carbonic Anhydrase II (CAII) in the cytoplasm of MA's suggests pH regulation during enamel mineralization is a necessary factor. Excess of fluoride ingestion during childhood impedes proper amelogenesis by an unknown mechanism, but the retention of matrix proteins and depressed mineral content of enamel in fluorotic teeth suggests proteolysis of the matrix is impaired. Hypotheses for this mechanism propose that excess fluoride 1. causes increased apoptosis in transition ameloblasts, 2. may influence pH regulation of the enamel by MA's, or 3. may inhibit formation of proteases MMP-20 and KLK-4 at different stages of ameloblast maturation. Understanding the timing, regulation, and function of ameloblasts is critical for revealing the methods of enamel formation and discovering the etiology of conditions that obstruct it, such as fluorosis.

Objective: The aim of this study was to compare apoptosis levels in rat fluorotic and normal incisors, determine the efficacy of MMP-20 and KLK-4 antibodies for future studies, and determine the presence and activity of CAII in developing teeth.

Methods: Rat and hamster jaw tissues were fixed with 5% paraformaldehyde, decalcified in EDTA for 6-8 weeks, embedded in paraffin, cut into 7µm sections, cleared, and rehydrated using standard techniques. Tissues from fluorotic sections were harvested from female rats grown on a 100 ppm F drinking water diet. Apoptosis screening was done by TUNEL staining for DNA fragments with peroxidase detection, by DNA staining with propidium iodine (PI) and by immunostaining with anti-Casp-3 antibody. Carbonic anhydrase activity assay was done using a modified Hansson's method. Cryosectioned sections were fixed with acetone, incubated in a 1.75mM CoSO₄, 5.85mM KH₂PO₄, 53mM H₂SO₄, 314mM NaHCO₃ solution, and developed with 1% Na₂S. Immunostaining for CA-II, MMP-20 and KLK4 was done with anti-CA-II, anti-MMP-20 and anti-KLK4 at varying concentrations for 1 hr at room temperature using ABC peroxidase or ABC alkaline phosphatase labeling (Vector kits).

Results and Conclusion: TUNEL staining revealed a positive staining in transitional ameloblasts. Immunofluorescent screening with PI showed positive reaction in multilobed nuclei, suggesting they were apoptotic bodies, but oblique sectioning makes this subjective. IHC methods for Casp-3 activity in control and fluorotic jaws were ineffective, likely due to poor tissue preservation during long decalcification. CAII assay and IHC staining showed presence and activity of CAII in the enamel organ, particularly in the MA, in agreement with published reports. The method does seem to be effective. IHC staining for KLK-4 was positive in MA's and negative in SA's, but background was intense. IHC staining for MMP-20 was positive apical to the Tome's processes of SA's, and negative in the MA's at concentration below 1:800. The IHC results for the proteases proteins could probably be improved by using an affinity purified Ab, as opposed to the total IgG fractions used. The data obtained serve as a starting point to investigate in detail the changes in protein location and activity in ameloblasts exposed to fluoride to unravel the mechanism of dental fluorosis.

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Smokers with Inattention or Hyperactivity/Impulsivity Symptoms: Response to Bupropion and Nicotine Patch Treatment

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Introduction: Attention Deficit Hyperactivity Disorder (ADHD) is a common childhood disorder that can continue into adulthood affecting approximately 4% of adults in the United States. Adults diagnosed with ADHD have higher smoking rates and a lower likelihood of successful quit attempts than the general population. Tobacco use is a major risk factor for dental pathoses such as periodontal disease and oral cancer, and systemic diseases such as lung cancer. As regularly visited health care providers, dentists have a special opportunity to recommend smoking cessation strategies to their patients. Therefore, dentists should be aware of the best treatment strategies for their patients.

Objective: The aim of the study was to determine the smoking cessation success rates in response to open label treatment with bupropion and nicotine patch therapy in participants with mostly Hyperactivity/ Impulsivity (HI) symptomatology and mostly Inattention (IN) symptomatology of ADHD compared to smokers without ADHD symptoms.

Methods: 583 adult smokers participated in an 8 week program run by Dr. Covey at the Columbia University Medical Center Smoking Cessation Clinic. Participants answered the ADHD Current Symptoms Scale, which measures symptoms of Inattention and Hyperactivity/Impulsivity. All participants were on an open treatment of bupropion for eight weeks and nicotine patch for seven weeks beginning on the day before quit day. Smoking abstinence was measured at weeks 1, 2, 4, 6, and 8 by different outcomes.

Results: 43 of 583 (7.4%) of participants had significant ADHD symptomatology. These participants were grouped into categories of IN alone (n = 20) or HI +/- IN (n = 23). The IN subgroup had a higher success rate than the HI +/- IN subgroup over the entire 8 week trial with a final success rate at week 8 of 55%, as compared to 35%.

Conclusions: HI +/- IN symptomatology is associated with a lower smoking quit rate during bupropion and nicotine patch treatment as compared to IN symptomatology of ADHD. This difference suggests that high impulsivity is a predictor for an unsuccessful quit attempt. This information suggests that symptoms of hyperactivity could be an adverse predictor of smoking cessation and that patients with these symptoms are not responsive to the treatment combination of bupropion and nicotine patch. Treatments that reduce impulsivity could act as helpful adjunct therapy when smokers who suffer from symptoms of impulsivity experience difficulty trying to stop smoking.

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Developing a Process for International Comparisons in Global Children's Oral Health

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Introduction: In 2005, 40 chief dental officers from around the world institutionalized a Global Child Dental Health Taskforce (GCDHT) with the specific aim of eradicating early childhood dental caries worldwide in the birth cohort of 2026. The initiative focuses on six key interventions in eight countries where national taskforces have been established: Australia, Brazil, China, India, Mexico, the Philippines, South Africa, and the United States. The six GCDHT interventions are: (1) community water fluoridation, (2) dental sealants, (3) sugar reduction, (4) improved access to dental care, (5) leadership and (6) oral health education.

Objectives: (1) To determine the availability of national-level data for each member country and (2) to identify indicators that can provide baseline comparisons of each member country's pediatric caries disease burden, oral health delivery system, and implementation of the six interventions.

Materials and Methods: An initial Medline search showed that scant national-level data are reported in the peer-reviewed literature. Epidemiological data was obtained from the World Health Organization through its Area Profile Programme. Workforce and dental system information reported by national dental associations was obtained through the Federation Dentaire International website. For the US, detailed information was obtained from Centers for Disease Control, the Association of State and Territorial Dental Directors, the American Dental Association, the US Department of Health and Human Services and Medicaid's EPSDT (Early Periodic Screening, Diagnosis and Prevention) program reports.

Results and Conclusions: A standardized data collection form was developed and disseminated to the GCDHT Taskforces. The form draws heavily on indicators for which data has descriptive power and demonstrates collection feasibility. Items were formulated to be as inclusive as possible of the diversity of funding sources, expenditures, and delivery systems of dental health services, as well as the avenues by which children access care. The form seeks to identify both the range of oral health treatments available and the types of providers qualified to deliver those services.

This investigation revealed that comprehensive pediatric caries epidemiologic data are available only for Australia, South Africa and the United States. Other member countries report only select indicators. These three countries also publish data on their oral health delivery systems for caries treatment and prevention. Various metrics for other countries may be available but are often incomparable. While some indicators of health have been standardized internationally, others (e.g. use of fluoride and sealants) remain variable. Data collection on non-standard measures necessitates the use of descriptive reporting that allows responses outside the parameters of a strictly quantitative measure. In addition, language barriers present a substantive challenge both in the development of a system to identify data for cross-country comparison and in developing measures that will elicit a focused but inclusive response.

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Study of Abnormal Remodeling Activities in *oim/oim* Mouse Using an *In Vitro* Osteoclast-Calvaria Co-Culture System

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Introduction: Osteogenesis Imperfecta (OI) is a heritable disorder, typically characterized by heterogenous mutations within the genes for type 1 collagen, the predominant protein within bone. Although osteoblast (OB) abnormalities have been identified in OI, investigations of osteoclast (OC) abnormalities in OI have been limited. This is especially important with the usage of bisphosphonates to control the level of bone turnover via inhibition of OC activity. Previous studies within our lab studied mouse model OI found OCs being greater in number, having a greater number of nuclei, and displaying increased bone-resorption activity in comparison to control OCs. Insight into the bone turnover abnormalities relating to the interaction of OBs and OCs in OI could be gained by studying co-cultures of OCs and OBs on actual bone matrix, but to date, no such studies in either OI patients or in animal models exist. This is the first in vitro cell/organ culture model to study the interaction among OB, OC, and bone matrix.

Objectives: The purpose of this project is to (1) characterize bone modeling in an invitro OC-Calvaria (CAL) Co-culture model (2) emphasize the function of OB and OC (3) investigate the interactions between OB and OC and (4) investigate the interactions between bone matrix and OC.

Methods: Bone marrow cells, the OC source, from either *oim/oim* or wildtype (+/+) mice, 5 to 8 weeks of age, were cultured on WT calvarial pieces 3mm in diameter (the OB source), in the presence of 10^{-8} M 1,25-(OH)₂D₃ and 10^{-6} M prostaglandine E₂. There are 4 groups based on the 2X2 table of OC and Cal from both *oim/oim* and wild type (+/+): WTCAL-WTOC, WTCAL-OIOC, OICAL-WTOC, and OICAL-OIOC group. Tartrate-resistant acid phosphatase (TRAP) staining using a commercial kit (Sigma Diagnostics) was used to study the OC function by identifying the multi-nucleated cells (MNCs). OB function was studied by histochemical semi-quantitative demonstration of alkaline phosphatase (ALP) in OB using a commercial kit (Sigma Diagnostics). In order to measure the actual amount of resorption the CALs in 12-well culture plates were harvested at day 7 and day 14. The whole surface of each CAL was scanned utilizing scanning electron microscopy. The images were analyzed using Image J v1.33 software (National Institutes of Health, USA). The resorption function was expressed as resorption percentage calculated by total resorption areas divided by total area of the CAL.

Results and Conclusion: ALP study showed that there was no difference in OB number at day 7 and at day 14 in each 4 groups. TRAP staining study indicated that osteoclastogenesis was significantly decreased in OICal-OIOC group comparing with +/+ group. Interestingly, the crossing groups such as OICal-WTOC and WTCal-OIOC were not significantly different from each other. The defect in osteoclastogenesis in this in vitro model is due to the combined defect in OB, OC, or matrix, not simply OB defects or OC defects. When the resorption percentage was normalized to OC number, the resorption function per OC was largely increased in the OICal-OIOC group comparing with 3 other groups.

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Identification of $\beta 6$ integrin cytoplasmic domain interacting proteins

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Introduction: Integrins are cell surface receptors that interact with the extracellular matrix (ECM) and mediate intracellular signals. Integrin signaling controls cell adhesion, migration, and cell proliferation. Structurally, integrins are heterodimers with an alpha and a beta subunit. The alpha subunit is the regulatory subunit, and the beta subunit is the signaling subunit. Both subunits are necessary for interaction with the ECM.

Previously, the lab generated the conditional knockout (KO) of integrin $\beta 1$ in the epidermis and successfully established $\beta 1$ KO cells in culture. The epidermis of the $\beta 1$ KO mice showed an increased expression of $\alpha v\beta 6$, an integrin that is upregulated during wound healing and in poorly differentiated tumors. When $\beta 1$ KO cells were cultured *in vitro* on fibronectin, the KO cells were able to attach due to the presence of $\alpha v\beta 6$; but were defective in cell spreading and cell migration. However, the increased expression of $\alpha v\beta 6$ *in vivo* did not rescue the basement membrane phenotype. This led us to ask the question: what is the difference between $\beta 1$ and $\beta 6$ containing integrins?

Objective: The goal of the study is to try to understand the functional and signaling differences between $\beta 1$ and $\beta 6$ integrins and, more specifically, to identify the intracellular proteins that interact with the cytoplasmic domain of $\beta 6$ but fail to interact with $\beta 1$. Previous studies identified 21 intracellular proteins that interact with the cytoplasmic domain of $\beta 1$; however, little is known about the proteins that interact with the cytoplasmic domain of $\beta 6$. Understanding the biology of $\beta 6$ integrin is important as it is highly expressed in cancers of various origins including lung, breast, pancreas, ovary, and skin.

Materials and methods: N-terminally tagged Glutathione S-transferase (GST) fusion proteins were generated with the $\beta 1$ and $\beta 6$ cytoplasmic domains. The proteins were expressed in BL21 bacterial cells and purified using the sarkosyl extraction method. For the *in vitro* pull-down assays, cell lysates were made from either from fibroblasts (AM12 cells) or keratinocytes. The cell lysates were incubated with GST- $\beta 1$, GST- $\beta 6$, and GST beads, washed and loaded onto 10% acrylamide gels, and stained with coomassie; interacting proteins were detected. The bands in the gel that represented proteins that bound to GST- $\beta 6$ and not GST alone were cut out and sent out for mass spectroscopy analysis (<http://cpmnet.columbia.edu/dept/protein/index.html>), which is a technique that uses the mass-to-charge ratio of ions in order to identify the composition of a physical sample by generating a mass spectrum.

Results and Conclusions: Five putative proteins that interacted with the $\beta 6$ cytoplasmic tail were identified using mass spectroscopy. These data are being analyzed.

Discussion: We were able to identify differential binding of proteins to the cytoplasmic domains of the two integrins $\beta 1$ and $\beta 6$, and the implications of these findings will be discussed.

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Role of Rac1 in the Disruption of TGF β Signaling in Cutaneous Squamous Cell Carcinoma

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Introduction: Squamous cell carcinoma (SCC) constitutes approximately 94% of all oral cancer. SCC has a relatively high propensity for invasion and metastasis although the biological basis for the aggressive behavior of cutaneous SCCs is poorly understood. One characteristic of epithelial tumors such as SCC with a high risk of tumor progression is inappropriate $\alpha 6\beta 4$ integrin expression. In transgenic mice, suprabasal expression of $\alpha 6\beta 4$ in the skin predisposes the epidermis to form chemically-induced tumors and perturbs transforming growth factor β (TGF β) signaling in basal cells via a mechanism that involves Rac1 activation. To determine whether a similar scenario exists in human SCC pathogenesis we examined the status of $\alpha 6\beta 4$ integrin, Rac1, and pSmad (as a readout for intact TGF β signals) in human cutaneous SCC specimens.

Materials and Methods: Samples of suspected epidermal SCCs obtained from Columbia University Medical Center were sectioned and fixed onto charged glass slides, fixed with acetone and incubated with 10% normal goat serum in PBS (NGS/PBS) for 30 min to block non-specific antibodies. The slides were stained with H&E to verify histopathological grade. Slides of those specimens exhibiting hallmarks of SCC were probed with antibodies to both $\alpha 6$ integrin (MPF410, 1:400) and Rac1 (1:1000) and correspondingly probed with fluorochrome-conjugate secondary antibodies to observe primary antibody staining. To visualize cell nuclei, slides were mounted in gelvatol medium, containing 1.5 $\mu\text{g}/\text{mL}$ diamidino-phenyl-indole, dihydrochloride (DAPI) and viewed using a Zeiss Axioplan II microscope with fluorescent capabilities.

Results and Conclusion: In a high percentage of human SCCs analyzed, co-localization of suprabasal $\alpha 6\beta 4$ and Rac1 was, indeed, evident in areas of the SCC exhibiting more intense suprabasal $\alpha 6\beta 4$ staining indicating there may be a link between the augmented Rac1 signaling. In addition, we found that expression of $\alpha 6\beta 4$ in human SCCs is due to a direct physical association between Rac1 and $\alpha 6\beta 4$ in the membrane of suprabasal cells.

These studies corroborate our previous findings in experimental mouse skin and indicate that the status of overexpression of $\alpha 6\beta 4$ in SCCs. Future applications of this research can focus on manipulating Rho GTPase signaling in these cells to determine its impact on TGF β -mediated growth inhibition to ascertain how changes in epidermal skin cell communication can impact on the development of potentially fatal human skin cancers. These studies illustrate how aberrations in a cellular system essential for normal function can influence the susceptibility for neoplasia, and therefore have broad implications for both oral and epithelial cancer.

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Adoptive Transfer of RAGE-Deficient T Lymphocytes Attenuates Atherosclerosis in Diabetic Apolipoprotein E Null Mice

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Introduction: Atherosclerosis is a chronic inflammatory response that causes the formation of plaques in the walls of arteries. It is associated with various immune responses and metabolic conditions such as diabetes. Receptor for advanced glycation end products (RAGE) is a part of the immunoglobulin family of cell surface molecules that plays a significant role in diabetic atherosclerosis. While there is an increased expression of RAGE in several pathological states, the correlation between RAGE and lesion development in atherosclerosis-prone mice has not yet been studied. In this study, we tested the hypothesis that increased levels of T lymphocyte RAGE expression contributes to lesion progression in established atherosclerosis in diabetic Apolipoprotein E (apoE) null mice.

Objective: To determine the role of T lymphocyte RAGE in atherosclerotic lesion progression in diabetic apolipoprotein E (apoE) null mice. To understand the mechanisms by which T cell RAGE contributes to this pathological process.

Materials and Methods: Male apoE null mice and male apoE null RAGE null mice, age 6 weeks, were rendered diabetic with streptozotocin. At 14 weeks of age, mice were sacrificed and aortas were collected for lesion analysis. The aortic mean lesion area in apoE null RAGE null mice was 2.3-fold lower compared to that in apoE null mice ($p < 0.05$). The apo E null RAGE null mice displayed no complex lesions compared to apoE null mice. To specifically dissect the role of T lymphocyte RAGE, we performed adoptive T cell transfers into sublethally-irradiated diabetic apoE null mice. We purified CD4 and CD8 positive T cells from apoE null mice and apo E null RAGE null mice and transferred these cells into diabetic apoE null mice at age 8 weeks. The chimeric mice were sacrificed at age 18 weeks.

Results and Conclusions: Quantitatively, mice receiving CD4+/CD8+ T cells from apoE null RAGE null mice displayed a 6-fold reduction in mean lesion area, compared with apoE null mice receiving T cells from apoE null RAGE-expressing mice, $50,039.00 \pm 9,435.43 \mu\text{m}^2$ ($n=7$) versus $304,275.20 \pm 76,797.69 \mu\text{m}^2$ ($n=6$), $p < 0.01$. Serum levels of glucose, cholesterol, and triglyceride did not differ between diabetic apoE null mice receiving apoE null RAGE-expressing or deficient T cells ($p > 0.05$). Analysis of cytokines in the aortas of mice receiving T lymphocytes devoid of RAGE revealed significantly lower levels of TNF-alpha.

Discussion: The results from the study provide evidence that increased expression of RAGE can be directly correlated with the acceleration of atherosclerotic lesion formation in the diabetic state. T lymphocyte RAGE expression is strongly implicated in this process, likely, at least in part, by mediating the expression of inflammatory cytokines. Targeting cell-specific RAGE may be a therapeutic strategy for treating the progression of atherosclerosis in diabetes.

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3D Co-Culture of Human Fibroblasts and Human MSC-Derived Chondrocytes

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Introduction: Fibrocartilage is a specialized type of cartilage that contains bundles of thick collagen fibers and endures shearing forces. It is present in temporomandibular joint, knee meniscus, and intervertebral disks. Trauma, congenital malformation, tumor resection and chronic diseases may place large demands for the surgical replacement of these joints. The use of allograft, synthetic, or autologous grafts is currently the primary treatment modality. However, these methods are associated with risks such as donor site trauma, immuno-rejection, poor integration and pathogen transmission. Tissue engineering using mesenchymal stem cells (MSCs), offers a significantly promising opportunity for future treatment modality. The purpose of this study is to understand the interactions between hMSC derived chondrocytes and fibroblasts, as one of the first steps toward tissue engineering of fibrocartilage.

Materials and Methods: Human mesenchymal stem cells (hMSCs) and human dermal fibroblasts. hMSCs were isolated from multiple bone marrow samples (AllCells), and culture-expanded in basic DMEM medium (10% FBS and 1% antibiotic-antimycotic) until confluence. Human dermal fibroblasts were culture-expanded in basic DMEM medium until 80-90% confluence. **hMSCs differentiated chondrogenic cells.** hMSCs were differentiated into chondrogenic cells (hMSC-Cy) using 10 ng/ml of TGF β 3 (R&D) in DMEM medium supplemented with 1% ascorbic acid, 0.9% sodium pyruvate, 0.1% L-proline, 1% +ITS, 1% penicillin/streptomycin, 1% dexamethasone, for about 2 weeks using our previous protocols. **Co-culture of fibroblasts and hMSC-derived chondrocytes in 3D gel.** Poly(ethylene glycol) diacrylated (PEG) hydrogel was used as a scaffold for the co-culture of fibroblasts and chondrocytes per our prior method. Human MSC-Cy and fibroblasts were trypsinized, and resuspended in PEG polymer /photoinitiator solution at density 2 million cells/ml. Cell/polymer suspension was exposed to UV light at 365nm to gel, washed by PBS solution and cultured at 37C with 5%CO₂ for 1, 2 and 4 weeks in incubator with medium change every 3-4 days. There were totally four groups: layered hMSCs/fibroblasts (in **A**: + TGF β 3 medium or **B**: - TGF β 3 medium) and mixed hMSCs with fibroblasts (in **C**: + TGF β 3 medium or **D**: - TGF β 3 medium). **Histology:** cartilage was characterized by Alcian blue and safranin O staining, whereas fibro-tissue stained by Trichrome and H&E. **Real Time-PCR** By Applied Biosystem 7300 to detect markers: col I, II, aggrecan, and fibronectin.

Results: 1. Alcian blue staining was visually more robust in group A & C than group B & D after 2 and 4 wks. 2. Aggrecan (AGG) mRNA expression by real time-PCR was significantly higher for hMSC-Cy in A & C than group B & D, which did not have detectable level of AGG. That indicates + TGF β 3 may play a major role for maintaining hMSC-Cy phenotype in vitro incubation. 3. Human dermal fibroblasts seeded in PEG hydrogel in all 4 groups are not remarkably different by trichrome staining after 2-4 wks in vitro incubation, and showed a lack of significant difference in mRNA expressions of collagen I and fibronectin, suggesting that human dermal fibroblasts may not readily change their lineages when co-culture with hMSC-Cy.

Discussion: In order to tissue engineering of fibrocartilage, we designed the current in vitro experiment to evaluate the interactions between hMSC-chondrocytes and human fibroblasts. The PEGDA gel used in the experiment is biodegradable. It allows for diffusion of nutrients through the medium to the encapsulated cells. Assigned cell location resulted in cell-cell physical contact or division within relevant optimized culture medium. Our results suggest that co-culture induces significant change for the expression of relevant markers. Aggrecan expression was detected only in + TGF β 3 for 2 and 4 wks, suggesting that the conditioned medium plays an important role for maintaining the phenotype of hMSC-derived chondrocytes in co-culture. The degree of expression of aggrecan in C was lower than that in A, implying that fibroblasts may suppress the matrix expression of hMSC-derived chondrocytes when the two different types of cells are in close vicinity. However, the lack of significant difference in mRNA expressions of collagen I and fibronectin may indicate fibroblasts may not readily change lineages even in conditioned media with co-cultured hMSC-derived chondrocytes. These data may be helpful in future co-seeding of fibroblasts and chondrocytes toward the tissue engineering of fibrocartilage.

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Creation of a Fetal Skull Atlas Using Cone Beam Computed Tomography

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Background: Since the late 1970s, little comprehensive research involving radiographic imaging of the fetal skull has been published. The Cranium of the Newborn Infant: An Atlas of Tomography and Anatomical Sections (by Robert H. Pierce, Michael W. Mainen, and James F. Bosma) was released in 1977, in anticipation of further fetal skull studies using more advanced imaging modalities. To date, only limited studies have been completed using computed tomography (CT).

Objective: A primary intent of this project is to update and improve the Pierce, Mainen, and Bosma atlas, which was limited to surface tomograms that could not capture the intricate anatomy of the fetal skull. Enhanced imaging capabilities of cone beam computed tomography (CBCT) make it possible to view internal skull structures previously difficult to visualize. An additional objective is to create an interactive video atlas of streaming annotated CBCT images in coronal, axial, and sagittal planes. Ultimately, it is our goal to add to the scientific literature a digital and printed fetal skull atlas that can be used as a reference for subsequent studies regarding prenatal growth and development.

Materials and Methods: Nine fetal skulls from a pre-existing collection in the Division of Oral Biology of Columbia University College of Dental Medicine were imaged. Nothing is known about the ages of the specimens or the circumstances preceding skull acquisition. Using Imaging Sciences International's Classic i-CAT™ Cone Beam 3-D Imaging System in Vanderbilt Clinic's Division of Oral & Maxillofacial Radiology, skulls were exposed to a standard 20 second scan (120 kV, 23.87 mAs, and 0.4 mm voxel size). i-CAT scans were reviewed with accompanying i-CAT Vision software and specific images selected for further study. Anatomic landmarks were labeled using the Image Annotation Tool created by the Columbia Center for New Media Teaching and Learning (CCNMTL). An interactive video atlas of annotated continuous CBCT images is currently being created in partnership with CCNMTL, using Camtasia Studio, Final Cut Pro, and QuickTime Pro softwares.

Discussion: Several studies (Neumann, 1997; Neumann, 1999) have concluded that CT is a preferred medium for fetal skull development and morphology research. The Cone Beam 3-D Imaging System allows exact anatomic imaging in virtually limitless planes, thereby minimizing the need for involved dissections that may damage or compromise the original specimens. Recent fetal skull research utilizing CT has mainly focused on specific regions, such as the developing skull base (Nemzek, 2000) and osseous labyrinth (Porowski, 2003). Missing from the literature is a complete atlas of CT images demonstrating the size, location, and approximation of the various fetal skull components. Highlights of our body of work include improved radiographic images of the developing teeth, optic canals, temporal bones, and temporomandibular joints. It is our hope that our project will increase understanding about prenatal developmental anatomy and encourage additional studies relating to normal and abnormal craniofacial growth in the fetus.

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Targeted Electronic Educational Intervention and Health Literacy Assessment of an Insured Population

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Background: Diabetes is a chronic metabolic disorder that can affect people of all age groups. The National Institutes of Health reports for the year 2005, 20.8 million people in the United States have Diabetes. This disease complex can lead to serious systemic complications such as retinopathy, neuropathy, nephropathy, coronary diseases and peripheral vascular disease. Patients with diabetes have a higher incidence of periodontal disease and other oral conditions. Evidence suggests that it can have deleterious effects on the periodontal and oral tissues. Thus people with diabetes are at an increased risk of developing periodontitis, oral infections and early tooth loss leading to impaired quality of life and oral disability. This is a very disconcerting fact as these oral health conditions can be prevented through personal and professional care. One of the national health objectives for 2010 is to increase the percentage of diabetic population who has an annual dental examination to 71%. The 2004 survey of the CDC found that this has been achieved in only seven states. This poor understanding of the oral-systemic linkages for people with diabetes contributes to diminished health outcomes. An increase in knowledge about oral-systemic linkages can improve the oral health status of diabetics. Furthermore, it has been observed that diabetics who maintain their oral health have better glycemic control.

Objective: The primary objective of this research project is to assess if a web based educational program targeted to diabetics can increase knowledge on the oral-systemic link for diabetes in a medically insured sample of persons with diabetes. The secondary objective of the study is to assess current health literacy, demographic, health history, and Internet usage information.

Methods: The participants for this study are medical insurance subscribers of a large national health insurer who have been diagnosed with diabetes. They will be recruited via direct mailing of a post card by the health insurance company. This project will consist of an initial survey to evaluate diabetes and oral health related knowledge, demographic, health history, and Internet usage information, followed by an online educational intervention in the form of a website, and a subsequent survey to assess for any change in knowledge and attitudes regarding diabetes and oral health and to determine the health literacy of the participants via an electronic assessment. The first 200 participants to complete the entire study will receive a \$20 gift card incentive. The intervention is a website designed to promote good oral health practices, create awareness about the oral complications of diabetes, and promote the effectiveness of home hygiene as well as regular dental visits. The target reading level for the information provided was 8th grade which has been found to be optimal for health information materials.

Results: The study is still under investigation.

Dental Care Service at Harlem United: A Needs Assessment Study

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Introduction: Harlem United Community AIDS Center in New York City is a community-based organization that provides comprehensive dental, medical, social, and supportive care to over 2,300 people per year. Most of the clients at Harlem United (HU) are HIV/AIDS positive, African-American or Hispanic, of lower socioeconomic status, and have had a history of mental illness and/or substance abuse. Previous research (Zabos et al., 2002) found that “problem with teeth or gums” was the most frequently reported complaint (30%) among Central Harlem adults. Thus it is of interest to investigate the extent to which new patients at HU present with a history of past oral health problems and irregular usage of dental care services.

Objectives: The goal of this study was to conduct a needs assessment on the new patients seen at the HU dental clinic between 2003 and 2007, by gathering information about their oral health-related behaviors and perceptions. Analyses of the results permit us (1) to identify patient needs and concerns with regard to prior oral health care experiences and conditions, and (2) to use this information to inform planning for the delivery of targeted dental services to address the patient needs and barriers identified.

Materials and Methods: At their initial visit at Harlem United Dental Clinic, patients were asked to complete a two-page survey describing their frequency of oral hygiene habits, smoking history, reasons for not seeing the dentist regularly, their oral health quality of life, and their self-assessed oral health status. A total of 506 patients filled out the survey over a period of approximately 4 years. Responses were input into a database and analyzed with the statistical analysis program SPSS, using both univariate and bivariate statistical techniques to describe basic patient characteristics at the time of the first visit and their associations.

Results and Conclusions: Of the 506 patients surveyed, 43% stated that they hardly ever floss, 54% identified themselves as current smokers, and 72% reported not going to the dentist regularly. Of this latter group (n=283), the most prevalent reason given for not going to the dentist regularly was fear of the dentist and/or pain (48%). Patients who viewed their oral health as very healthy were less likely to express fear of the dentist and/or pain as a reason for not going to the dentist regularly than were those who viewed their oral health as not healthy (31% vs. 60%, p=0.008). They were, on the other hand, more likely to think they did not need a dentist than did those who perceived themselves as unhealthy (31% vs. 5%, p=0.002). When asked to assess their oral health status, 37% of the patients reported that their teeth were not healthy or in pain, and 35% reported their last visit to the dentist was because of pain. Fifty-nine percent of the patients said that they were embarrassed by the appearance of their teeth. Patients who reported going to the dentist regularly were less likely to have ever felt embarrassed by the appearance of their teeth relative to those who did not go regularly (38% vs. 69%, p<0.001) and were less likely to assess their oral health as not healthy than those not attending regularly (22% vs. 47%, p<0.001). This assessment indicates that relatively large percentages of new HU patients floss infrequently, smoke currently, go to the dentist irregularly and often with pain, fear going to the dentist and/or its associated pain, perceive their teeth as unhealthy, and are embarrassed by the appearance of their teeth.

Discussion: This initial needs assessment identified a number of barriers to accessing dental care, as well as need for improved oral health maintenance among new patients at the HU dental clinic. Findings from this study suggest that disparities in rates of HIV infection between minority and non-minority populations also exist with respect to oral health status and access to dental care for HIV-infected minorities. Informed by the needs and concerns expressed by new dental patients at HU, focus groups are planned to understand in greater depth: what previous experiences underlie their fear of the dentist and/or pain, and their feelings of embarrassment due to the appearance of their teeth, as well as their strategies for coping with previous episodes of pain and what it means to be a regular user of dental services among this group of patients. More understanding of each of these areas can help to frame future delivery of dental care at HU, motivating consideration of whether or not standard patterns of providing oral hygiene instruction, preventive care and treatment should be modified, or new programmatic initiatives developed, to best serve the dental patient population at HU.

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Comparison of Endo-Eze AET™ and Manual Root Canal Instrumentation Techniques in Primary Teeth: An *In Vitro* Study

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Background: Pediatric endodontic procedures can be challenging given both the microanatomy of primary teeth and the child's ability to cope with extended treatment times. Recent advances in endodontic techniques are reported to help overcome such limitations, thus preventing the needless extraction of deciduous teeth. Conservation of primary teeth is essential for the developing occlusion and preservation of arch length. Current literature shows encouraging success rates towards achieving this goal using endodontic therapy (75%-96%¹) versus the alternative: extraction followed by a space maintainer (72%²).

Objectives: (1) To evaluate cleaning capacity of two root canal instrumentation techniques in extracted primary teeth and (2) to compare instrumentation time in each group.

Methods: 90 root canals of extracted primary teeth were injected with India Ink and divided into two groups for instrumentation: (A) stainless steel hand instruments and (B) Endo-Eze AET™. Instrumentation time was recorded per tooth and root canals were sectioned into cervical, middle, and apical thirds. The degree of dye removal was assessed using the ProScope HR™ digital microscope under 100x magnification.

Results: Objective (I): Hand instrumentation with K-files showed a greater extent of dye removal than Endo-Eze AET™ instrumentation. The differences in cleaning capacity for the cervical and middle thirds were statistically significant ($p < 0.05$); the differences in the apical third showed greater dye removal with hand instrumentation, although not statistically significant ($p = 0.069$). Objective (II): Less time was needed for Endo-Eze AET™ instrumentation (7.06 min/tooth) than hand instrumentation (10.48 min/tooth), requiring 32.6% less time on average.

Conclusion: Pilot data suggests that conventional root canal hand instrumentation techniques perform better than Endo-Eze AET™ reciprocating technology in terms of cleaning capacity of primary root canals. However, the latter requires significantly less time which may be a consideration when treating children. Larger studies are warranted to confirm above trends.

¹ Holan G, Fuks AB. A comparison of pulpectomies using ZOE and KRI paste in primary molars: a retrospective study, *Pediatric Dentistry* 15:403, 1993.

²Moore TR, Kennedy DB. Bilateral space maintainers: A 7-year retrospective study from private practice. *Pediatric Dentistry* 28:6, 2006.

Protein Kinase G Activation and Chronic Orofacial Pain

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Introduction: Chronic inflammatory pain in conditions such as osteoarthritis, cystitis, irritable bowel syndrome, etc, is a common and serious medical concern. One component of such pain is a burning sensation that is termed thermal hyperalgesia. Research in the Ambron/Sung lab has focused on the molecular basis of thermal hyperalgesia using rat models with the goal of developing effective pharmacological solutions in the future.

Pain due to inflammation is associated with the appearance of a transcription-dependent, long-term hyperexcitability (LTH) in the cell bodies of peripheral 1st order nociceptive neurons. Recent studies have shown that the LTH arises in response to the activation and retrograde transport of Protein Kinase G (PKG) from the site of the inflammation to the cell bodies of the affected neurons. The PKG in the cell bodies ultimately alters transcription, resulting in LTH. A useful model to study the role of this pathway in the induction of thermal hyperalgesia is to inject complete Freund's adjuvant (CFA) into the hindpaw of the rat. The CFA produces an inflammation, persistent pain, and LTH.

Objective: Previous studies show that injecting CFA into perioral skin leads to conditions of chronic inflammatory pain similar to that which develops after injection of the hindpaw. If PKG plays a role in the induction of chronic thermal hyperalgesia in the trigeminal nerve, then increased PKG activation in the trigeminal ganglia (TG) will be seen post-CFA injection to the perioral region. These findings would indicate a similar role for PKG in the cranial sensory system as in the somatic sensory system.

Materials and Methods: Male Sprague–Dawley rats (200–250 g) were anesthetized intraperitoneally with ketamine and xylazine and then injected with CFA or saline in the perioral region. One day later, the rats were again anesthetized with ketamine and xylazine, and the trigeminal ganglia were harvested. PKG activity in the tissue samples was then assessed.

Results: The relative PKG activity in the trigeminal ganglia 24 hours after CFA injection was not greater than those that received saline, 0.094 ± 0.023 (n=12) and 0.092 ± 0.03 (n=12) respectively.

Conclusions: Previous work has demonstrated the presence of PKG in 1st order nociceptive neurons in the trigeminal nerve. If the factors producing LTH are the same in the cranial sensory system as in the somatic sensory system, then PKG should play a role in pain signaling pathways in the head and neck. However, this initial experiment did not demonstrate an appreciable difference in PKG activation between CFA-injected subjects and controls. Efforts will be made to refine the research protocol. Inflammation has an uncertain temporal onset, and it is possible that 24 hours is not the optimum window to see the effect. Moreover, the behavioral response to pain is readily determined after CFA injection into the hindpaw, but it is more difficult to determine a state of hyperalgesia in the face. We will closely monitor the nociceptive behavior after CFA injection in the perioral region such as rubbing the orofacial region and flinching the head.

Given the results above, we must entertain the idea that PKG activation leading to LTH may occur in another location rather than in the TG or that the LTH model in the cranial sensory system is different from that of the spinal sensory system; further studies will determine whether or not this is the case.

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Physician and Dentist Attitudes Towards Medicaid and Medicaid Patients

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Introduction: Medicaid beneficiaries have significantly less access to medical and dental services than patients with private insurance. Numerous surveys have addressed influences on physicians' and dentists' willingness to participate in Medicaid. This study reviews that literature to (1) determine providers' attitudes toward Medicaid and Medicaid beneficiaries; (2) compare medical and dental providers' attitudes toward Medicaid and Medicaid beneficiaries; and (3) assess findings for their applicability to a recognized theory of decision making (the Theory of Reasoned Action or TRA) that may explain provider's participation in Medicaid.

Methods: A structured MEDLINE search of studies from 1966 through 2007 was conducted using keywords *physician(s)*, *dentist(s)*, *Medicaid*, and the MESH term *Attitude of Health Personnel and Medicaid*. Additional sources were identified through a secondary search using the references from identified studies. Explanatory literature on TRA was collected from texts on health behaviors and from the web.

Findings: The primary search identified 124 citations of which 76 met inclusion/exclusion criteria based on the abstract and 18 were retained after focused reading. An additional 12 were identified through the secondary search. The resulting 19 MD surveys and 11 DDS surveys were of three types: studies of the decision whether to participate, the extent of participation, and attitudes toward proposed changes in program financing and administration. Regarding the program, providers report that reimbursement is too low (100% of all providers in all studies); paperwork is burdensome (68% MD, 27% DDS in 13 and 3 studies respectively); payments are sometimes denied (47% MD, 36% DDS in 9 and 4 studies). Regarding Medicaid patients, providers report unreliability (42% MD, 63% DDS in 8 and 7 studies) and non-compliance with professional recommendations (16% MD, 18% DDS in 3 and 2 studies). Three MD and three DDS surveys reported that providers believe that Medicaid patients upset private patients and make private patients feel uncomfortable. Questions asked in these surveys generally fail to address key elements of recognized theories that explain provider decision making.

Conclusion: Questionnaires about MD's and DDS's attitudes toward Medicaid and Medicaid patients are few given the 41-year period studied. Findings for MDs and DDSs tend to be more similar in direction than in size. TRA suggests that a person's behavior is determined by their attitudes towards the outcome of the behavior and the opinions of person's in their social environment. TRA is partially informed by these survey findings as significant percentages of both MDs and DDSs believe that participating in Medicaid will result in unfavorable outcomes (low reimbursement and "hassle") and therefore do not participate or participate actively. Studies based on intended behavior theory could improve understanding of provider's participation in the Medicaid program and help point out changes needed to increase access for these patients.

State Medicaid Program Orthodontics Criteria

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Introduction: Publicly funded orthodontic programs are faced with the difficult task of deciding which cases to fund with limited resources. Under the Early and Periodic Screening and Diagnostic Testing (EPSDT) program, Medicaid provides orthodontic coverage when there is a “medically necessary handicapping malocclusion.” To determine what meets the federal guideline of a “handicapping malocclusion,” states have set criteria to meet this definition.

Objective: The purpose of this study is to 1) obtain information from each state’s Medicaid program regarding its criteria for approval of orthodontic treatment; 2) compare different standards applied by states in defining a “handicapping malocclusion” and 3) make recommendations for future research and improvement in this area.

Methods: Information regarding each state’s program was solicited from the state dental Medicaid office. In states where this information could not be attained by phone, information was found using online Medicaid provider manuals.

Results: Information was obtained for all 50 states, the District of Columbia, and the New York City Medicaid programs. Fourteen (14) of the states cover orthodontic treatment for malocclusions associated with cleft palate. Twenty six (26) states use a scoring index which measures the severity of the malocclusion. If the malocclusion is severe enough to reach a certain number, children and adolescents having this type of malocclusion qualify for treatment. Fifteen (15) states use the Handicapping Labio-lingual Deviation (HLD) index. Nine (9) states use the Salzmann index, one (1) state uses the Peer Assessment Rating (PAR) index, and one (1) state has its own index. Each index studies certain characteristics of the malocclusion. The HLD index scores on millimeters of overjet, openbite, and anterior crowding. A score is given for selected traits of the malocclusion (i.e. 1 point for each millimeter of openbite), and the sum of all scores defines the final score of the particular malocclusion. The Salzmann index focuses on the position of each tooth in relation to the rest of the dentition by measuring the tooth’s degree of impaction, rotation or tipping. In addition, this index emphasizes the aesthetic handicap. For example, additional scores are given if the maxillary anterior teeth are missing, rotated or impacted.

Even states that use similar indices may differ in the cut off scores for approval depending on the state’s funding ability. The remaining twelve (12) states pay specific attention to one trait of the malocclusion. For example, if a child has an overjet of greater than 9 millimeters, the child will automatically qualify for treatment. Finally, eleven (11) states approve orthodontic treatment based on an index or on a trait of the malocclusion particularly if this trait is severely deviated from normal.

Conclusion: It is evident that there is great disparity between state Medicaid orthodontic programs regarding the definition of a “handicapping malocclusion.” We conclude that more research is needed to determine what defines a handicapping malocclusion today based on functional, aesthetic and psychological factors affecting the child with a malocclusion.

Post-Doctoral Student Abstracts

Periodontal Therapy Alters Systemic Inflammatory Mediator Levels

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Objectives: We investigated prospectively the effect of periodontal therapy on levels of several systemic biomarkers potentially relevant to cardiovascular disease risk.

Methods: Patients (n=29) with advanced periodontal disease and non-contributory medical history were comprehensively treated with non-surgical and surgical periodontal therapy within a 6-week time period. Clinical parameters were obtained at enrollment and 4 weeks after completion of therapy. Blood samples were obtained at 4 time points: one week prior to therapy (T1), at start of therapy (T2), immediately after completion (T3) and 4 weeks after therapy (T4). Assessments of serum concentrations of 19 biomarkers were performed using multiplex assays and of IgG antibodies against 11 periodontal bacteria by checkerboard immunoblotting. At T2 and T4, dental plaque samples were obtained from 6-8 interproximal sites/person and analyzed with respect to 11 species using checkerboard DNA-DNA hybridizations.

Results: At T3, CRP was elevated while soluble E-selectin, PAI-1, MMP-9, myeloperoxidase (MPO), sVCAM and a composite inflammatory score including all analyzed mediators (SIS) were reduced ($p < 0.05$). At T4, sE-selectin, sICAM, and serum amyloid P were reduced ($p < 0.05$). Greater probing depth reductions were associated with an increase in adiponectin ($p = 0.05$), MMP-9 ($p = 0.01$) and MPO ($p = 0.003$) at T4. There were no significant associations between changes in bacterial levels and serum mediator levels. Relatively low baseline values of a combined IgG antibody titer against *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, and *T. forsythia* predicted larger decreases in sE-selectin, sICAM and sVCAM at T3.

Conclusions: Periodontal therapy altered the levels of circulating inflammatory mediators, but the responses were complex, inconsistent across subjects, and more pronounced immediately after completion of therapy.

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Investigation of Labially vs. Palatally Impacted Canines in the Transverse Dimension of the Maxilla

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Introduction: Ectopic eruption or impaction of the maxillary canine is a frequently encountered tooth malposition in orthodontics. Due to their tortuous path of eruption, maxillary canines are the second most common teeth to be impacted in the dentition, following third molars. The incidence of maxillary impacted canines is approximately two percent in the general population, with palatal impaction two to three times more common than labial impaction (Thilander *et al.*1968, Fournier *et al.*1982). As one of the more common complex orthodontic issues, it is in our best interest to fully understand the conditions of impacted canines. Thus, thorough studies are needed to determine the spatial and transverse dimensions for patients with this problem.

Objective: To explore the transverse aspect of the maxilla and compare the interpremolar and the intermolar widths of labially impacted canine patients (LIC) and palatally impacted canine patients (PIC). Furthermore, a comparison of each of the two groups will be examined with a comparison group of skeletal and dental Class I patients (SDI).

Materials and Methods: A search of 4646 records stored in the Dolphin Imaging 10.5 Premium database of the postgraduate orthodontic clinic at Columbia University was performed for patients with impacted maxillary canines, and for the comparison group that meet our inclusion criteria. Through the search of radiographs and intra-oral photographs, 158 patients were selected and were divided into 3 groups of LPC (38 subjects), PDC (37 subjects) and SDI (83 subjects). All patients in each group were at least 12.5 years of age at initial record appointment and are non-syndromic and without congenital defects. Interpremolar and intermolar widths were measured using OrthoCAD™ 2.9.0.7 software. Initial models were measured for inter-arch widths at gingival margin of first premolars (or primary first molars) and first molars. Continuous variables were summarized with means and standard deviations. To compare groups, the two-sample t-test assuming unequal variances was used. The difference between the means of each group was reported along with its confidence interval.

Results: The two groups were not statistically different when assessed in the transverse dimension. The difference was -1.0, with a 95% confidence interval of (-2.5, 0.4), p-value=0.16, in premolar width and -0.3, with 95% confidence interval of (-1.7, 1.2), p-value=0.7 for molar width.

These groups were also compared to the SDI group in both interpremolar and intermolar widths. Here, the only statistically significant difference was between the LIC and SDI groups in premolar width. The difference was 2.1, with a 95% confidence interval of (0.8, 3.3), p-value=0.002. The comparison between PIC and SDI groups in premolar width achieved marginal statistical significance, with difference of 1.0, at 95% confidence interval of (-0.03, 2.1), p-value=0.057.

Discussion: Recent studies discuss different treatment modalities for labially and palatally impacted canines due to their different etiologies. It has been suggested that the treatment of palatally impacted canines should not involve expansion therapy since these patients do not present with constricted maxillas (Langberg *et al.* 2000). Clinicians may apply this type of approach in treating patients without considering their actual condition. This study has shown that the transverse dimension of the maxillas of patients with labially and palatally impacted canine is not different at the premolar and molar locations. A treatment option such as expansion should remain a modality to gain space and improve the maxillary arch shape on those patients with labially impacted canines as well as those with palatally impacted canines.

Closing Congenitally Missing Mandibular Second Premolar Space with the Sabbagh Universal Spring – A Case Report

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Introduction: The Sabbagh Universal Spring (SUS²) is a telescope unit with a spring for universal intermaxillary use. It produces constant, mainly horizontal forces when the mouth is closed. The SUS² is gentle on the temporomandibular joints and is ideal for patient with poor cooperation. The SUS² is commonly used for molar distalization, space closure, dentoalveolar compensation of the occlusion, and temporomandibular dysfunction.

Objective: To close the congenitally missing mandibular second premolar space by enhancing anterior mandibular anchorage with SUS².

Materials and Methods: A 11 year 2 month old male with congenital aplasia of both mandibular second premolars presented for orthodontic treatment at the Columbia University Post-Doctoral Orthodontic Clinic. The patient's chief complaint was "my front teeth are not lined up with the other teeth." Clinical exam and study models revealed an Angle Class III (Subdivision left) occlusion, Class II canine relationship, bilateral posterior molar crossbite, deep overbite, mild mal-aligned maxillary teeth, and atrophic dentoalveolar ridge where the mandibular second premolars are missing. Cephalometric analysis revealed Skeletal Class I tendency, normodivergent mandibular angle, slightly retroclined lower incisors. The Haas Expander was used to correct the transverse discrepancy followed the SUS² which was used to enhance the mandibular anterior anchorage in order to close the spaces. This treatment goal was chosen because the patient did not wish to receive an implant as a restorative option. The patient was treated with a standard fixed orthodontic therapy in conjunction with the SUS².

Results and Conclusions: With the SUS², the anterior anchorage in the mandible was improved and the congenitally missing second premolar spaces were successfully closed. More importantly, periodontal health of the mandibular first premolar and mandibular first molar were maintained. Also, Class I canine relationship was achieved, which is important in function. Overall, a stable dental correction was obtained while maintaining patient's natural facial profile. For patients who are congenitally missing mandibular second premolars, successful closure of the second premolar space can be accomplished with the SUS². This method is an attractive alternative to implant therapy, which requires growth of the patient to be completed.

Discussion: Unlike the traditional functional appliances such as the Herbst appliance, the SUS² is an attractive alternative device that gives patient comfort not requiring patient cooperation. SUS² is well accepted by patients due to its delicate design and its slow and gentle force delivery system. With the SUS², the mandibular spacing was slowly closed by protracting molars forward into atrophic dentoalveolar ridge which is a similar method described by Kokich *et al.* The Kokich group reported that if one can move another tooth into the atrophic space, alveolar cortical bone will be deposited ahead of and around the tooth, if done slowly.

Investigation of Unilateral vs. Bilateral Canine Impaction in the Context of Maxillary Width

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Introduction: The maxillary canines erupt into the dental arch at 10-12 years of age following the eruption of the premolars and the incisors. Maxillary canines have the longest period of development and have the longest course to erupt into the occlusion. The normal path of eruption could be altered by various etiological factors such as ankylosis, prolonged retention or early loss of primary canine, the presence of an alveolar cleft, tooth size/arch length discrepancies, congenitally missing or peg shaped lateral incisors, supernumerary teeth, cystic or neoplastic formation, and other mechanical determinants that may interfere with the normal process of eruption. Canines play a role in functional occlusion and in an aesthetic smile. However, these are compromised if maxillary canines are impacted. Impaction can occur unilaterally, bilaterally and labially or palatally.

Objective: Maxillary transverse discrepancy has been implicated in canine impaction. The aim of this study was to verify this correlation and examine maxillary width in unilateral versus bilateral impaction groups.

Materials and Methods: A search of all records stored in the Dolphin (Dolphin Imaging Systems™ 10.5, Valencia, CA.) database of the postgraduate orthodontic clinic at Columbia University was performed for patients with impacted maxillary canines. Selected patients were divided into unilateral and bilateral impaction groups. Subjects were chosen based on the following inclusion criteria: 1) minimum age of 12.5 years; 2) unerupted maxillary canines; 3) no previous orthodontic treatment; 4) non syndromic patients or patients with congenital defects. A Class I, non-impaction, comparison group was also established. All initial models were measured for inter-arch width at gingival margin of first premolars and first molars (via a 3D digital model software: OrthoCAD™ 2.9.0.7, Cadent Ltd, Carlstadt, NJ.) For the premolars, this distance was measured from the point of intersection at the enamo-gingival junction formed by a projected bisecting perpendicular line drawn from the widest mesio-distal width of the premolar, to its antimere on the contra-lateral side. For the inter-arch width at the first molar region, the width was measured from the gingival extent of the lingual groove of one first molar to the other. Age range for the unilateral sample (66 subjects) was from 12.5 to 28.5 years with a mean of 15.4 and a median of 14.3. The bilateral group (35 subjects) was from 12.6 to 26.8 with a mean of 15.2 and median of 14.0. The comparison group (83 subjects) ranged from 12.5 to 21.2 with a mean of 14.9 and median of 14.4. Statistical analysis consisted of the two-sample t-test assuming unequal variances. Continuous variables were summarized with means and standard deviations. The difference between the means of each group was reported along with its confidence interval (CI).

Results and Conclusions: The primary research question was whether the unilateral and bilateral impaction groups differed in transverse dimensions. The two groups were not statistically different when assessed in the transverse dimensions that were measured. The difference between those two groups was -1.3 with a 95% CI of (-2.9, 0.3), p-value = 0.1, in premolar width. For molar width, the difference between these groups was -0.6 with a 95% CI of (-1.8, 0.7), p-value=0.4. These groups were also examined against the comparison group. Here, the only statistically significant difference was between the comparison group and the unilateral group in the premolar width. The difference was 1.7 with a 95% CI of (0.7, 2.6), p-value = .001. Much of the literature regarding canine impaction has been focused on its association with arch length discrepancies and tooth anomalies. This study showed that there is no difference in the transverse dimension of the maxilla between unilateral versus bilateral impactions at the premolar and molar locations. This comparison had not heretofore been explored. Arch-width does not contribute to the occurrence of canine impaction unilaterally versus bilaterally.

Mandibular Distraction Osteogenesis in Pediatric Patients for Relief of Upper Airway Obstruction

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Introduction: One manifestation of Pierre Robin Sequence is micrognathia that may clinically manifest as moderate to severe upper airway obstruction (UAO). Traditionally this airway compromise has been managed with non-invasive methods such as prone positioning and nasopharyngeal intubation or with endotracheal intubation. In the most severe cases, where prolonged intubation is necessary and there is a clinical inability to maintain a patent airway, a tracheostomy is performed to maintain a safe airway. Distraction Osteogenesis (DO) has become an increasingly common technique for the relief of UAO in patients with craniofacial syndromes characterized by glossoptosis and/or micrognathia. We report on our multicenter experience with mandibular DO for UAO relief in patients with craniofacial syndromes.

Objectives: 1) Discuss treatment options for patients with craniofacial syndromes and severe upper airway obstruction; 2) Evaluate Mandibular distraction osteogenesis as a treatment option for relief of upper airway obstruction. 3) Compare treatment protocols and algorithms with current literature.

Materials and Methods: Retrospective study evaluating 5 patients treated for severe UAO with mandibular distraction osteogenesis in the period from 2003-2007. In all patients Distraction latency, rate and consolidation time periods were compared. Successful treatment outcome was noted as ability to extubate, decannulate a tracheostomy and clinical amelioration of UAO.

Results: Of ten patients treated, 2/5 patients had Pierre Robin Sequence, 1/5 Treacher Collins Syndrome, 1/5 presented with VATER malformation, and 1/5 Goldenhar Syndrome. Clinical follow-up ranges from 6months to 5 years. In 5/5 patients treated the mandible was distracted 20mm. In 4/5 patients complete relief of the obstructive symptoms were obtained, allowing for decannulation of tracheostomy tubes or extubation; 1/5 patients has completed the consolidation phase and has had clinical improvement of his airway obstruction but has yet to be decannulated. Complications included wound infection (1/10), Distractor intrusion (1/10), device failure without clinical failure of distraction (1/10), loss of suckling reflex (2/10).

Conclusion: Mandibular Distraction Osteogenesis is a valid treatment of upper airway obstruction with a low incidence of complications. Results are consistent with published success rates.

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