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**Birnberg Research Program
April 5-6, 2006**

**Editor-in-Chief
David A. Koslovsky '06**

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“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

Table of Contents

A Message from the Editor David Koslovsky, Class of 2006	6
Letter from the Dean Ira B. Lamster, DDS, MMSc	7
Letter from the Academic Dean Letty Moss-Salentijn, DDS, PhD	8
History of the William Jarvie Society An excerpt from the <i>Dental Columbian</i>, 1933	9
Description of the Birnberg Research Award	10
Birnberg Research Award Recipients	11
The 2006 Birnberg Award Recipient and Lecturer Jeffrey D. Hillman, DMD, PhD	12
Birnberg Research Program	13
A Message from the Jarvie President Aaron Schwartz, Class of 2007	14
2006 William Jarvie Society Membership	15
<u>PRE-DOCTORAL STUDENT ABSTRACTS</u>	
Preparation of an Innovative Apex Barrier Using Portland Cement and a Resorbable Matrix to Prevent Leakage into the Periapical Area N. Ahman, E. Christensen, B. Greenberg, R. George, Y. Huang, N. Jeong, J. Mourokh, J. Oh, D. Radulescu, Y. Shon, J. Levi	18
The Education of the Dental Student: Factors Influencing Dental Education R. Ansong, N.W. Johnson	19
Influence of Medicaid Fee Schedules on Dental Services Rendered J. S. Bae, B. Edelstein, S. Kauer	20
Down-Regulation of RAGE in Human Oral Squamous Cell Carcinomas C. Bailey, L. Huang, V. Woo, C. Pulse, A.M. Schmidt, R. Landesberg	21
A Review and Comparison of the Odontogenic Potential of Stem Cells from Human Exfoliated Deciduous Teeth (SHED) and Dental Pulp Stem Cells (DPSC) M. Baptiste, S. Lal	22
Egr-1 Plays an Important Role in Cell Death in an Experimental Model of Toxin Induced Cataract P. Belusko, T. Nakajima, R.D. Walkup, M. Azuma, T.R. Shearer	23
The Presence of Depression and Catastrophizing in Individuals Diagnosed with Myofascial Pain Syndrome and TMJ Arthralgia L. Cohen, J. Uyanik	24
Electrotransformation of <i>Streptococcus gordonii</i>, <i>Streptococcus mutans</i>, and <i>Veillonella parvula</i> Cells Using Electroporation R.M. Davidson, D.M. Deng, W. Crielaard, J.M. ten Cate	25
The Role and Functional Significance of Mast Cells in CNS Angiogenesis H. Erickson, A.J. Silverman	26

Oral Cancer Related Knowledge, Opinions, and Practices Among South Asian Older Adults in NY, NJ, and CT	27
S. Goel, S. Kaur, K.P. Ahluwalia	
Trends in United States Legislations for Adult Oral Health	28
N. Katchen, M.J. Ro	
Heat Shock Protein of <i>Tannerella Forsythia</i>	29
J.S. Kim, H.C. Kim, W. Zhu, S.W. Lee	
Reconstruction of the Temporomandibular Joint with Distraction Osteogenesis in the Minipig Animal Model	30
D. Koslovsky, H. Israel, S. Drew, T. Plansky, C.J. Langevin, D. Kim, J. Abend, L. Stewart, D. Behrman	
Social Determinants of Health: A Definition for Oral Health	31
R. Laughlin, B. Edelstein, C. Kunzel	
Controlled Delivery of Growth Factors Derived from Platelet-Rich Plasma	32
S. Lin ¹ , R. Landesberg ² , H. Chin ³ , J. Lin ¹ , S. B. Eisig ² , H. H. Lu ³	
Functional Analysis of 5'-Flanking Region of Human MUC7 gene	33
F. Liu, S. Li, L.A. Bobek	
Susceptibility of Bacteria Collected from Deep Dentinal Caries Against Three Antibiotics	34
C. Lo, D. Jha, H. Lu, G. Hasselgren	
Late-Phase Hemorrhagic Shock: Diagnostic Technologies and Therapeutic Interventions	35
L. Nikoghosyan, J. Macdonald, S.X. Deng, M.N. Stojanovic, D. Landry	
Northern Manhattan Oral Health Project	36
S. Nistar, Y. Quintana, J. Martinez	
S-100-Stimulated SUMOylation of RAGE: A Mechanism to Trigger Activation of NF-κB	37
J. Oh, W. Kim, A.M. Schmidt	
Inhibition of Nuclear Factor kappa B (NFκB) Activity in Oral Tumor Cells Upregulates RANTES Levels Under Treatment of TNF-α and IFN-γ	38
N. Panchal, A. Jewett, A. Paranjpe, P. Vakil	
Maternal-Child Caries Transmission and Infant Oral Health Promotion: Need for Clinical Practice Guidelines	39
T. Rubin, B. Edelstein	
The Ability of Periodontal Bacteria to Elicit Serum IgG Responses	40
A. Schwartz, T. Huang, J. Paik, M. Herrera-Abreu, R. Celenti, J. Yang, P.N. Papapanou	
Knock down of NELL2 in Wilms' Tumor Cell Line	41
Y. Shen, B. Tycko	
Hemolytic Transfusion Reactions Following Transfusion of Red Blood Cells Obtained from Human Glycophorin A Transgenic Mice	42
R. Sternberg, S. Spitalnik	
Neuronal Pain Pathway Modulators	43
T. Suranyi and D. W. Landry	
Dental Healthcare in Elderly Persons with Alzheimer's Disease and Related Disorders	44
T. Vani, L. Honig	

POST-DOCTORAL STUDENT ABSTRACTS

A Clinical Study of the Transmucosal Herbal Periodontal Patch (THPP) on Gingival Inflammation: The Effect of Monitoring on Gingival Inflammation in Control Sites J. Altman, J.T. Grbic, R. Celenti, A. Saffer	46
Effect of Nicotine on the Growth and Protein Expression of <i>Porphyromonas gingivalis</i> O. Baek, S.W. Lee	47
A Case Report of Apert Syndrome: Diagnosis and Treatment P. Chiang, J. Starobinets, K. Jarjoura, S. Eisig, J. Ascherman, M. Santoro, M. Meistrell, M. Yuan, T. Cangialosi	48
Cephalometric Evaluation of Posterior Airway Space in Patients Undergoing Superior Repositioning of the Maxilla P. Feibish, C. Choi, E. Eisig	49
Matrix Extracellular Phospho-glycoprotein: Effects on Mineralization <i>In Vitro</i> A. Fermanis, A. Boskey, P. Rowe, L. Spevak, Y. Fujimoto, J. Hui, L. Moss-Salentijn, M. Santoro, M. Yuan, T. Cangialosi	50
Infection with a Periodontal Pathogen Increases Leukocyte Adhesion to Human Aortic Endothelial Cells S.J Huang, G.A. Roth, B. Moser, F. Roth-Walter, M.B. Giacona, E. Harja, P.N. Papapanou, A.M. Schmidt, E. Lalla	51
Identification and Characterization of the Genes Encoding a Surface (S-) Layer of <i>Tannerella forsythia</i> H.C. Kim, W. Zhu, S.W. Lee	52
Identification of Individuals at High Risk for Osteoporosis Using Digital Panoramic Analysis: A Pilot Study A. Koch, R. Landesberg, E Siris, R. Tsay, S. Eisig, D. Michaeli	53
Multi-disciplinary Treatment of a Patient with Crouzon's Syndrome A. Rudnicki, D. Luk, J. Ascherman, S. Eisig, M. Santoro, M. Meistrell, M. Yuan, T. Cangialosi	54
Phenotyping and Clinical Ascertainment of a Cohort of Class III Patients for Genetic Linkage Analysis R. Sandman, D. Agarwal, E Michailidis, J. Dubin, P. Jain, K. Rogers, A. Christiano, S. Eisig, L. Moss-Salentijn, M. Santoro, M. Yuan, T. Cangialosi	55
Effects of Periodontal Therapy on Mediators Relevant to Cardiovascular Risk M.H. Sedaghatfar, D.L. Wolf, R. Celenti, G.A. Roth, E. Lalla, P.N. Papapanou	56
Severe Periodontitis as a Rheologic Modifier K. Sung, J.T. Grbic, S. Engebretson, R. Celenti, A. Schwartz, C. Rodrugeuz, H. Chang	57
An Overview of Muscular Dystrophy: Clinical Manifestations and Treatment Modalities P. Yang, R. Memory, S. Patel, S. Eisig, M. Santoro, M. Meistrell, M. Yuan, T. Cangialosi	58
Acknowledgements	59

A Message from the Editor

The William Jarvie Society is the official student research group of the College of Dental Medicine. The goal of our society is to promote research among students and faculty for the advancement of dentistry and most importantly, to provide the best service to others. This is achieved by hosting the Jarvie Luncheon Seminar Series, presenting individual research on Birnberg Day, and publishing the annual Jarvie Journal.

The Jarvie Journal contains abstracts submitted by pre-doctoral and post-doctoral student researchers at the College of Dental Medicine. The combined efforts of students and faculty from various departments of Columbia University as well as other institutions worldwide, are demonstrated throughout this journal. Seeing the student poster presentations on Birnberg Day reminds us of the importance of research in keeping our standards high and our profession innovative.

For the advancement of the Jarvie Society and the publication of this journal, we would like to express our gratitude to those who have helped us. We would like to thank Dr. Richard Abbott, Dr. Heera Chang, Dean Ira Lamster, Dean Letty Moss-Salentijn, and Dean Martin Davis for their guidance and continued support of our goals.

For sponsoring the Student Clinician Award, we sincerely thank Dentsply. The members of the William Jarvie Society appreciate their support and recognition. Finally, we want to express our gratitude to the officers of the Jarvie Society for their enthusiasm and continued efforts in making this year's Jarvie Journal a success.

David A. Koslovsky
Class of 2006



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March 19, 2006

Members of the Jarvie Society,

A research experience can be a rewarding and valuable part of predoctoral or postdoctoral dental education. Being involved in research allows one to learn about the process of scientific discovery, from concept through experimentation and on to outcomes and interpretation. Research also teaches you to read and interpret the literature, as the need to consider the existing knowledge base in a field is essential when trying to ask pertinent questions and develop an appropriate research plan. Further, a research experience strengthens your application for residency and postdoctoral programs, because program directors appreciate how challenging it is to be involved in research at the same time that you are negotiating the dental school curriculum.

The College of Dental Medicine places an emphasis on biomedical research. Recruitment of new faculty and development of new research programs that run the gamut from basic laboratory research to health services research is an ongoing process. These initiatives are often planned in collaboration with faculty at other schools at Columbia University. Each of these new programs represents a new research training opportunity.

I hope that everyone that attends the 2006 Birnberg Student Research Day finds the experience to be rewarding. We celebrate the accomplishments of our presenters, and hope that they enjoy the opportunity to report on the results of their work.

Sincerely yours,

Ira B. Lamster, D.D.S., M.M.Sc.
Dean



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March 10, 2006

Dear Members of the William Jarvie Society,

Research has been one of the core missions of our College from its very beginnings. William Gies, who was instrumental in establishing the dental faculty at Columbia University, was a founder of the Journal of Dental research, still one of the premier dental publications in the world.

The importance of research in maintaining and advancing the intellectual base of our profession is well understood by the 60 active members of the Williams Jarvie Society. Your interest and, more importantly, your robust participation in the various research activities in the laboratories of dental faculty, medical faculty, as well as other laboratories in the University and indeed internationally, holds great promise for the future.

The papers that are abstracted here for presentation on Birnberg Day indicate the level of the research activity of pre- and postdoctoral students. I look forward to your presentations on Birnberg Day. Research is very much alive and well at Columbia's College of Dental Medicine.

My warmest congratulations on another successful year!

Sincerely,

Letty Moss-Salentijn, D.D.S., Ph.D.
Associate Dean for Academic Affairs

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. Forty-nine outstanding scientists and teachers have been honored as the Birnberg Lecturer since the first Birnberg Award was presented in 1954.

Birnberg Research Program 2005



2005 Birnberg Lecturer Dr. Bruce Pihlstrom (3rd from left) along with
CDM Periodontics Resident
Dr. Dana Wolf (Postdoctoral Research Award recipient, 2nd from left)
CDM predoctoral students
Keith Da Silva (1st Place Research Award recipient, 3rd from right) and
Eleni Michailidis (2nd Place Research Award recipient, 2nd from right),
Dr. Richard Abbott (left) and Dean Ira Lamster (right).

Birnberg Lecturers and Award Recipients

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

2006 Birnberg Lecturer

Jeffrey D. Hillman, DMD, PhD

Dr. Jeffrey Hillman received his DMD degree from the Harvard School of Dental Medicine (1973) and his PhD from the Department of Microbiology and Molecular Genetics, Harvard University (1976). From 1976 to 1991 Dr. Hillman was Clinical Instructor in Oral Biology at the Harvard School of Dental Medicine while also holding various appointments at the Forsyth Dental Center, including Head, Department of Molecular Genetics (1982-91).

Since 1992 Dr. Hillman has held the rank of Professor in the Department of Oral Biology at the University of Florida College of Dentistry. Dr. Hillman was a founder, in 1996, of the biopharmaceutical company Oragenics, Inc., located in Alachua, FL, and he presently serves as its Chief Scientific Officer.

Dr. Hillman has made numerous contributions to the scientific literature, and he has served as a reviewer for manuscripts submitted to the *Journal of Dental Research*, *Advances in Dental Research*, *Journal of Bacteriology*, *Archives of Oral Biology*, and other similar journals.

Dr. Hillman's research interests include the microbial ecology of the oral cavity, the molecular biological construction of effector bacterial strains for use in replacement therapy, the purification and characterization of lantibiotic agents (a type of antimicrobial peptide produced by bacteria), and the development of novel *in vivo* expression systems for use in the identification of bacterial virulence factors. In addition to teaching and research, Dr. Hillman has been awarded US patents related to replacement therapy and antimicrobial agents and methods.

Birnberg Research Program

WEDNESDAY, APRIL 5, 2006, 2:00-5:00 P.M.

THURSDAY, APRIL 6, 2006, 12:00-2:00 P.M.

WEDNESDAY, APRIL 5, 2006

2:00-5:00 P.M.

Table Clinic Presentations

Hammer Health Science Center

Riverview Lounge

HHSC-Fourth Floor

THURSDAY, APRIL 6, 2006

12:00-1:00 P.M.

Birnberg Lecture

Jeffrey D. Hillman, DMD, PhD

Professor, University of Florida

College of Dentistry

Founder and Chief Scientific Officer,

Oragenic, Inc.

HHSC-301

“Replacement Therapy for the Prevention of Dental Caries”

1:00-2:00 P.M.

Award Presentations and Luncheon

Riverview Lounge

HHSC-Fourth Floor

A Message from the Jarvie President

This academic year, the William Jarvie Society highlights research as a progressive, proactive enterprise in science. Research serves as a vehicle for scientific growth in all aspects of medicine

Our lunch seminars highlighted concepts that served to stimulate and urge those in attendance to participate in research and be proactive in dentistry not only as a student, but also after we graduate and become more involved with our families and the business aspects in the field. In our first seminar, Dr. Heera Chang, Jarvie Society faculty advisor and Oral Surgery clinical professor, along with Dr. Linda Huang, Chief Resident in the CU-CDM/NYPH Oral and Maxillofacial Surgery program, spoke about current concepts in oral surgery. They both shared their invaluable personal experiences in research, and Dr. Huang's slide presentation of her time in China performing cleft lip/palate repair was tender and inspiring. Dr. Burton Edelstein, Professor of Clinical Dentistry and Health Policy/Management, spoke in our second lunch seminar. His lecture, titled "Activist Research in Support of Public Policy," passionately stressed the importance of taking a proactive, collectively responsible stance in dentistry. By strategizing and implementing oral health policies with our community leaders, the goal of caring for the underserved can better be accomplished. In the beginning of March, the Jarvie Society co-sponsored the first Dean's Research Seminar of 2006, titled "Dental Medicine: From Repair to Regeneration". Dr. Jeremy Mao, Associate Professor in the Section on Growth and Development, spoke about the latest technologies in biomedical engineering in dentistry, including stem cell regeneration of oral and craniofacial tissues. We welcome Dr. Mao to CDM with the utmost regard.

Also this year, the Dean's Office covered the expenses of professionally printing all pre-doctoral students' posters for display at Birnberg Day. With a professionally printed poster in hand, we hope students will present their work at other seminars in the future.

The Jarvie Society would like to especially thank Dr. Richard Abbott, Director of the Office of Research Administration, for his continued dedication to our society. We would also like to thank our faculty advisor Dr. Heera Chang, and Dean Lamster, Dean Davis, and Dean Moss-Salentijn for their continued support. Thank you, Mrs. Marlene Sanchez, for organizing another successful Birnberg Day. Lastly, I want to thank Matthew Fien, Tina Vani, Fred Liu, and James Kim, including our Editor-in-Chief, David Koslovsky, for their outstanding commitment as members of the Executive Board.

Our student body and faculty at the College of Dental Medicine at Columbia University continually demonstrate that we do indeed value the significance of scientific/epidemiologic research and by doing so, support and contribute to the evolving nature of dentistry. This journal celebrates the work of our fellow colleagues. It has been a pleasure serving as President.

Aaron Schwartz
Class of 2007

2006 William Jarvie Society Membership

Officers:

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Dr. Heera Chang

Staff Advisor:

Mrs. Marlene Sanchez

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Merissa Blais	Steve Huang	Joseph Sanghyun
Eklea Cakuli	Eugene Ko	Neeru Singh
Michael Castagna	Bernard Lam	Tasneem Rangwala
Abraham Chahine	Robert Laughlin	Derek Park
Angie Chin	Thao Le	Kristina Rodriguez
Evan Christensen	Shihpin Lin	Talia Rubin
Sung Cho	Ben Liu	Peter Trinh
Keith DaSilva	Robert Laughlin	Trent Tucker
Carmel Dudley	Tiffany Madison	Joshua Wolf
Helamen Erickson	Rimpy Manchanda	Benjamin Yagoubian
Derrick Flint	Philip Mann	Robin Yang
Ajay Gianti	Victor Kwame Marfo	
Sheenu Goel	Oana Opariuc	

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Pre-Doctoral Student Abstracts

Preparation of an Innovative Apex Barrier Using Portland Cement and a Resorbable Matrix to Prevent Leakage into the Periapical Area

N. Ahmed¹, E. Christensen¹, B. Greenberg¹, R. George¹, Y. Huang¹, N. Jeong¹,
J. Mouroukh¹, J. Oh¹, D. Radulescu¹, Y. Shon¹, J. Levi²

¹*College of Dental Medicine, Columbia University, NY, NY;*

²*Division of Endodontics, College of Dental Medicine, Columbia University, NY, NY*

Background: Abnormal anatomic or pathological entities, such as periradicular resorption, open apex root development, and over instrumentation beyond the apical constriction, may result in the lack of an apical dentin matrix. When this apical constriction becomes altered, it becomes extremely difficult to obtain proper apical seal during root canal therapy. The loss of a hermetic seal potentiates the possibility of microbial leakage through the gutta percha from the coronal aspect of the tooth towards the periapex as a result of poor adaptation and seal of the gutta percha against the dentinal tubules thus permitting a pathway for microbial leakage. To combat this problem, a pioneering apical barrier technique has been developed using Portland cement, which has identical properties to mineral trioxide aggregate (MTA) which is commonly used as a retrograde filling material in apicoectomies. By using a resorbable matrix (surgicel) which is placed beyond the apex, and 3mm of Portland cement as an apical barrier, an innovative apex can be reconstructed in order to prevent overextension, overfilling, and microbial leakage beyond the apex. Placing an additional material at the apex with better dentinal bonding properties than gutta percha and sealer will inhibit the ingress of oral microorganisms into the periradicular area.

Objective: The purpose of this study is to investigate the effectiveness of an innovative Portland cement apical barrier technique in order to reduce the penetration of India Ink dye from the coronal aspect of the tooth to the apex. If the apex barrier protects the periapical area from dye penetration, we can infer that it will also be an adequate barrier to oral fluid penetration. A novel apex can be prepared by placing a master gutta percha point 1mm into the center of a soft pre-set barrier, leaving an indentation that will facilitate placement and retention of gutta percha points during obturation of the canal.

Materials and Methods: Random operators used 33 clear plastic incisors and 27 extracted human first and second maxillary (palatal root) and mandibular (distal root) molar teeth as experimental and controls. All teeth were mounted in a ball of carting wax at the cemento-enamel junction (CEJ). The root canals of all teeth were instrumented using a standardized crown-down technique with nickel titanium rotary files. The canals were irrigated with NaOCl during the procedure. Excessive instrumentation beyond the apical constriction was intentionally performed, which resulted in the loss of a dentin matrix, thus giving a parallel open apex. The 14 plastic control teeth and 10 extracted human teeth were obturated with gutta percha and Columbia cement using the cold lateral condensation technique. A coronal portion of the gutta percha was then removed leaving approximately 3mm of gutta percha remaining at the apex of the tooth. No matrix was placed at the apex. For the experimental teeth, a resorbable matrix (surgicel) was extruded through the canal apex until a firm apical stop was obtained. These teeth were then filled with 3mm of Portland cement using a Messing Gun syringe. The canals of all teeth were filled with India ink and penetration of the dye was aided by suction at the apex. A mesh suction trap with filter paper in the suction tip enabled the visualization of dye penetration. The absence of dye stain indicated no leakage through either the gutta percha or Portland cement, while the presence of staining indicated leakage through the material.

Results: Initial results indicate that the dye penetrated significantly more through the gutta percha and less through the Portland cement. Of the 14 plastic teeth filled with gutta percha, 71% showed either partial or full leakage compared with the 19 experimental teeth filled with Portland cement, which showed 26% either partial or full leakage. The extracted human molar teeth showed a similar pattern with 60% of the 10 molar teeth filled with gutta percha showing partial or full leakage compared to only 41% of the 17 molar teeth filled with Portland cement showing partial or full leakage.

Conclusion: The innovative Portland cement apical plug will have a beneficial effect in preventing microbial leakage in those complex endodontic cases which have lost the apical dentin matrix necessary for an apical seal.

The Education of the Dental Student: Factors Influencing Dental Education

R. Ansong¹, N.W. Johnson²

¹*College of Dental Medicine, Columbia University, NY, NY;*

²*School of Dentistry and Oral Health, Griffith University, Australia*

Background: Many dental students are plagued by subtle difficulties and decisions in their dental school career. From an objective point of view, it is quite significant how personality characteristics, demographic influences, and psychological factors, determine to a large extent the decisions dental students make, and its subsequent educational and clinical outcomes for the entire dental team. The above major categories will also have substantial subcategories that may play some roles in shaping the dental student including the following: demographic influences such as socioeconomic backgrounds and previous educational backgrounds; personality characteristics spanning from study strategies, perceptions to health issues, coping with stress and burnout; psychological factors including drives and motivations, career goals and expectations. All of these notwithstanding the fact they may overlap in one way or another, have consequences in the life of the dental student.

Objective: The purpose of this study is to describe from literature, profiles of dental students and the way they vary by time, country, culture and its possible impact on educational or clinical outcomes. We hypothesise that the typical dental student is one who has been exposed to dentistry and medicine, chooses dentistry, and is willing to adapt to the challenges of dental education by a variety of coping mechanisms.

Methods: A search was done using the Griffith University library database. Databases used mainly were Medline via Ovid (1966-July 2005), ProQuest educational journals (1988-July 2005), ProQuest career and technical education (1991-July 2005), PubMed (Medline), and Google search. Priority was given to educational journals, e.g. American Journal of Dental Education, British Dental Journal, and European Dental Journal. Trade magazines that expressed special or extreme interests were ignored.

Results and Conclusions: From this review, we can clearly see that a lot of factors affect the education of the dental student. These include demographic influences (socioeconomic backgrounds and prior educational background), personality characteristics (study strategies, perceptions to health issues, coping with stress and burnout), and psychological factors (motivations, career goals and expectations). The one obvious problem with this review is that it is not possible to generalize to the overall dental student population from a limited number of studies specific to their own cultures and institutions. We also know from this review that extensive pre-professional science education is a big confidence booster. Reinforcement for students whose parents are dentists can be very helpful. One area that needs more research is the issue of integrating the education of dental technician training and dental student training. More so, reasons why students choose dentistry are mostly influenced by family and culture, even though, major motivations are the financial and social status afforded by the dental profession. Students survive on study strategies tailored for them as individuals, despite ethical issues regarding academic work that leaves much to be desired. Many students cope with either substance abuse in varying degrees of toxicity and frequency of use, religion, emotional support, positive reframing, planning, humor, religion, suppression of competing activities, and instrumental support. Being an introvert or an extrovert may have its own effects on how students relate to their patients. However, based on this review, we can conclude that despite the hardship and reality of dental school, the dental student is willing to try to adapt by a variety of coping mechanisms

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Influence of Medicaid Fee Schedules on Dental Services Rendered

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Introduction: Dental Medicaid programs for low-income children provide for comprehensive dental care that includes preventive and restorative services. Each state program individually establishes fee schedules that are adjusted in part by states' desire to influence dentists' care-giving behaviors.

Objective: The purpose of this study is to assess the impact of Medicaid reimbursement rates on biasing dentists' provision of dental services. We hypothesize that compensation incentives do not affect dentists' service provision.

Methods: State Medicaid payment rates for 15 dental procedures, expressed as the percentile of usual and customary regional fees for each state based on 2002 reported fee schedules, were retrieved from an American Dental Association 2003 report entitled, "State Innovations to Improve Access to Oral Health Care for Low-Income Children: A Compendium" (www.ada.org/prof/advocacy/issues/medicaid_issues.asp). We selected 5 common preventive services and 3 common restorative pediatric dental services. All possible ratios of preventive to restorative payments were calculated—15 total for every state—to construct an index number representing the relative market value of preventive to restorative Medicaid payments by state. For the ratios that included multiple preventive or restorative procedures, averages were used. State Medicaid dental program performance for 2003 was obtained from the Federal Centers for Medicare and Medicaid Services (www.cms.gov/epsdt, form 416, lines 12b,c). For the three restorative variables, [1] stainless steel crowns (SSC), [2] SSC + pulpotomy, and [3] SSC + pulpotomy + extraction, age restrictions were made (1-9 year-olds for [1] and [2] and under 6 years of age for [3]) to include only the range of age when these procedures are most frequently performed. Ratios of preventive to restorative visits were calculated for all ages (birth through age 20 for preventive and appropriate ranges for treatment) and for children under 6 by state.

Results: The relationship between payment ratios and treatment ratios was investigated by calculating Pearson's correlation coefficients using the Statistical Package for the Social Sciences program 13.0 (SPSS). R and R² values were calculated to determine significance and degree of significance. Ratios of preventive to restorative payments ranged from 0.03 to 45.00 for 'all ages' and 'under 6'. Ratios of preventive to restorative visits ranged from 0.54 to 5.20 for 'all ages', and 0.46 to 6.71 for 'under 6'. For the 'all ages' group, correlations were negative but weak and statistically insignificant, whereas for the 'under 6', correlations were positive and negative, again weak, and again not statistically significant.

Conclusions: Weak correlations of all 30 trials suggest that the distortion in Medicaid reimbursements, which were designed to promote and reward preventive procedures, do not affect dentists' clinical behavior. These findings imply that either Medicaid-accepting dentists are practicing ethically and individualizing treatment plans for each patient regardless of varying fee schedules or that the Medicaid incentives are not high enough to warrant the alteration in behavior. The Medicaid program's goal to encourage prevention by distortion of payments seems largely unsuccessful. Further analysis comparing states with the highest and lowest ratios of preventive to restorative payments will be conducted to determine whether correlations between payment incentives and provider behaviors do exist at extreme levels of payment distortion.

Down-Regulation of RAGE in Human Oral Squamous Cell Carcinomas

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Background: The Receptor for Advanced Glycated End-products (RAGE) is linked to tumor growth and metastasis, and appears to be up-regulated in prostate, renal, gastric, colorectal, and pancreatic cancers. In contrast, RAGE is down-regulated in non-small cell lung carcinomas. RAGE expression, however, has not been investigated in Oral Squamous Cell Carcinoma (OSCC). Our aim was to examine a series of OSCCs to determine RAGE expression and its correlation with tumor differentiation. Additionally, Proliferating Cell Nuclear Antigen (PCNA), a nuclear protein synthesized in late G1 and S phases of the cell cycle, was examined to determine the fraction of proliferating cells.

Methods: Paraffin-embedded, formalin-fixed tumors were graded as well-differentiated (n=12), moderately-differentiated (n=15), and poorly-differentiated (n=12). RAGE and PCNA expression were examined by immunocytochemistry (ICC). Normal human oral mucosa served as a control (n=12). Results were graded by an oral pathologist and analyzed by Tuckey-Kramer HSD to determine statistical significance. To confirm antibody specificity, anti-RAGE antibody was depleted by absorption with soluble RAGE (sRAGE) and used for ICC. In addition, western blot analysis of several frozen OSCCs was used to confirm ICC results.

Results: PCNA was weakly expressed in well-differentiated tumors and expression increased as the tumors de-differentiated. Inversely, RAGE was strongly expressed (>50% positive reactivity) in normal oral epithelium and in well-differentiated tumors, showed moderate expression (25-50% positive reactivity) in moderately-differentiated tumors, and showed minimal immunoreactivity in poorly-differentiated tumors. Normal oral epithelium was negative for RAGE staining when sRAGE-absorbed antibody was used. Western blot analysis confirmed the ICC findings.

Conclusion: As OSCC de-differentiates, PCNA expression increases, while RAGE expression is down-regulated. Whether the decrease in RAGE expression is due to the location of the tumor (i.e. aerodigestive system), chronic exposures to toxins (i.e. tobacco), and/or cellular derivation of the tumors (epithelial vs. non-epithelial derived) has yet to be determined.

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A Review and Comparison of the Odontogenic Potential of Stem Cells from Human Exfoliated Deciduous Teeth (SHED) and Dental Pulp Stem Cells (DPSC)

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Introduction: In a paper written by S. Gronthos et. al. it was recently shown that stem cells were isolated from the pulp of deciduous teeth. These cells were found to be distinctively different from DPSCs in respect to odontogenic differentiation and odontogenic induction. In this study, we looked at the set of experiments that quantified the properties of these stem cells to differentiate into odontoblasts, osteoblasts and neural cells and looked at their efficiencies at which they differentiate. To receive a quantitative measurement we looked at the ability for these cells to express specific cell molecules expressed specifically by odontoblasts, osteoblasts and neural cells. With this information we were able to analyze the odontogenic potential and proliferation rates between the two cell lines. Here we show that SHED based on their ability to differentiate indirectly by measuring their cell markers, ability to proliferate and their convenience and accessibility are a much more efficient line and warrants the bulk of future investigations.

Objectives: The objective of this analysis is to evaluate the relative efficiencies of these unique stem cells to differentiate into odontoblast, osteoblasts and neural cells.

Materials and Methods: Normal exfoliated human deciduous incisors were collected from 7- to 8-year old children for SHED and normal human third impacted third molars were collected from adults for DPSC. Tooth surfaces were cleaned and cut around the cemento-enamel junction. Pulp tissue was gently separated from the crown and root and this tissue was then digested in a solution of collagenase and dispase for 1 hour at 37°. Single suspensions were obtained by passing the cells through a 70-µm strainer. Next, in order to test for SHED/DPSC's ability to form odontoblasts *ex vivo*, these cells along with hydroxyapatite tricalcium phosphate were transplanted into immunocompromised mice. They examined the transplants for dentin sialophosphoprotein (DSPP), a protein found exclusively in dentin. In this study, we compared the data relating to these two lines and looked and derived three criteria that we set to indirectly measure their efficacy to differentiate which were their cell marker/dentin-like tissue expression, proliferation rates and convenience/accessibility.

Results and Conclusion: When comparing the SHED and DPSC one must pay attention to the differences in developmental processes, function, age differential and structure between primary and adult teeth. Further investigation is needed to elucidate the important signaling molecules and growth factors essential for the final differentiation into odontoblasts and osteoclasts. Based on this data, we can see that although SHED might not be as efficient in differentiating into dentin-like complexes or DSPP expression, but in sheer number and availability makes it more efficacious for use and further investigation. Finally, when looking at the sources of these cell lines, primary teeth is easily accessible and the most novel approach to harvest tissue that can someday be used and stored early in life and then used later on in life if needed and as cell differentiation signals become elucidated.

Discussion: Looking solely at their ability to express DSPP and their ability to form dentin-like tissue in the mouse model, DPSC seems to be the better stem cell line as 6 out of 12 (66%) single colony lines were shown to form dentin complexes with HA TCP, compared to 3 out of 12 (33%) as shown in SHED. DSPP expression seems to be non-existent in SHED while J Lin reports to be 20% relative expression of DSPP in DPSC. Although, it seems as if DPSC would be the more efficient stem cell line to warrant further investigation, one also has to look at the ability of these cell lines to proliferate and double. S. Gronthos et al. looked at the ability of these two lines to proliferate. Using BrdU 5-bromo-2-deoxyuridine (BrdUrd) which is an immunohistochemical marker to measure cell proliferation, they showed, using the Student's t test, that SHED showed a significantly higher proliferation rate in comparison to DPSCs. In another paper written by Gronthos, they report that single colony derived strains of human DPSCs showed that most of the colonies (80%) failed to proliferate beyond 20 population doublings (PD). SHED was shown to be able to proliferate to 140 population doublings, which was significantly higher (P 0.05, Student's t test). The final criteria comparing the two lines is accessibility/convenience, a subjective measure looking at the sources and practicality of isolating these lines. SHED clearly is at an advantage based on its availability and number, but also these teeth are available as a result of a natural exfoliative process.

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Egr-1 Plays an Important Role in Cell Death in an Experimental Model of Toxin Induced Cataract

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Introduction: Cataracts are caused by proteolytic degradation of lens proteins and precipitation of these protein fragments which cause opacities of the lenses. One model of human cataracts is a toxin induced cataract of neonatal rats by selenite injection. Selenite acts through many mechanisms, leading to lens epithelial cell death and cataract formation. In previous work we have identified many genes that undergo changes in expression due to selenite exposure. Early growth response protein-1 (Egr-1) was found to experience up regulation after selenite injection.

Objectives: Our current investigation attempts to further elucidate the role of Egr-1 expression in selenite induced lens epithelial cell death. Previous implication of Egr-1 in selenite induced cell death was shown by DNA microarray, therefore confirmation of a change in Egr-1 protein expression is a key aim of the present study. We hope to identify the time course of Egr-1 mRNA expression after selenite exposure. Egr-1 protein expression post selenite exposure will be modulated using antisense oligonucleotide suppression. Lactate dehydrogenase (LDH) leakage post selenite exposure will be measured as a surrogate for cell death and possible indicator of cataract formation.

Materials and Methods: Neonatal rat lens epithelium was separated from the dense fibrous core of the lens. These thin sheets of cells were then cultured in serum-free DMEM/F12 for 24 hours followed by serum-free DMEM/F12 with 10-100 μ M sodium selenite. The mouse α -TN4 immortal lens epithelial cell line was cultured as described in previous protocols, followed by exposure to 10-300 μ M sodium selenite in serum-free medium. Cultured α -TN4 cells were also exposed to either 2 μ M antisense or control oligonucleotides, followed by selenite exposure. Protein and total RNA was extracted from cultured lens epithelium and α -TN4 cells after selenite exposure. Proteins were analyzed by western blot using an anti-Egr-1 protein antibody. Total RNA was subjected to reverse transcription-polymerase chain reaction (RT-PCR) and analyzed by polyacrylamide gel electrophoresis. LDH leakage was assessed using LDH Cytotoxicity Detection Kit (Takara, Shiga, Japan). Statistical significance was computed with the student's t-test.

Results: Both cultured neonatal rat lens epithelium and mouse α -TN4 cells demonstrated a time and dose dependent increased LDH leakage and altered Egr-1 mRNA expression post selenite exposure. Egr-1 protein expression closely followed mRNA expression in mouse α -TN4 cells. Egr-1 antisense oligonucleotide inhibited expression of Egr-1 protein as compared to control oligonucleotide exposed cells. Furthermore, Egr-1 antisense oligonucleotide inhibited selenite induced cell death of mouse α -TN4 cells as compared to control oligonucleotide exposed cells treated with selenite.

Conclusion: Our results support the hypothesis that Egr-1 is an active player in cell death induced by selenite exposure. The decrease in cell death following addition of Egr-1 antisense oligonucleotide and subsequent inhibition of Egr-1 protein expression suggests a protein induced cell death. This observation should be repeated in tissue explants and whole animal models to clearly define the role of Egr-1 in the pathophysiology of cataract formation.

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The Presence of Depression and Catastrophizing in Individuals Diagnosed with Myofascial Pain Syndrome and TMJ Arthralgia

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Background: Temporomandibular disorders (TMD) refer to a subgroup of orofacial pain disorders of multifactorial etiology that can be further classified into two non-mutually exclusive groups, myogenous and arthrogeous TMD. Clinicians and researchers have recognized that psychological factors play a significant role in the development and maintenance of temporomandibular disorders, finding higher levels of emotional disturbances in these patients than in the general population. Furthermore, while some studies support the theory that psychological factors (i.e. depression) tend to play a more prominent role in patients with myogenous TMD than in patients with arthrogeous TMD, other studies have failed to reproduce this relationship.

Objectives: To determine if there is a higher prevalence of depression and a higher tendency to catastrophize in both individuals with arthrogeous and myogenous temporomandibular disorders (TMD) as compared to healthy controls and to determine if there is a higher prevalence of depression and a higher tendency to catastrophize in individuals with myogenous TMD as compared to those with arthrogeous TMD.

Materials and Methods: Fifty participants, 18-65 years of age, will be recruited from the TMD center at the Columbia University Medical Center. After a thorough assessment by a certified clinician, the participants will be diagnosed according to the Research Diagnostic Criteria of Temporomandibular Disorders (RDC/TMD) and will be assigned into the following diagnostic groups: myofascial pain disorder (MPD) (n=25) and TMJ arthralgia (n=25). Healthy controls (n=25) will be recruited from the College of Dental Medicine. All participants will be administered two surveys, the Beck Depression Inventory (BDI) and the Pain Catastrophizing Scale (PCS).

Electrotransformation of *Streptococcus gordonii*, *Streptococcus mutans*, and *Veillonella parvula* Cells Using Electroporation

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Introduction: In the dental field of microbiology, gram positive *Streptococcus* species have become well studied due to their roles in early colonization of the tooth surface and formation of dental plaque. Among these are *Streptococcus gordonii* and *Streptococcus mutans*, which have been shown to play a very critical role in disease processes of the oral cavity. Another bacteria, *Veillonella parvula*, is studied not because of its pathogenicity, but because it is a normal part of the flora of the mouth, even thought to play a helpful role. When *Streptococcus mutans* feeds on carbohydrates, it produces lactic acid, leading to tooth destruction. *Veillonella parvula* can break down lactic acid to produce acetic acid and propionic acid, two acids of normal food digestion. If it is possible to further develop existing protocols for gene transfer into these bacteria, it could be a very valuable tool for bacterial study and manipulation.

Objectives: The objectives of this research are to further develop existing protocols for the electrotransformation of dsDNA into *Streptococcus gordonii* and *Streptococcus mutans*, and to develop a general protocol for dsDNA transfer into *Veillonella parvula*.

Materials and Methods: In order to complete the electrotransformation, electrocompetent cells were first prepared via centrifugation and resuspension methods. The electrotransformation of the foreign DNA into the host cell was carried out using the following plasmids:

- pVA 838- this is an expression vector only for *Streptococcus*; no fluorescence
- pDM 15- this is a construct created from pVA 838; modified with constitutive promoter and green fluorescence protein fusion
- pCM 18- expression vector for both gram positive and gram negative bacteria, but used for *Veillonella parvula* transformation; modified with constitutive promoter and green fluorescence protein fusion

Results: The results for *Streptococcus gordonii* and *Streptococcus mutans* are presented as transformation efficiency. Unfortunately, our method failed to achieve the overall goal of improving upon previous protocols. Due to poor DNA transfer in *Veillonella parvula*, the results of a series of growth curves are presented.

Conclusions: The development of an efficient protocol for DNA transfer into bacteria is an arduous task. While invaluable lessons have been learned along the way, it is apparent that there is a great deal to learn about the conditions in which bacteria desire to take up the foreign vectors. It was found that higher transformation rates were achieved when the process of forming electrocompetent cells was initiated when cells were at a higher level on the exponential portion of their growth curve. Also, a series of growth curves were established for *Veillonella parvula*. These discoveries could be of great use in future attempts to advance our knowledge of electrotransformation.

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The Role and Functional Significance of Mast Cells in CNS Angiogenesis

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Background: It is well established that the central nervous system (CNS) contains mast cells located on the brain side of the blood brain barrier. Mast cells (MCs) are members of the immune response and function in profound ways, having both properties of the innate and acquired-immune system. As such, MCs are characterized by a large complement of secretory granules which store a wide variety of mediators including biogenic amines, neuropeptides, cytokines, sulfated proteoglycans and neutral serine proteases. MCs secretory products can influence and alter the function of both neural and vascular elements, participating as a component of the neuro-immune-vascular unit. During development of the CNS, blood vessels enter the brain parenchyma from the pia. In many brain regions, especially the thalamus, MCs appear to enter with growing blood vessels. Most recently, molecules that distinguish presumptive arteries and veins have been discovered and appear to guide blood vessel growth and patterning. They may also play a similar role in axonal guidance. It has been shown that Ephrin B2 is expressed in arteries but not veins and conversely, that the ephrin receptor EphB4 is expressed in veins but not arteries. Thus, arteries and veins are molecularly distinct at early stages of CNS development.

Objective: The goal of this research was to elucidate the complex ways in which the component cells of the neurovascular unit interact in the normal development of the mammalian brain. Other vascular associated proteins are to be explored, including: HEY 1, Jagged, NP-1, NG-2, Notch-4, DLL-4, Occludins, and OX-42. These will further elucidate the pattern of developing vasculature.

Methods: The physical relationship of MCs, presumptive arteries, and veins during blood vessel growth in neonatal rat pups was accomplished by fixing brain tissue, post-natal day 5-15, and cutting 50µm frozen sections. Free floating sections were then incubated with avidin-Cy2 (1:1000, Sigma, fluorescent green) for 24hrs. Avidin binds specifically to the heparin in mast cell granules. After extensive washing, sections were incubated in antibodies with either EphB4 or Ephrin B2 for 48 hrs. Sites of antibody binding were then visualized with a secondary antibody conjugated to a fluorophore. Digital images were analyzed for distribution of MCs relative to blood vessel type over developmental time. Focus was placed on the thalamus where mast cells are most numerous. Images were obtained by double fluorescent immunohistochemistry and scanning confocal microscopy.

Results: The preferential location of MCs along blood vessels and at sites of new vessel formation sustains an association between MCs and angiogenesis. The relationship is attributed to the aforementioned guidance molecules which provide precise attractive and repulsive cues to the growing vessels.

Discussion: Blood vessels were largely thought to grow along the path of least resistance without active guidance. However, there is an association between the MCs and the growth of the vasculature as identified by numerous blood vessel association guidance markers, including: HEY 1, Jagged, NP-1, NG-2, Notch-4, DLL-4, Occludins, and OX-42. Future experiments involving these proteins will elucidate how the vasculature responds to these cues.

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Oral Cancer Related Knowledge, Opinions and Practices Among South Asian Older Adults in NY, NJ and CT

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Introduction: Although oral cancer is relatively rare in the United States (for 2005 it is estimated that oral cancer accounted for less than 3% of new cancers diagnosed and less than 1.4% of cancer deaths), it accounts for 40% of all malignancies in South Asia. The high incidence of the disease in South Asia is attributed to the culturally-sanctioned practices of areca (betel) use, as well as to the use of smokeless tobacco. Areca, which comes as a quid or nut, has been known to be a risk factor for sub-mucous fibrosis. However, recent data suggests it is also an independent risk factor for oral cancer. Despite increasing immigration from South Asia, the oral cancer risk of South Asians in the US is not known. This pilot study is conducted to determine the oral cancer related knowledge, opinions and behaviors of South Asian older adults in NY, NJ and CT.

Materials and Methods: This pilot study uses a cross-sectional study design. A 140-item face-to-face survey is conducted in a convenience sample of South Asian Adults (n = 150), aged 50 and older. Subjects, recruited from religious and cultural organizations, immigrated to the United States directly from the Indian Subcontinent (India, Pakistan, Bangladesh). The data is managed and analyzed in SPSS.

Results: Preliminary data suggests that although South Asian older adults are, on the average, well-educated (61.3% reported having a college degree), almost 30% continue to practice the indigenous risky behavior of areca (without tobacco) use. Furthermore, the prevalence of tobacco use (1.3% smoke cigarettes and 2.0% use areca with tobacco) is low, suggesting that cultural and religious ties continue to be strong post-immigration. The population is not well informed about oral cancer, and a majority of subjects do not believe that areca products are risk factors for oral cancer (66% do not believe areca leaf is a risk factor and 60% do not believe areca nut is a risk factor). Interestingly, increased time in the US is directly associated with increased utilization of areca products. This may suggest improved access to areca products in the US, primarily due to improved financial capacity. Furthermore, while public health efforts in South Asia have been directed toward increasing knowledge of the potential risks of areca product use, the US has largely ignored areca product use. Although this population is at high risk for oral cancer, utilization of dental services is poor – only 50% reported having dental insurance and average time since the last dental visit among those with dental insurance being 24 months. Those without dental insurance were more likely to see a practitioner of Eastern medicine or return to their home country for care. This is significant because early detection is crucial in the management of morbidity from oral cancer.

Conclusions: Although this population is relatively well-educated, oral cancer related knowledge remains low, and indigenous risky behaviors are high. Given the high morbidity and mortality associated with oral cancer, it is important to raise awareness of the risks associated with using alcohol, areca leaf, and areca nut, with and without tobacco in this population. In addition, it is important to improve the awareness of the risk posed by areca product use among the medical and dental community. Lastly, South Asian older adults should be targeted for oral cancer early detection because they are likely to continue risky behaviors after migrating to the United States.

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Trends in United States Legislations for Adult Oral Health

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Introduction: On May 25, 2000, Surgeon General David Satcher released *Oral Health in America: A Report of the Surgeon General*. It was a ground breaking report due to its long overdue look at the condition of America's oral health. The report provided clear evidence that oral health is not separate from overall health and showcased that while the oral health of many Americans is improving, there is a disparity in the quality of oral health that falls along racial and socio-economic lines. In regards to the poor quality of oral healthcare that many vulnerable populations are currently receiving, the report was of a sobering nature. However, a wake-up call was directed to elected officials that they need to begin to implement change for the purpose of improving the oral health of all Americans.

Objective: To monitor and document recent legislation dealing with the improvement of access to oral health care for underserved populations.

Materials and Methods: The internet was utilized to record legislative activity from January 2004 to August 2005. The Library of Congress' website Thomas, (<http://thomas.loc.gov/>) and the governmental websites of ten states (MD, NY, NM, CA, NC, WV, MI, GA, FL, and CO) were searched for legislation recently passed or undergoing debate. The 10 states were selected due to their participation in the W.K. Kellogg Community Voices: HealthCare for the Underserved Initiative. Legislative bills were sorted by issue and by state.

Results: The categories of issues addressed are as follows with parenthetical totals of the number of bills under each category: Oral health of veterans (3), Native Hawaiians (1), impoverished populations (1), and homeless (1); Client payment (6); Tax deductions for receiving oral health care (3); Increasing the number of Medicaid service providers (9); Increasing the loan repayment for new dentists serving in underserved areas that have low reimbursement rates (5); Decreasing licensure requirements to allow more dentists to serve in underserved areas (5); Increasing reimbursement rates to increase participation in Medicaid (2); Expanding the duties of dental hygienists (3); Increasing access to dental education (1); Considering oral health care as part of overall health (3); Improving cultural awareness (1); Including dental care needs in the Family and Medical Leave Act of 1993 (1); Federal and state employee benefits (3); Prison oral health (3); Prenatal care (1); HIV/AIDS patients (1); Expanding oral healthcare for children (2)

Conclusions: While there are many bills proposed that would improve access to oral health care, it still remains to be seen if they will be implemented. Widening the scope of access is a two part process. First, federal and state governments need to improve funding to make it more economically feasible to provide increased coverage. Second, people in underserved areas must be made aware of their eligibility for Medicaid and the increase in coverage (number of dentists, expansion of eligibility, etc). Many proposed bills have addressed monetary issues; however, finances are usually not the only issue. One bill, NY A06239, deals with the issue of cultural awareness. It is these cultural issues that can isolate members of a community. So while the financial aspect of limited access to care appears to be in the process of being addressed, cultural education is at a minimum. What are most needed are proposals that deal with increasing dental education on a one on one basis. Only then, will the number of people receiving oral care be at a maximum.

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Heat Shock Protein of *Tannerella forsythia*

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Introduction: Bacterial heat shock proteins (HSPs) are immunodominant antigens that can induce potent and protective immune responses. The previous studies suggest that HSPs are implicated in the pathogenesis of human periodontal disease. *Tannerella forsythia* (*Tf*) (formerly *Bacteroides forsythus*), a Gram-negative, filament-shaped, and non-motile anaerobe, has been implicated as a crucial periodontal pathogen. So far however, the nature and potential roles of *Tf* HSP have not yet been adequately characterized.

Objective: The putative HSP gene termed *groEL* has been identified from *Tf* based upon the sequence homology to other well-known bacterial HSPs. The purpose of this project was to express the *Tf groEL* gene in *E. coli* and produce recombinant GroEL (rGroEL) to assess humoral immune responses in periodontal patients against the *Tf* HSP.

Materials and Methods: The *groEL* gene was cloned into *E. coli* as follow. The *groEL* gene was amplified from *Tf* genomic DNA by PCR using forward and reverse primers containing the *Bgl*III and *Xho*I restriction sites, respectively, to facilitate an insertion procedure. After confirming a size of the PCR product (1635bp), the DNA fragments were directly purified from the gel. Both *groEL* PCR product and the pET-30c(+) vector (Novagen) have been digested with *Bgl*III and *Xho*I. Subsequently, the *groEL* DNA fragments were ligated with the linearized pET-30c(+), constructing pET-30c(+)/*groEL*. The pET-30c(+)/*groEL* vector was transformed into *E. coli* cells, and the transformants were selected on LB-kanamycin plates. After confirming the presence of *groEL* in the vector by direct DNA sequencing, the pET-30c(+)/*groEL* was again transformed into *E. coli* BL21(DE3) cells for protein induction. The *E. coli* cells were grown in LB-kanamycin broth at 37°C overnight. One ml of the overnight culture was inoculated into 9 mL of fresh LB-kanamycin medium and grown until when the growth reaches at OD₆₀₀ = 0.4 - 0.5. Then, 1 mL of 1M IPTG was added in order to induce protein expression. The expression of the recombinant proteins was analyzed by SDS-PAGE.

Results and Conclusion: The 1.6 kb *groEL* gene of *Tf* was cloned into the expression vector pET-30c(+), and the 60 kD recombinant GroEL was successfully expressed in *E. coli*. In the future study, humoral immune responses in periodontitis patients will be analyzed by ELISA using rGroEL as antigen.

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Reconstruction of the Temporomandibular Joint with Distraction Osteogenesis in the Minipig Animal Model

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Introduction: Tissue engineering techniques are now being developed for the reconstruction and replacement of diseased human tissues. Distraction osteogenesis has potential to provide autogenous reconstruction of the temporomandibular joint, using an in vivo tissue engineering technique. A major advantage of this technique is that the mandibular condylar transport segment has a blood supply from the medial pterygoid muscle. Although there are anecdotal reports of the use of distraction osteogenesis for human temporomandibular reconstruction; there is a paucity of literature on this subject.

Objective: The purpose of this investigation was to determine the feasibility of reconstructing the temporomandibular joint with distraction osteogenesis using a minipig model.

Materials and Methods: Three female Yucatan minipigs were used as the animal model for this investigation. The mean age at the start of the investigation was 6 months, and the mean weight was 27.5 kg. All animals underwent injection of a sclerosing solution (3% Sotradecol or 23.4% Sodium Chloride) into the left temporomandibular joint to induce osteoarthritic changes. At 6 weeks left mandibular condylectomy and discectomy were performed, creating a gap between the glenoid fossa and remaining mandible of 15 – 20 mm. An L- shaped osteotomy was made from the sigmoid notch vertically and inferiorly, and then horizontally and posteriorly, to create a mandibular bone transport segment. A distraction device with two four hole plates (KLS Martin, LP) was secured to the transport segment and remaining mandible with 2.0 mm titanium screws. The condyle and disk tissues, which were removed, were submitted for histologic examination (hematoxylin and eosin staining). Following a latency period of 7 days, the distraction device was activated 1 mm per day for 20 days, until the transport segment was docked into the glenoid fossa. The minipigs were fed a normal diet for two months at which time the distraction devices were removed. The animals were monitored for food intake and weight while eating a normal diet for an additional three months. The animals were euthanized 5 months following the docking of the condylar transport segment into the glenoid fossa. Block sections of the experimental left temporomandibular joint, as well as the bone in the distraction gap were performed for histologic examination. The tissue from the experimental left side was compared to the contralateral right side histologically.

Results: The initial condylar and disc specimens that were removed 6 weeks following injection of sclerosing solution demonstrated articular cartilage with fibrillation, splitting and necrosis, consistent with degenerative joint disease. The bone transport segments that were functioning as new condyles, all demonstrated a fibrous tissue layer over the condyle. A dense band of fibrous tissue resembling discal tissue was articulating over the new condyle. There was an intervening joint space between the superiorly located dense fibrous tissue and the inferiorly located new condyle. An additional surprising finding was the presence synovial tissue. The bone from the distraction gap demonstrated normal trabecular bone of significant and variable thickness. All animals gained weight throughout the 7 months of the experiment, with the mean initial weight being 27.5 kg and the mean final weight being 41.3 kg.

Discussion: This pilot study demonstrated that reconstruction of the temporomandibular joint using a distraction osteogenesis technique is feasible. A functional temporomandibular joint was formed in the minipig model, with the creation of a joint space, fibrous tissue surfaces and synovial tissues. It is anticipated that further studies using the minipig model, are likely to result in a new and improved technique of autogenous reconstruction of the temporomandibular joint using distraction osteogenesis. Additional human clinical trials using distraction osteogenesis for temporomandibular joint reconstruction are required and will offer the potential for restoring mandibular function in patients with severe temporomandibular joint disease.

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Social Determinants of Health: A Definition for Oral Health

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Introduction: Social Determinants of Health have been extensively explored by medicine but not dentistry. The term “Social Determinants of Health (SDH),” coined in 1996 (WHO Commission on Social Determinants of Health, 2005) is now used to describe the public health discipline interested in the impact of social factors on health outcomes. SDH has ready application to dentistry because of a growing attention to oral-systemic relationships and the increase in oral disease among populations made vulnerable by age, poverty, or other factors. However, SDH has several limitations, including: (1) a lack of consensus on definition, (2) difficulty relating social determinants to biological processes, and (3) an inability to prioritize specific social determinants for prevention (Syme, 2005, Baker et al, 2005, and Thisted, 2003). As a result, confusion remains concerning the discipline’s scope in reference to oral health.

Objectives: 1. Develop a definition for “Social Determinants of Health” based on specific qualifying criteria.
2. Assess the quality of the dental literature on SDH by applying these qualifying criteria.

Materials and Methods: Definition of Social Determinants of Health - A definition and set of five qualifying criteria for SDH was developed based on: (1) a systematic Pubmed and CLIO search of the medical literature which yielded thirty articles and (2) five face-to-face structured interviews with New York City area social research experts. Dental Literature Analysis - Using a similar systematic search as above, seventeen dental articles with apparent relevance to SDH were identified, of which, five were excluded on the basis that they were not primary studies. The remaining twelve were categorized as: (1) those that meet one or more of the qualifying criteria but pre-date the coining of the term “SDH”, (2) those that meet all five qualifying criteria and use the term SDH, (3) those that meet one or more qualifying criteria and use the term SDH, and (4) those that meet none of the criteria and use the term SDH.

Results: Definition of Social Determinants of Health - The 5 qualifying criteria of SDH are: (1) reference to socioeconomic status, psychosocial factors, and/or societal or community attributes, (2) bi-directional and independently causal relationships between social factors and health, (3) findings within all populations, but, with uneven distribution, (4) multi-factorial modeling, and (5) inclusion of the “Life-Course Approach.” Dental Literature Analysis - Of the twelve qualifying papers, two pre-dated the term SDH but met one or more qualifying criteria and ten postdated the term SDH. Of those that postdated the term, two met all five qualifying criteria, eight met some of the qualifying criteria, and zero failed to meet any qualifying criteria.

Conclusions: By understanding SDH as a discipline that identifies social factors directly and independently impacting oral health, the dental community can begin to incorporate social concepts into its understanding of dental health. The present study, which establishes objective criteria for SDH studies and characterizes relevant dental literature, facilitates consideration of the two additional challenges facing SDH as a discipline: relating social determinants to biological processes and prioritizing social interventions that can improve oral health. If accomplished, the implications of an SDH approach in dentistry are numerous, with the potential to impact various oral diseases in an effort to alter the course of disadvantage in an individual’s oral health. Therefore, it is necessary for dental professionals to step back from the long established “dental treatment” model and begin to consider the underlying social causes of oral illness. Only then may poor oral health be successfully tackled and the dental disease burden reduced.

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Controlled Delivery of Growth Factors Derived from Platelet-Rich Plasma

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Introduction: Platelet-rich plasma (PRP) contains growth factors including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-beta), and insulin-like growth factor (IGF) that have the potential to augment bone regeneration in oral and maxillofacial surgery. The efficacy of PRP-derived growth factors is dependent upon dosage, distribution, and temporal sequencing. PRP prepared with thrombin releases over 90% of growth factors within 24 hours. To control the bioavailability of PRP, we have designed an alginate hydrogel-based PRP-delivery system. The objective of this study was to determine the bioactivity of growth factors released from alginate carriers. To delineate the effects of the released growth factors from those of the serum-supplemented media, a preliminary study was also conducted to determine the effects of decreasing serum concentration on the osteoblastic phenotype.

Methods: PRP Alginate Beads and Capsules: PRP was prepared following Landesberg *et al.* For alginate beads, PRP was mixed with 2% alginate (Sigma) and dispensed into a 6% CaCl₂ solution. To form capsules, PRP in 6% CaCl₂ was dropped into a 1% alginate solution.

Serum Concentration Effects: Human osteoblast-like cells (SaOS-2) were pre-seeded (5x10⁴ cells/well) and Dulbecco's Modified Eagles Medium supplemented with 0%, 1%, 2% or 10% of Fetal Bovine Serum (FBS) was added 24 hours later. Cell proliferation (n=6) was quantified using PicoGreen assay. Alkaline phosphatase (ALP) activity (n=6) was measured using a colorimetric assay.

Evaluation of Bioactivity: SaOS-2 cells were pre-seeded (5x10⁴ cells/well) in 10% FBS and alginate carriers were added 48 hours later. Beads without PRP and a monolayer of SaOS-2 served as controls. Cell growth (n=6) and ALP activity (n=6) were determined as above.

Results and Discussion: Effect of Serum on Osteoblasts: The SaOS-2 cells proliferated in all groups examined, however, no statistically significant differences in proliferation rates were found between the groups. For ALP activity, the 10% FBS group was significantly higher than all the other conditions on Day 7; otherwise, the differences in ALP were not statistically significant. Therefore, 10% FBS supplemented media was used for the rest of the studies to ensure the maintenance of the osteoblastic phenotype.

Effects of Released Growth Factors on Osteoblasts: The SaOS-2 cells proliferated in all groups examined, with a significant increase in cell number at day 7 in cells cultured with PRP-beads and day 14 for cells cultured with the PRP-alginate capsules. Cell ALP activity was significantly lower for the PRP alone group at day 1, while it peaked for both the PRP-bead and PRP-capsule groups at the same period. A higher ALP activity was measured for the capsule than the bead group at day 3, suggesting that different factors may be influencing cell response in these carriers.

Conclusion: The results of this study demonstrate the feasibility of controlling bioavailability of PRP-derived growth factors using hydrogel carriers. Moreover, the released factors maintained their bioactivity and had a positive effect on osteoblast differentiation. Future studies will focus on *in vitro* and *in vivo* testing of the efficacy of these PRP-carriers.

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Functional Analysis of 5'-Flanking Region of Human MUC7 gene

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Introduction: The MUC7 gene encodes a low molecular weight human salivary mucin glycoprotein. This salivary mucin comprises a major portion of mucous secretion from human submandibular and sublingual glands. In order to characterize the transcriptional regulation of MUC7 gene, the current MUC7 research has focused on the functional analysis of its 5'-flanking region. A 2.7 kb DNA fragment and sequentially truncated fragments were subcloned into a pGL3-Basic vector that contains a reporter luciferase gene. The constructs were transiently transfected into human lung carcinoma cells (A549), and the luciferase expression, which was under the control of *MUC7* promoter, was measured. The large increase in luciferase activity between base pairs 90 to 168 suggests that the transcription factor binding sites are located within this sequence of DNA. This research project focused on discovering the specific transcription factor(s) binding sequence(s) that is (are) involved in the promotion of salivary mucin production by the MUC7 gene.

Objective: This research project focuses on discovering the transcription factor that is specifically involved in the promotion of salivary mucin production by the MUC7 gene.

Materials and Methods: 261, 121 & 134 bp DNA fragments were amplified by PCR and cloned into pGL3-Basic vectors. The resulting constructs were transformed into *E. coli* cells and allowed to multiply. Vectors containing the desired DNA fragments were screened using gel electrophoresis and DNA sequencing. The constructs were then introduced into human carcinoma cells and luciferase activity (reflecting the MUC7 promoter activity) was measured.

Results and Conclusion: By DNA sequencing, it was determined that the 121 and 134 bp fragments failed to be cloned into the plasmid. The bands that appeared on the gel that were initially thought to be the correct fragments are most likely produced by the primers. The 261 bp fragment was successfully cloned into a plasmid as shown by gene sequencing, and subsequently transfected into A549 cells.

Discussion: Finish current research by measuring luciferase activity in lung carcinoma cells containing the cloned 261 bp construct. Attempt to clone the 121 bp and 134 bp DNA fragments by running PCR product on low melting point 4% agarose gel which will hopefully separate of the DNA fragments from their primers.

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Susceptibility of Bacteria Collected from Deep Dentinal Caries Against Three Antibiotics

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Objective: This study was carried out to evaluate the susceptibility of bacteria collected from deep dentinal caries to the antimicrobial action of three antibiotic agents.

Materials and Methods: *Metronidazole*, *Ciprofloxacin*, *Minocyclin* were tested at concentrations of 0.5µg/ml, 1.0µg/ml, 2.0µg/ml, 4.0µg/ml and 8µg/ml by the disk diffusion method. Carious, infected dentin was collected from teeth with deep cavities and culture in Thioglycolate and Brain heart infusion broth for 48 hours anaerobically and aerobically. The infected broth was tested for adequate growth against the McFarland turbidity standard. The infected broth was then spread on to Brucella and Brain heart infusion Agar plates. Discs with different antibiotics were placed onto the inoculated agar plates. Each concentration was tested three times. The control group consisted of infected agar plates without antibiotic. All agar plates were then placed in aerobic or anaerobic incubators for 48 hours. The plates were checked for zone of inhibition after 48 hours.

Results: A zone of inhibition was seen in all concentrations of *Ciprofloxacin* in both aerobic and anaerobic environments. *Metronidazole* and *Monocyclin* did not show any zone of inhibition. All control groups had bacterial growth.

Conclusion: *Ciprofloxacin* shows promise as an antimicrobial agent against deep dentinal caries.

Late-Phase Hemorrhagic Shock: Diagnostic Technologies and Therapeutic Interventions

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Introduction: Hemorrhage is characterized by a decline in cardiac output and by compensatory vasoconstriction that supports perfusion of vital organs. Vasoconstriction worsens the tissue perfusion in hemorrhagic shock and if the bleeding is not staunched and volume restored rapidly, hemorrhage will proceed to a late phase which is unresponsive to restoration of intravascular volume. We have discovered that late-phase hemorrhagic shock does not reflect general collapse of the contractile machinery, but rather the deficiency of the hormone vasopressin. However, the therapeutic index of vasopressin is relatively narrow with serious side effects; bolus administration is not desirable and vasopressin is best administered by low-dose continuous infusion. Such a continuous infusion can possess the danger of inadvertent overdose and results in admission to the intensive care unit. A partial V1a agonist would permit bolus administration with an intrinsic limit on the magnitude of vasoconstriction.

Objective: Identification of a partial agonist for the human vasopressin (V1a) receptor. We sought to screen libraries of peptides and small molecules.

Materials and Methods: Partial agonism reflects a defect in binding and signal transduction and we sought to screen using a cell-based assay. Partial agonism is frequently species specific and thus we screened in mammalian cells transfected with human V1a receptor. The vasopressin-induced Ca-transient was a convenient marker for degree of agonism. The peptide library was constructed from known V1a agonists with full agonism at nonhuman V1a receptors. The small molecule library was a commercial collection of molecules of molecular weight <500 Da.

Results and Conclusion: A focused 15-peptide library of agonists for V1a receptor in nonhuman species yielded two potential partial agonists. A screen of a 20,000 compound small-molecule library was undertaken, and preliminary hits are now under investigation.

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Northern Manhattan Oral Health Project

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Introduction: The primary goals of the Northern Manhattan Community Voices Oral Health Project is to reduce racial and ethnic disparities in oral health care and improve the oral health of the residents of northern Manhattan. The oral health community assessment will essentially disclose some key information about the status of the community oral health and provide guidance on how to steer the necessary policy changes to eliminate barriers to oral health care. Working together, the Northern Manhattan Community Voices Collaborative and the College of Dental Medicine will identify solutions to eliminate these oral health disparities.

Objectives: The overall objective of this project is to determine if the Medicaid population of Northern Manhattan is using their coverage to access oral health care. According to New York State Medicaid utilization data, out of the 1,334,776 Medicaid enrollees only 276,955 (21%) use Medicaid to obtain oral health care. The general health care status and oral health in particular is assessed subjectively by the survey. This is accomplished in three ways: 1) Review the existing data on dental services and oral health status and conduct surveys in order to determine whether or not residents were getting access to care and whether they were utilizing the care they need, 2) Assess the approach necessary for a Northern Manhattan Oral Health Plan, and 3) Community dialogue to develop a strategy that enables the population of northern Manhattan to access oral health care.

Materials and Methods: The material that was used to undertake this research was a survey created in order to assess the health care needs of individuals in Washington Heights. The survey was developed by a committee of people from the School of Public Health, Northern Manhattan Community Voices Collaborative, and the College of Dental Medicine. Initially the survey was administered as a test while waiting for IRB approval. This involved approaching voluntary participants to provide information about their own health care. The survey gauged how many individuals utilized Medicaid for their oral health care needs.

Results: In the allotted time, we were unable to complete the project. Only eleven surveys were conducted during the pilot study. The results we obtained do not have any significant statistical value however the study served to measure the effectiveness of the survey. We expect to find in the upcoming months that people are not utilizing Medicaid coverage to obtain dental services and identify the main reasons why. This will enable us to inform the community and affect policy in a way that the population can have better access to oral healthcare.

S100-Stimulated SUMOylation of RAGE: A Mechanism to Trigger Activation of NF- κ B

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Background: RAGE is a signal transduction receptor for certain S100/calgranulins, whose roles are related to homeostatic properties such as calcium binding [2-4]. Once released into the extracellular milieu, however, these molecules (such as S100A12 and S100B) activate EC, MP, SMC and peripheral blood mononuclear cells (PBMC) via RAGE, thus triggering activation of signaling cascades and generation of cytokines and pro-inflammatory adhesion molecules with important links to atherosclerosis initiation and progression[1]. Although our studies in this application will be focused on SMC, these ligands have been demonstrated to activate other cells as well, including EC and MP. However, the precise molecular mechanism for the initiation of cell signaling by RAGE has remained to be elucidated. Since its discovery in 1996, Small ubiquitin-related modifier (SUMO) have been investigated for its important role in various processes such as chromosome segregation and cell division, DNA replication and repair, nuclear protein import, protein targeting and formation of certain subnuclear structures, and the regulation of a variety of processes including the inflammatory response in mammals and the regulation of flowering time in plants. The process of SUMOylation results in lysine modifications of proteins by the addition of a polypeptide group to the backbone protein. The Sumo activating enzyme E1 transfers activated SUMO to the E2-conjugating enzyme Ubc9 that directly catalyzes the isopeptide bond formation between the C-terminal glycine residue of the activated SUMO to the lys residue of the target protein [15,16]. E3 SUMO ligases attach SUMO to the target protein.

Objective: RAGE has multiple potential SUMOylation sites, especially in its tail region, which is its cytoplasmic domain. Studying this novel interaction, we may be able to answer the exact molecular mechanism for the initiation of cell signaling by RAGE with SUMOylation in SMC biology and explore its potential role in the pathogenesis of diabetic vascular complications.

Materials and Methods: Streptozotocin-treated ApoE null mice aortic vessel atherosclerotic plaque samples were stained with anti-SUMO1, anti-Ubc9, anti-RAGE antibodies and visualized for colocalization by confocal microscopy facility in Surgical Science Division.

Conclusion: To examine the interactions between SUMO and RAGE in the cells, CHO parent cells and CHO cells expressing RAGE were harvested after RAGE stimulation to S100b for 0 min, 20 min, and 10 hours. Cell lysates were immunoprecipitated with anti-RAGE antibodies, immunoblotted with anti-SUMO1 antibodies, and visualized by ECL. Compared to the control with the endogenous RAGE expression, a marked increase in signals in RAGE-SUMO interaction was observed at 20 min and 10 hours after RAGE stimulation to S100b. Colocalization of RAGE and SUMO1 were also shown in confocal microscopy. The aortic root atherosclerotic plaque in ApoE null mice rendered Diabetic with streptozotocin was stained with anti-RAGE antibodies and anti-SUMO1. As expected, colocalization of RAGE and SUMO1 were shown in cytoplasm of SMC and more strongly around the nucleus region. From the previous observations on Ubc9 and SUMO [23-26], a noncovalent Ubc9-SUMO complex is formed and induces further subsequent transfer of SUMO to the target protein resulting in formation of an isopeptide bond between the C-terminal carboxy group of SUMO and the ϵ -amino group of a lysine residue in the target protein. Our data together with previous observations, suggest that SUMO1 with Ubc9 may play a role in subnuclear localization and signaling of RAGE upon stimulation with S100b and triggering activation of NF- κ B. We are still investigating the use of different mutants on consensus SUMOylation sites in the cytoplasmic tail domain of RAGE. With this novel idea, we may be able to elucidate the precise mechanism of RAGE with SUMO for the initiation of cell signaling by RAGE with SUMOylation in SMC biology and explore its potential role in the pathogenesis of diabetic vascular complication

Inhibition of Nuclear Factor kappa B (NFkappaB) Activity in Oral Tumor Cells Upregulates RANTES Levels Under Treatment of TNF-alpha and IFN-gamma

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Introduction: Oral carcinoma is one of the 5 most common cancers in the United States. The percentage of people affected by this type of carcinoma is on the rise. HEP-2 tumor cells are a stable line of human oral laryngeal cells. It would be of great importance to delineate the effects of chemokine secretion on this particular cell line. If chemokine secretion does have an effect on the HEP-2 tumor cells then we could apply these results to other oral carcinoma cell lines. This could be an effective method of treatment for oral carcinomas. Of the chemokines, RANTES (regulated on activation normal T-cell-expressed and secreted) levels plays a significant role in a number of diseases. RANTES is regulated by two important transcriptional factors, Nuclear Factor kappa B (NFκB) and active protein-1 (AP-1). In this study we will focus on NFκB. By looking at this system we will be able to analyze the manipulation of NFκB and its regulation of chemokines within oral carcinoma.

Objectives: Nuclear factor-κB (NF-κB) is an important anti-apoptotic transcription factor that plays a key role in immune response. NF-κB regulates the transcription of a variety of genes for anti-apoptotic peptides, cytokines and chemokines. In this study we will measure the levels of chemokine secretion by the vector alone transfected HEP2 cells and HEP2-IκB_(S32AS36A) cells when treated with tumor necrosis factor-α (TNF-α), Interferon -γ (IFN-γ), and a combination of both. The aim of our study is to compare the levels of chemokine secretion in the above mentioned cell lines and correlate these results to the presence or absence of NF-κB in these cells. This study will look at the synergism between TNF-α (20ng/ml) and IFN-γ (200 units/ml) leading to increased secretion of RANTES in HEP2 tumor cells transfected with an IκB super-repressor.

Materials/Methods: Cell lines, plasmids and reagents - HEP2 tumor cell lines were obtained from ATCC and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FCS. RPMI 1640 supplemented with 10% FCS was used for the cultures of NK and T cells. Generation of vector-alone and IκB_(S32AS36A) transfected stable cell lines were described previously in our lab. Recombinant IL-2 was obtained from Chiron Corporation (Emeryville, CA). IFN-γ was a generous gift from Dr. Yoichi Mizutani. TNF-κ was purchased from Peprotech. (Rocky Hill, NJ). The fluorokine MAP cytokine multiplex kit was purchased from R & D Systems.

Results: HEP 2 cell transfectants were left untreated or treated in the presence of IFN-γ and TNF-α and the levels of RANTES were determined. Treatment with IFN-γ and TNF-α increased the levels of RANTES substantially. However, greater effects were seen when treating the cell line with both IFN-γ and TNF-α. The blocking of NFκB in HEP2 cells and treatment with IFN-γ and TNF-α was the most potent combination in upregulating RANTES.

Conclusions: NFκB is an important anti-apoptotic transcription factor. As an important transcription factor our study here shows that blocking of NFκB in HEP2 cells and treatment with IFN-γ and TNF-α is advantageous for immune response with increased levels of RANTES. These results can be used to help in the treatment of oral carcinoma.

Maternal-Child Caries Transmission and Infant Oral Health Promotion: Need for Clinical Practice Guidelines

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Background: Early childhood caries is the most prevalent chronic disease among children. It has been well established that the cariogenic mutans Streptococci bacteria are implanted by mothers in their children by direct salivary transmission. Evidence suggests that preventive oral health measures in mothers, initiated in the prenatal period and continued throughout early childhood, both delay mutans transmission and result in a decreased caries rate among offspring. Despite this evidence, the number of pregnant women seeking dental care remains low and oral health counseling about maternal-child caries transmission is not typically provided. Surveys show that pregnant women lack awareness of oral health care as an aspect of prenatal care. Given the burden of this preventable disease, the adequacy of the current clinical guidelines must be assessed. This paper reviews existing recommendations for prenatal interventions to reduce postnatal vertical transmission of cariogenic organisms and promote infant oral health.

Objectives: The purpose of this study is to determine the quality of current prenatal recommendations and the need for new and improved prenatal guidelines.

Materials and Methods: A literature search was conducted using MEDLINE, Google, dental journals, web-based guideline clearinghouses, and professional organization sites. A secondary search was conducted using relevant articles found in the original search. Included in the review were references that address maternal oral health, early childhood caries, and oral bacterial colonization, as well as references that offered preventive oral health recommendations for the mother or primary caregiver.

Results: Ten sources were included in the final review, based on relevance to the selected topics. These included recommendations for pregnant women in the following six areas: oral hygiene, fluoride, diet, professional dental care, parental education, and xylitol chewing gum. The majority of recommendations were based on expert opinion or Type III evidence. One source cited evidence from a randomized controlled trial but failed to acknowledge flaws in the trial. None of the ten reviewed sources rated the quality of evidence cited.

Conclusions: The existing recommendations for prenatal interventions to reduce postnatal vertical transmission of cariogenic organisms and provide preventive counseling about the oral health of infants and toddlers do not meet the standards of a clinical practice guideline. There is, however, a variety of information that would support elements of such a guideline. A new guideline must therefore be developed.

The Ability of Periodontal Bacteria to Elicit Serum IgG Responses

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Background: Checkerboard immunoblotting is a useful means for assessing antibody responses to multiple bacterial species in epidemiologic studies. The variability in the capacity of different oral bacterial species to elicit serum IgG responses has not been adequately explored.

Objective: The purpose of the present study was to compare the potential of a number of bacterial species in the dental plaque to induce serum IgG antibody responses in a large, nationally representative subject sample.

Materials and Methods: Serum samples collected during the second phase of the Third National Health and Nutrition Examination Survey (NHANES III) obtained from 8,153 subjects ≥ 40 years old were analyzed in our laboratory. The level of serum IgG responses against 19 subgingival species was determined by checkerboard immunoblotting as earlier described (Sakellari et al. 1997). Standardized bacterial suspensions from the following species were produced using ATCC type strains: *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Prevotella melaninogenica*, *Tannerella forsythia*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponema denticola*, *Peptostreptococcus micros*, *Campylobacter rectus*, *Eikenella corrodens*, *Eubacterium nodatum*, *Streptococcus intermedius*, *Streptococcus oralis*, *Streptococcus mutans*, *Capnocytophaga ochracea*, *Veillonella parvula*, *Selenomonas noxia*, and *Actinomyces naeslundii*. Using a miniblotted device, these suspensions were immobilized on nitrocellulose membranes and allowed to interact with 1:1000 serum dilutions. After appropriate washing steps, immunocomplexes were detected by using Fab fragments of anti-human IgG conjugated with horseradish-peroxidase and subsequent incubation with a horseradish-peroxidase substrate. Quantification of titers was performed by evaluating chemiluminescent signals in an automated, computerized workstation (LumiImager, Roch-Boehringer-Mannheim) in comparison to a standard curve created by serial dilutions of human IgG interacting with protein-A .

Results: Based on the entire cohort, median serum IgG responses against each of the investigated species, expressed in $\mu\text{g/ml}$ by descending order, were as follows: *E. nodatum* (927), *A. actinomycetemcomitans* (718), *P. gingivalis* (359), *A. naeslundii* (312), *P. intermedia* (286), *P. melaninogenica* (223), *P. nigrescens* (204), *E. corrodens* (197), *S. intermedius* (158), *P. micros* (142), *C. ochracea* (127), *T. denticola* (125), *T. forsythia* (123), *S. mutans* (92), *C. rectus* (84), *F. nucleatum* (83), *S. oralis* (74), *V. parvula* (40), and *S. noxia* (31).

Conclusion: We documented a substantial variation in the ability of different periodontal species to elicit serum antibody responses. These observations should be taken into account in the assessment of immune responsiveness in different population samples and/or different forms of periodontal disease.

Knock Down of NELL2 in Wilms' Tumor Cell Line

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Introduction: Wilms' tumor (WT) is a pediatric malignant kidney tumor containing blastemal, stromal and epithelial derivatives. Due to the distinctive genetic defects or pathways to the formation of Wilms' Tumors, WTs can be classified into either sporadic or syndromic. In a recent study by Yuan et al (1), it has been shown that the loss of heterozygosity (LOH) event in WTs was found on all chromosomes, except chromosome 12. Yuan et al has further demonstrated that trisomy (+12) at chromosome 12 is one of the common genetic events in the WTs, and confirmed that overexpression of NELL2 mRNA in +12 Wilms tumors by northern blotting.

NELL2 (neural epidermal growth factor like 2), with six EGF-like repeats, is highly expressed in brain and weakly in fetal kidney (2). NELL2 plays important roles in regulating neural cell growth and differentiation by interacting with PKC in an isoform specific manner (3). It has also been suggested that NELL2 may play oncogenic activities in other non-neural cancer cells such as colorectal adenocarcinoma cells (3). As the first step to study its potential oncogenic activities, here we report that we can knockdown *NELL2* activity in a WT cell line by introducing a stably integrated doxycycline-inducible shRNA plasmid to the cells.

Materials and Methods:

Construction of pshNELL2 The pshNELL2 plasmid was constructed by inserting the ds-oligonucleotides with Bgl II and Hind III sticky ends to pTER vector. The double strands used were: 5' to 3' GATCCCGTATTTCTCTGTCCTAGCCATTCAAGAGATGGCTAGGACAGAGAAATATTTTGGAAA and AG CTTTTCAAAAATATTTCTCTGTCCTAGCCATCTCTTGAATGGCTAGGACAGAGAAATACG.

Generation of stable cell line A derived cell line, TR13, stably expressed the repressor of tet operator was established from a WT cell line, CCG99-11, by the other member of our research group. The created pshNELL2 plasmid with a zeocin selectable marker was introduced into TR13 cell line by using lipofectamineTM 2000. After 48 hours, complete DMEM solution with blasticidin (2 µg/cc) and zeocin (150 µg/cc) was added to the cell line and selected for 2 weeks.

Northern blotting 5 µg of total RNA was resolved in 1% formamide-denaturing agarose gel and transferred the Nytron membrane. The RNA membrane was hybridized with NELL2 cDNA in Untrahyb solution at 42°C for overnight and washed in 0.1xSSC/0.1%SDS at 65°C for 1 hour after 10 minutes of rinsing in 2xSSC/0.1%SDS at room temperature.

Results and Conclusion: Only the cells that had successfully integrated the pshNELL2 plasmid are found to survive under the selection pressure of zeocin. After these cells are exposed to doxycycline, our northern blotting results demonstrate knockdown of NELL2 in both mixed pool and single clone, indicating the NELL2 mRNA is degraded. Normally the shRNA of NELL2 in the construct is inhibited from transcription, because a Tet operator binding to a Tet repressor resides in between the RNA polymerase III H1 promoter and the gene of interest (4). In the presence of doxycycline, the Tet repressor is removed from binding to Tet operator, enabling the RNA polymerase III to drive the expression of the hairpin NELL2 RNAs. As mentioned by Almeida and Makeyev (5-6), these hairpin structures can exert silencing effect by inducing cleavage of the posttranscriptional product of the target gene. Our northern blotting results consistently demonstrated this effect. Currently our lab is investigating the knock down effect on a mouse model to examine whether *NELL2* is a potential oncogene. We expect that the tumor progression and growth may be inhibited in WTs based on this initial finding.

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Hemolytic Transfusion Reactions Following Transfusion of Red Blood Cells Obtained from Human Glycophorin A Transgenic Mice

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Background: Hemolytic transfusion reactions (HTRs) are a serious consequence of blood transfusions. They are mediated either through IgM (acute-type), in which there is a mismatch of the ABO blood group, or by IgG (delayed-type), the result of an incompatibility with minor antigens. Both have serious complications such as shock, disseminated intravascular coagulation, and ultimately death. The mechanisms of these reactions and the steps in their pathophysiology are not clearly understood. However, insight into the relevant cause(s) will illuminate the need for differing therapeutic interventions at different points in a patient's course. An interesting adjunct is to create a mouse model displaying a human element in its blood group. To this end, we used the human glycophorin A glycoprotein as a representative novel blood group antigen in our transgenic mice. With this protein we can simulate HTRs as they would occur in humans. This will allow for further study into the mechanism of HTRs, a review of current therapies, and inevitably the creation of new treatments.

Objective: The purpose of this research is to replicate HTRs in recipients of blood transfusions using donor cells from transgenic mice. Some of the speculations behind these antibody mediated reactions suggest that these could be from a "cytokine storm," defined as a continuous cycle of cytokine signaling leading to massive inflammation, breathing constriction and possibly death. Another aspect is to explore the role of Fc receptors and the complement system. Each of these mechanisms present the possibility of affecting therapeutic treatments. The goal is to understand the sequence of events from the initiation of an incompatible transfusion to the final pathways that can result in serious illness or death.

Materials and Methods: The experiments were conducted using an animal model, and involved transfusions between transgenic donor and wild type recipient mice. The recipient mice are passively immunized with a monoclonal mouse IgG antibody overnight to allow for complete circulation throughout the bloodstream. This step is necessary since unimmunized mice do not have antibodies recognizing the hGPA antigen. To insure the utility of the purified antibody, a hemeagglutination assay is used to see if the antibody recognizes the antigen. The concentration of antibody used depends on analogy to human transfusion in a clinical setting and then is applied to mice. The blood from the donor mice is retrieved and labeled with chromium-51. The incompatible transfusion is performed through the jugular vein, and retro-orbital bleeding is done (t=0) immediately following the surgery. After a set time (e.g. t=4, 8, 12, 24 hours), another bleed is taken. The retro-orbital bleed is a small amount of blood collected in a capillary tube that allows for calculation of the red blood cell survival after the transfusion. With radioactive labeling the blood is placed in a gamma counter to count the number of surviving cells and thus gives a measure of hemolysis. Organs (liver, spleen, lung, kidney) are collected at autopsy for microscopic analysis to find evidence of erythrophagocytosis and inflammatory infiltrate and, thus, the extent of the hemolytic reaction.

Discussion: Currently, the experiments have shown that the cytokines have increased in the transfused mice, while the entire population of negative control mice appeared healthy. Evidence of the hemolytic reaction appeared in the histologic sections where it was seen that RBCs coated with antibody were phagocytosed by Kupffer cells of the liver. Erythrophagocytosis and inflammatory infiltrate were also observed in the spleen.

Neuronal Pain Pathway Modulators

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Background: The present research relates to the discovery of novel modulators of a molecular pathway involved in long-term hyperexcitability of sensory neurons, which, in higher animals is associated with persistent pain. Peripheral pain receptors are located on free nerve endings, which can respond to mechanical, thermal or chemical stimuli. Pain can be acute or chronic. Acute pain is typically transmitted from the receptor through A δ sensory nerve fibers, which are thinly coated with the insulating compound myelin, which facilitates impulse conduction. Chronic pain typically travels through C fibers, which are unmyelinated and transmit impulses slowly, leading to the characteristic dull, diffuse nature of chronic pain. Chemical mediators of inflammation such as bradykinin and prostaglandins stimulate pain receptors. These mediators are important agents in chronic pain syndromes, such as the persistent pain associated with arthritis or nerve inflammation. Regarding chronic pain associated with nerve injury in the peripheral nervous system (PNS), persistent neuropathic pain is a major clinical problem that has mostly resisted effective treatment. In humans and mammalian model systems, persistent pain after nerve injury is associated with long-term hyperexcitability (LTH) of sensory neurons (SNs) having axons in the injured nerve. LTH is manifested as increased sensitivity to electrical stimuli in the SN cell body and axon at the injury site. These changes result in discharge of action potentials from SNs at rest or during innocuous stimulation, leading to continuing excitation of higher order neurons in the CNS and to secondary, or spinal hyperalgesia and persistent pain. Because the appearance of LTH involves alterations in gene expression, a central question is, how are such changes in the nucleus induced by an injury that occurs far from the cell body? In the PNS, following injury to an axon of a sensory neuron, an increase in nitric oxide synthase (NOS) activity results in increased nitric oxide (NO) production, which, in turn, activates guanylyl cyclase (GC), thereby increasing levels of cyclic guanosine monophosphate (cGMP). Increased cGMP results in activation of protein kinase G (PKG), which is then retrogradely transported along the axon to the neuron cell body, where it phosphorylates mitogen-activated protein kinase-erk (MAPKerk). The activated MAPKerk then translocates into the cell nucleus, where it modulates expression of pain-related genes.

Objective: Balanol, a compound capable of PKG inhibition, has been used as a template for synthesis of derivatives, in the hopes of discovering new molecules with equal, if not greater, inhibitory activity. Each new derivative has the potential to act as a LTH inhibitor, and thus as a possible modulator of neuronal pain pathway(s). Ultimately, our goal is to develop a drug capable of blocking PKG, thereby down regulating expression of pain-related genes.

Dental Healthcare in Elderly Persons with Alzheimer's Disease and Related Disorders

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Introduction: The US population is increasingly elderly. And among the elderly, we are seeing an increasing incidence of dementia disorders, such as Alzheimer's Disease, affecting memory and thinking. The CDC reported that during 1999 in New York State, 74.0 percent of people ages 45-64, and 67.6 percent of people ages 65 and over visited the dentist. Yet, there is little known about how these disorders affect aspects of oral healthcare, such as ability to brush teeth, chewing or swallowing problems, frequency of dental visits, and ability to access to dental care.

Objective: The objective of this study is to obtain information on aspects of dental hygiene and dental healthcare in elderly persons with neurological cognitive symptoms.

Methods: We created a dental questionnaire consisting of 18 questions. IRB approval was obtained for the use of this questionnaire. This study was performed at the Memory Disorders Center at the Neurological Institute at Columbia University. The questionnaire was delivered to patient caregivers by a skilled examiner. Out of 48 caregivers approached for possible participation, 42 agreed to participate, resulting in a participation rate of 88 percent. 57 percent of subjects were female and 43 percent male. Average age was 73 +/- SD of 13 (range 50 to 95). Ethnicity included 83 percent non-Hispanic White, 2 percent non-Hispanic Black, and 10 percent Hispanic. Language distribution was 74 percent English, 10 percent Spanish, and 2 percent each of Italian, German, Yiddish, and Polish. Caregivers all spoke English.

Results: 88 percent of subjects have a dentist and 86 percent visit the dentist once or more per year. 17 percent need to be reminded to brush and 12 percent brush only with assistance. 15 percent have problems chewing and 12 percent have problems with swallowing. 85 percent of caregivers are satisfied with the subject's dental care. 33 percent have a dental plan or dental insurance. 10 percent have problems accessing dental care due to the following: lack of transportation, difficulty finding the right dentist, high periodontal costs, and difficulty getting insurance. Major differences observed since the onset of neurological symptoms include taking less care of one-self, forgetting to brush, less frequent visits to the dentist, refusing to see the dentist, poorer breath, and increase in caries.

Conclusion: The goal of the study was met. Based on the results of the study, subjects were found to visit the dentist with a higher frequency than the New York State average. Overall, the majority of caregivers were satisfied with the subject's dental care. Improvement in care may be aimed towards ensuring proper functions such as chewing and swallowing.

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Post-Doctoral Student Abstracts

A Clinical Study of the Transmucosal Herbal Periodontal Patch (THPP) on Gingival Inflammation: The Effect of Monitoring on Gingival Inflammation in Control Sites

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Introduction: Use of medicinal botanicals is widespread with the unique advantage of multiple chemical constituents acting together in synergism to produce a desired effect. Oral hygiene products containing herbal components have been used to decrease plaque and gingivitis primarily by targeting bacteria (i.e. Listerine® antiseptic mouthrinse). We are examining the effect of an herbal product which modulates the local host response in the periodontium. The product evaluated contains a combination of three herbs which have traditionally been used as anti-inflammatory agents with beneficial effects on wound healing and skin disorders. The herbal compound is delivered to the gingiva by a direct delivery mucosal adhesive called the transmucosal herbal periodontal patch (THPP). We are conducting a Food and Drug Administration (FDA) Phase 2 double-blind randomized controlled clinical trial to evaluate the THPP.

Objectives: The purpose of this trial is (1) to determine the safety of the patch upon application to the gingiva; and (2) to determine the efficacy of the THPP on gingival inflammation. In this report, we evaluated the effect of patient monitoring on gingival inflammation as measured by the levels of the pro-inflammatory molecule, β -glucuronidase (β G).

Materials and Methods: 52 healthy patients with gingivitis or mild periodontitis were recruited. Placebo and experimental patch application was randomized between two phases of the study, each lasting 14 days. Patches were applied on day 1, 2 and 3 of each phase to the buccal attached gingiva of the posterior maxillary teeth. The posterior maxillary teeth on the contralateral side were examined as controls. Clinical examinations were performed on days 1, 2, 4, 8, 15, 16, 18, 22 and 29, and included Plaque Index (PI), Gingival Index (GI) and gingival crevicular fluid (GCF) collection. GCF strips are being analyzed for the PMN lysosomal enzyme, β G, the pro-inflammatory cytokine, Interleukin-1 (IL-1) and Matrix Metalloproteinase-8 (MMP-8). Our initial analysis focused on a potential beneficial effect of monitoring (the “Hawthorne effect”) on GI, PI and β G in the control side.

Results: Initial analysis was performed on 35 subjects who completed the study and had data available for all time points during the first week of patch placement (days 1, 2, 4, & 8). Our data indicated a statistically significant decrease in mean GI (1.422 ± 0.038 to 1.314 ± 0.043 ; $p=0.0004$), mean PI (0.557 ± 0.070 to 0.406 ± 0.078 ; $p=0.0012$) and mean β G (72.94 ± 4.46 to 61.58 ± 3.95 ; $p<0.0001$) between day 1 (baseline) and day 2 (1st day after patch placement) on the control (non-patch) side. Because of blinding, analysis comparing experimental and placebo patches is not yet available.

Conclusions: Our results confirm that a significant Hawthorne effect occurred immediately after the initiation of the study but remained stable thereafter. The decrease in gingival inflammation was noted on both clinical and biochemical signs of inflammation.

Effect of Nicotine on the Growth and Protein Expression of *Porphyromonas gingivalis*

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Background: Tobacco smoking appears to be one of the most significant environmental risk factors for the initiation and progression of destructive periodontal disease. The effect of smoking on the periodontopathic microbiota has not yet been elucidated, as epidemiological studies have not identified the concrete relationship between periodontopathic microorganisms and smoking. It is likely that smoking, as an environmental stress factor, may affect the behavior (physiology) of dental plaque microorganisms, ultimately leading to a modification of host-parasite interaction.

Objective: The goal of this study was to examine the effect of nicotine, a major component of tobacco, on the growth and protein expression of a crucial periodontal pathogen *P. gingivalis*.

Materials and Methods: *P. gingivalis* 381 was grown on blood agar plates and liquid broth containing varying nicotine concentrations using two different schemes. Bacterial cells were exposed to (1) a single dose of nicotine (0, 1, 2, 4, and 8 µg/ml), and (2) five consecutive doses of nicotine (1, 2, and 4 µg/ml) every 48 hrs. The growth of bacterial cells was measured by optical density and the protein expression was analyzed by 1-D and 2-D gel electrophoresis.

Results: It was observed that the growth of *P. gingivalis* was inhibited by nicotine in a dose-dependent manner. However, 1-D and 2-D gel electrophoresis did not reveal any significant differences in the protein expression of *P. gingivalis* after exposure to nicotine in either protocol.

Conclusions: Our study showed that the growth of *P. gingivalis* was inhibited by nicotine as expected. However, the protein expression of *P. gingivalis* was not affected by nicotine exposure. These results suggest that nicotine alone may not significantly modulate the physiology or virulence of *P. gingivalis*.

A Case Report of Apert Syndrome: Diagnosis and Treatment

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Background: Apert syndrome is a genetic defect that occurs in approximately 1 per 160,000 to 200,000 live births. It is thought to result from a mutation in a Fibroblast Growth Factor Receptor (FGFR I-IV) which is essential for proper head and extremity development. It falls under the broad classification of craniofacial/limb anomalies and is characterized by specific malformations of the skull, midface, hands and feet. Patients with Apert syndrome typically have premature fusion (craniosynostosis) of multiple cranial sutures. The coronal suture is most commonly affected. As a result of this fusion, the head of these patients is shortened from front to back (brachycephalic) and elongated from top to bottom (turricephalic). The eyes of these patients appear to "bulge out" due to the fact that their skull base and mid-face fails to grow in a normal fashion. The palate of these patients is typically high arched and narrow. The fusion of the fingers and toes, also known as syndactyly, along with the craniofacial problems mentioned above is what differentiates Apert syndrome from other similar ones.

Objective: The purpose of this presentation is to display the diagnosis and treatment of a medically compromised patient with Apert syndrome. These patients should always be treated by a craniofacial team, as their syndrome involves multiple regions of the body. A multidisciplinary approach is used to treat this patient. A craniofacial anomalies team may consist of a craniofacial surgeon, neurosurgeon, ENT, audiologist, speech pathologist, oral surgeon, psychologist, ophthalmologist, pediatric dentist and an orthodontist.

Materials and Methods: A clinical case will be presented including full diagnostic records, diagnosis, treatment objectives, treatment performed, and final records. The patient was operated on the cranial and facial regions in several stages to improve the growth and development as well as to correct the deformity.

Results and Conclusion: The satisfactory outcome is the proud result of a joint work effort of the craniofacial team. The treatment of Apert syndrome begins at birth with the proper diagnosis, identification of the child's individual needs, and the proper facilities to administer what is needed.

Cephalometric Evaluation of Posterior Airway Space in Patients Undergoing Superior Repositioning of the Maxilla

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Background: Vertical Maxillary Excess (VME) is a facial deformity that is seen in patients with “long face syndrome.” Patients with this condition typically present with a long anterior facial height and proportionately long lower anterior facial third. They often exhibit lip incompetence, narrow alar bases, and a steep mandibular plane angle due, in part, to counterclockwise rotation of the mandible secondary to the maxillary excess. VME can exist alone or in conjunction with any other skeletal dysmorphology. It is not uncommon for patients to show an anterior open bite, which may be attributed to the rotation of the mandible. Diagnosis of VME is made via cephalometric analyses by evaluation of the relationship of maxillary skeletal base to dental units and soft tissue analysis of upper incisor relative to the upper lip in repose. Clinically, excessive gingival display reaching the posterior segment is characteristic of VME. The surgical correction of choice for VME is at least a Le Fort 1 maxillary osteotomy with superior repositioning of the maxilla (also described as maxillary impaction or intrusion). Often, a mandibular procedure (IVRO or BSSO) is carried out concurrently and is referred to as double jaw surgery. Functionally, VME patients have similar skeletal patterns as patients suffering from obstructive sleep apnea (OSA), thus predisposing them to this condition. OSA is an extremely disabling disease characterized by sleep-induced obstruction of the upper airway that results in cessation of airflow, depriving the afflicted patient of sleep. In light of this debilitating condition, there has been an interest in effects of the movements of skeletal structures in orthognathic surgical procedures on the airway space. Maxillomandibular advancement surgery has been shown to be very effective in the treatment of OSA because it enlarges the PAS and tightens the upper airway muscles and tendons (velopharyngeal and suprahyoid muscles) by advancement of their bony origins. Conversely, mandibular setback surgery is known to cause a narrowing of the PAS, sometimes resulting in sleep-related breathing disorders. However, there are no studies that examine the changes that occur in the PAS after correction of VME via maxillary impaction.

Objective: The purpose of the study was to use cephalometric radiographs to analyze the changes in the posterior airway space of patients undergoing either isolated maxillary impaction or double jaw surgery for the correction of vertical maxillary excess.

Materials and Methods: Twenty patients, who were referred to Columbia University’s Department of Oral & Maxillofacial Surgery for correction of dentofacial deformity, were included in this study. Selection criteria included patients diagnosed with vertical maxillary excess and treated with at least a Le Fort 1 osteotomy within the past 2 years by one of two surgeons. Some patients underwent concurrent mandibular procedures or genioplasty. Lateral cephalometric radiographs were taken pre-operatively and immediately postoperatively in natural head position with teeth in occlusion. Cephalometric radiographs were digitized, traced using 12 skeletal and 22 soft tissue measurements and evaluated using Dolphin Imaging version 10. Each measurement was performed twice by one operator and the mean values were used for computations.

Results: The study is still under investigation.

Matrix Extracellular Phospho-glycoprotein: Effects on Mineralization *In Vitro*

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Background: Matrix extracellular phospho-glycoprotein (MEPE) is an extracellular matrix protein thought to be part of a regulatory system for phosphate homeostasis and bone metabolism. MEPE is expressed in osteoblasts, osteocytes, and odontoblasts. It was first cloned from a tumor resected from a patient with oncogenic hypophosphatemia (OHO) and later found to be elevated in autosomal dominant hypophosphatemic rickets (ADHR) and X-linked hypophosphatemia (HYP). *In vivo* experiments with bolus infusion of recombinant MEPE in mice induced phosphaturia (Rowe *et al.*, 2004). MEPE knockout mice have increased trabecular bone mass and an increase in osteoblastic number and activity. These studies suggest that MEPE functions as a mineralization inhibitor.

Objective: The goal of this study is to determine the *in vitro* effects of intact MEPE and of the MEPE peptide on hydroxyapatite (HA) proliferation in a gelatin-gel system (Boskey, 1989).

Materials and Methods: One concentration of intact phosphorylated MEPE, and three concentrations of the MEPE C-terminal ASARM peptide in phosphorylated form (25µg/mL, 12.5µg/mL, 50.0µg/mL) were pre-incubated overnight with HA seed crystals (0.5mg/mL or 0.25mg/mL) to determine effects on crystal growth. These coated HA-seeds were placed on the gelatin-gel system for 4.5 days and compared to controls containing HA seed crystals in buffer solution.

Results: Intact MEPE protein tended to inhibit HA seeded growth while 12.5µg/mL and 50.0µg/mL ASARM peptide significantly decreased mineral accumulation. Results at 25µg/mL were equivocal and showed phosphate promotion.

Conclusion: MEPE has multiple effects on *in vitro* mineral crystal proliferation, inhibiting crystal growth when the protein is phosphorylated and intact, with the C-terminal ASARM peptide inhibiting growth when phosphorylated. Future studies will examine the effects of lower concentrations of MEPE-ASARM peptide in phosphorylated and un-phosphorylated forms on HA seeded growth.

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Infection with a Periodontal Pathogen Increases Leukocyte Adhesion to Human Aortic Endothelial Cells

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Introduction: A link between periodontal infections and an increased risk for vascular disease has been demonstrated. *Porphyromonas gingivalis* (Pg), the principal etiologic agent of chronic periodontitis, is a Gram (-) anaerobic coccobacillus that has the ability to adhere to and invade various cell types, including endothelial cells. Pg can gain access into the systemic circulation and has been identified in human atherosclerotic plaques.

Objective: To explore the effects of Pg infection on endothelial cells with respect to leukocyte adhesion, an important step in atherosclerotic plaque infection.

Methods: Confluent primary human aortic endothelial cells (HAEC), passages 4-8, were infected with either Pg strain 381 or its non-invasive fimbriae-deficient mutant, DPG3. Non-adherent bacteria were removed by washing and HAEC were incubated with fluorescence-labeled U937 monocytes, polymorphonuclear leukocytes (PMN), or Jurkat T cells. Non-adherent leukocytes were removed by washing and cells were lysed. Fluorescence was measured and the number of adherent cells was calculated using standard curves. The ability of Pg 381 to adhere to/invade HAEC was confirmed by antibiotic protection assay and immunofluorescence. Expression of adhesion molecules was assessed by flow cytometry and levels of pro-inflammatory cytokines were measured in supernatants by ELISA. Data are reported as mean \pm SD and n represents the number of experiments.

Results: Pg 381-infected HAEC demonstrated significantly increased adhesion of U937 cells ($0.69 \pm 0.08 \times 10^5$), compared to non-infected (NI) ($0.48 \pm 0.07 \times 10^5$), and DPG3-infected ($0.49 \pm 0.04 \times 10^5$) cells ($p < 0.01$ for both, $n=4$). Results were similar for PMN and T cell adhesion. Expression of VCAM-1 was significantly increased in Pg 381-infected (34 ± 5 %) compared to NI (20 ± 1 %) and DPG3-infected (21 ± 2 %) HAEC ($p < 0.05$ for both, $n=2$). Similarly, infection with Pg 381 significantly increased expression of ICAM-1 and E-selectin compared to NI and DPG3-infected cells. Moreover, Pg 381 infection significantly enhanced production of pro-inflammatory cytokines and chemokines. Levels of MCP-1 were 764 ± 203 pg/ml for Pg 381 vs. 379 ± 229 pg/ml for NI and 360 ± 122 pg/ml for DPG3 ($p < 0.05$ for both, $n=3$). Levels of IL-6 and IL-8 were also significantly upregulated in a similar manner.

Conclusions: *P. gingivalis* 381, unlike its non-invasive mutant, increases immune cell adhesion to HAEC and upregulates HAEC expression of adhesion molecules and production of pro-inflammatory cytokines and chemokines. Taken together, these data demonstrate that *P. gingivalis* elicits a pro-atherogenic response in HAEC.

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Identification and Characterization of the Genes Encoding a Surface (S-) Layer of *Tannerella forsythia*

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Introduction: Most prokaryotic organisms possess surface (S-) layers on their outermost surface. The S-layers are composed of regularly arranged protein or glycoprotein subunits and thought to form a protective coating, functioning as molecular sieves and ion traps, or promoting cell adhesion and surface recognition. *Tannerella forsythia* (formerly known as *Bacteroides forsythus*) is a Gram-negative, filament-shaped, anaerobic bacteria which is associated with advanced forms of periodontal diseases. In previous studies, it was found that *T. forsythia* possesses the S-layer which consists of two proteins, 200 and 210 kDa protein.

Objective: In our previous work, the S-layer from *T. forsythia* was first isolated and its functions, both *in vitro* and *in vivo*, were subsequently determined. It was shown that the S-layer alone was able to mediate hemagglutination and involved in attachment and invasion to KB cells. This data indicates that the S-layer of *T. forsythia* is a potential virulence factor. However, the genetic and biochemical aspects of the S-layer have yet been elucidated. The objective of this study is to explain the nature of the S-layer of *T. forsythia* by characterizing the 200 and 210 kDa proteins biochemically and genetically.

Materials and Methods: *T. forsythia* 43037 was grown anaerobically on blood agar medium. *Escherichia coli* XL-1Blue Supercompetent cells and BL21(DE3)/pLysS cells were grown in LB broth medium aerobically. *T. forsythia* genomic DNA was isolated and used as a template to amplify the 200kDa and 210kDa protein genes (*tfsA* and *tfsB* respectively) by PCR. Genes *tfsA* and *tfsB* were cloned into pET-30c(+) vector and were directly sequenced from the recombinant plasmids using gene-specific sequencing primers. For Northern blot analysis, mRNA was isolated from *T. forsythia* total RNA, DNA fragments that are complementary to a portion of *tfsA* and *tfsB*; regions flanking the intergenic segment were produced by PCR. Reverse transcriptase-polymerase chain reaction was performed using the total RNA as a template. For a genetic analysis of *tfsA* and *tfsB* genes, Vector NTI 7.1 software was used. Recombinant TfsA and TfsB proteins were induced from *E. coli* BL21(DE3)/pLysS cells with IPTG.

Results and Conclusion: Sequence analysis has revealed the presence of two S-layer protein encoding genes, *tfsA* (3537bp) and *tfsB* (4092bp) in *T. forsythia*. Further genetic analysis has indicated putative promoter sequences present in the upstream region of *tfsA* and putative rho-independent transcription terminator in the downstream region of *tfsB*. Northern blot and RT-PCR analyses have shown that *tfsA* and *tfsB* form an operon structure. Recombinant rTfsA and rTfsB proteins were successfully induced and expressed in *E. coli*, and SDS-PAGE analysis has revealed that the molecular weights of rTfsA and rTfsB proteins are 135 kDa and 152 kDa, respectively. An apparent discrepancy in the molecular weight between native and recombinant TfsA and TfsB proteins is due to extensive glycosylation on the native surface layer proteins. Sequence analysis has shown four putative promoter sequences for *tfsAB* operon. Using a reporter gene system, each putative promoter is being analyzed. In order to confirm the role of *T. forsythia* S-layer as a key virulence factor, attempts to knock out either *tfsA* or *tfsB* and both genes are underway.

Identification of Individuals at High Risk for Osteoporosis Using Digital Panoramic Analysis: A Pilot Study

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Introduction: Osteoporosis is a systemic metabolic bone disease that affects the hip, wrist, and vertebrae leading to significant morbidity and mortality. It is estimated that over 20% of postmenopausal Caucasian women have osteoporosis, making this disease a significant health issue. Osteoporosis is characterized by low bone mass and reduced bone quality. Decreased bone content in the hip, lumbar vertebrae, iliac crest, and forearm positively correlate with an increased risk of fracture. Early diagnosis is critical as several treatments have been shown to slow progressive bone loss and reduce the risk of fracture. Several procedures are currently available that aid in the diagnosis of osteoporosis. Bone mineral density (BMD) is most commonly evaluated using Dual Energy X-ray Absorptiometry (DEXA) of the spine, hip, and forearm. DEXA requires expensive equipment, specialized operator training, and a large space requirement. Screening for osteoporosis with a panoramic radiograph would be a low cost alternative; however the relationship of bone mineral density of the craniofacial bones to systemic bone loss has not been well established. Additionally, there has been an increase in the number of patients at risk for osteoporosis receiving panoramic screenings, due to the increasing popularity of dental implants. Therefore, screening for osteoporosis through dental panoramic radiographs can provide convenient, low cost accessibility to those patients who might not otherwise have access to a screening by conventional methods. The aim of this study was to examine the correlation of maxillofacial BMD with hip, spine, and forearm BMD using digital panoramic radiography.

Materials and Methods: This study consisted of 10 women between the ages of 40 and 80. Subjects on bisphosphonates, steroids, or hormone replacement therapy were excluded from participation. This study was approved by the Columbia University Institutional Review Board. All patients received a hip, lumbar spine (L1-4), and forearm (non-dominant arm) bone mineral density scan on a Hologic QDR 45000C DEXA following the official guidelines of the International Society for Clinical Densitometry. A digital panoramic exam was performed on a Schick Technologies CDR PANX4792. A unique aluminum phantom using nine ball bearings of three known BMDs was specially designed for panoramic radiology in a checkerboard pattern and was placed below the inferior border in the region of the mental foramen prior to each exam. The same operator conducted each panoramic radiograph. The Pearson Correlation efficient was determined between all sites.

Results: In all panoramic images, we were able to obtain good quality, reproducible images of the maxillary and mandibular structures, with good visualization of the aluminum ball bearings. Furthermore, the ball bearings were very easily placed with tape at the inferior border of the mandible in the region of the mental foramen (See Figure 1). In this small pilot study, the BMD of the mental foramen region was positively correlated with the BMD of the femoral neck ($r=0.83$, $p=0.016$), lumbar spine ($r=0.95$, $p=0.00$), and forearm ($r=0.79$, $p=0.042$). The standard anatomic sites were all positively correlated with each other as well. For instance, the Pearson correlation coefficient between the lumbar spine and femoral neck was 0.84 ($p=0.015$) (See Table 1).

Conclusion: Osteoporosis according to a recent NIH consensus panel is a major threat to Americans (3). The major consequence of osteoporosis is fracture, often resulting in significant financial losses as well as a profound impact on quality of life. Furthermore, the numbers of patients being screened for the disease are insufficient. A low cost and easily accessible method of screening will provide a broader segment of the population an opportunity for identifying those at risk for osteoporosis. In this pilot study BMD of the mandible highly correlated with densities of the hip, spine, and forearm. Digital Panoramic analysis using a phantom of known density in the mental foramen area of the mandible may be useful in the identification of individuals with low bone mineral density. A large scale correlation study, and development of a normative database will be necessary to evaluate whether digital panoramic radiographs can predict the overall risk of osteoporosis and its associated sequelae.

Table 1: Pearson Correlation Matrix between all sites				
	Femoral Neck	Lumbar Spine	Forearm	Mental Foramen
Femoral Neck	1.000	-----	-----	-----
Lumbar Spine	0.837	1.000	-----	-----
Forearm	0.742	0.836	1.000	-----
Mental Foramen	0.834	0.945	0.787	1.000

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Multi-disciplinary Treatment of a Patient with Crouzon's Syndrome

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Background: Crouzon syndrome, also known as craniofacial dysostosis, and acrocephalosyndactyly type II, has phenotypic features similar to those of Apert syndrome – both of which are genetic diseases. This syndrome has been mapped to chromosome locus 10q25-10q26 and is caused by a mutation in the fibroblast growth factor receptor 2 (FGFR2) gene. It may be due to transmission of an autosomal dominant genetic condition, or it may appear as a new mutation, without having affected parents. There is a wide range of clinical presentations of the disease, varying from subtle to severe forms. Crouzon syndrome is manifested by premature closure of coronal sutures of the infant's head. As a result, the brain cannot develop normally and expands in the direction of the open sutures, causing an abnormally shaped head and increased pressure on the brain. Consequently, hypertelorism and a very flat recessed forehead will develop. Exophthalmos is due to fusion of the sutures/bones of the cranial base and midface in conjunction with shallow orbits, giving the appearance of protruding eyes and a flat midface. Other signs include eye muscle problems, beaked nose, short upper lip, and a small and retrusive upper jaw. People with this syndrome may also present with respiratory difficulties, feeding problems, and mental retardation.

Objective: This case study is to exemplify the diagnosis and treatment planning of a patient with Crouzon syndrome. Management of the Crouzon patient involves a multi-disciplinary approach. It is imperative to address the neurological factors, respiratory problems, cleft palate and speech difficulties, orthodontic and orthognathic concerns, ophthalmologic and cosmetic considerations.

Materials and Methods: A clinical case is presented with a full compliment diagnostic record including physical examination, photographs, models, radiographs, and cephalometric analyses. Treatment progress consisted of orthodontics and Lefort III surgery with midface distraction osteogenesis to correct the skeletal discrepancy. Computer generated treatment simulations were performed to predict and later, compare with the actual treatment outcome.

Results and Conclusion: The successful outcome of this case is a result of a teamwork approach and demonstrated the collaborative efforts of multi-disciplines.

Phenotyping and Clinical Ascertainment of a Cohort of Class III Patients for Genetic Linkage Analysis

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Background: Class III Skeletal Pattern is the forward positioning of the mandible relative to the maxilla. The prevalence of this phenotype and associated malocclusion is race and age dependant. Although Class III Skeletal Pattern is one of the least common malocclusion variants, it is one of the most recognizable cranial and dentofacial phenotypes in orthodontics and dentofacial orthopedics. The relative contribution of environment and inheritance to non-syndromic Class III Skeletal Pattern is still unclear. The lack of significant progress in elucidating the cause is most likely due to the limited knowledge related to the genetic mechanisms involved during cranial and facial development, especially in the maxillomandibular region. Some past studies have suggested genetic models that indicate that Class III skeletal pattern could be inherited as an autosomal recessive trait. Others have suggested an autosomal dominant mode of transmission, while still others have theorized that the Class III Skeletal Pattern is polygenic. Environmental etiologies are thought to range from endocrine disturbances to dental eruption pattern deviations.

Objective: The purpose of this study is to investigate various genetic models of Class III Skeletal Pattern in better understanding the role of genetics in the causation of this maxillomandibular saggital discrepancy. This paper will highlight relevant historical research and demonstrate operational laboratory protocols.

Materials and Methods: After clinical examinations are completed and patients' histories are documented, blood samples are taken from subjects and their relatives with a family history of Class III Skeletal Pattern. Several genetic markers will be utilized to determine linkage.

Results and Conclusion: Investigation is a work in progress. The knowledge about the underlying genetic contributor(s) to Class III skeletal pattern will be an important advance in the field of orthodontics, dentofacial orthopedics, and oral & maxillofacial surgery as this will improve the accuracy of diagnosis and provide novel treatment modalities for affected individuals.

Effects of Periodontal Therapy on Mediators Relevant to Cardiovascular Risk

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Objectives: Epidemiological studies suggest that periodontal infection is associated with increased risk for cardiovascular disease. We assessed pre- and post-treatment levels of systemic inflammatory mediators relevant to atherosclerosis in patients with moderate/severe periodontitis.

Methods: To date, 9 patients with moderate/severe periodontitis (≥ 2 teeth/quadrant with ≥ 6 mm pockets and concomitant attachment loss of ≥ 3 mm) have been enrolled. All participants contributed with blood samples at four time points: 1 week prior to periodontal treatment (#1); at treatment initiation (baseline, #2); 6 weeks post-baseline (#3); and 10 weeks post-baseline (#4). Full-mouth clinical periodontal status was recorded at baseline and 10 weeks. Periodontal therapy, including periodontal surgery and extractions but no systemic antibiotics, was completed within a 6-week period. Serum concentrations of 14 biomarkers (C-reactive protein, serum-amyloid-A, serum-amyloid-P, haptoglobin, ICAM, VCAM, E-Selectin, MMP-9, myeloperoxidase, PAI-1, IL-12, IP-10, Eotaxin, and MCP-1) were determined using multiplex assays for Luminex technology. A 'favorable treatment response' was defined as a $>20\%$ reduction in mediator concentration at draws #3 ('early') or #4 ('late') from the mean of the pre-treatment levels. An individual response score (IRS) was calculated for each patient, based on the number of favorable (score +1), unfavorable (score -1), and stable (score 0) individual mediator responses.

Results: Thus far, 9 patients have completed the protocol. Post-treatment clinical measurements showed substantial reductions in bleeding on probing, average pocket depth, and number of deep pockets. The mean 'early' IRS score was -0.03 (0.27 SD; range -0.43 to 0.36) and the mean 'late' IRS score was -0.05 (0.32 SD; range -0.43 to 0.57), indicating a highly variable treatment effect among patients.

Conclusion: In a sample of moderate to severe periodontitis patients, comprehensive periodontal therapy had inconsistent effects on systemic biomarkers.

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Severe Periodontitis as a Rheologic Modifier

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Introduction: Chronic periodontitis is associated with increased risk for heart attack and stroke, but the mechanism for this association has not been fully defined. Gram negative bacteria and associated endotoxins are found in great abundance around the teeth of patients with severe chronic periodontitis. Frequent transient bacteremia is common following dental manipulations. Experimental endotoxemia models in humans indicate that cytokine and coagulation profiles are altered acutely following endotoxin administration. We propose that transient bacteremia from the oral cavity represent a chronic challenge to hemostatic parameters via an endotoxin driven cytokine cascade, resulting in a transient hypercoagulant state, which may predispose the patient to thrombotic events.

Objectives: The purpose of this study is to determine whether scaling and root planing can cause an acute increase in the clotting factors thrombin-antithrombin (TAT), plasminogen activator inhibitor-1(PAI-1), pro-inflammatory and endotoxin, in the blood of patients with severe untreated chronic periodontitis.

Materials and Methods: A total of 8 male subjects will be enrolled, 6 with severe chronic periodontitis, and 2 healthy volunteers. Echocardiograms will be used to screen subjects for any undiagnosed heart problems. Subjects will be admitted to the in-patient unit of the General Clinical Research Center (GCRC) the night prior to the procedure. The following morning, having refrained from oral hygiene procedures for 12 hrs, fasting subjects will be catheterized by venipuncture for repeated blood draws and transported to the outpatient dental unit of the GCRC. Following the baseline blood draw, subjects will receive conservative periodontal therapy consisting of full mouth scaling and root planing (SRP) for not more than two hours under local anesthesia, using hand instruments and ultrasonic scalers. Blood will be drawn at 5 min, 45 min, 90 min, 2, 4, and 6 hours following the treatment. Vital signs will be continuously monitored for the first 6 hours. Subjects will return to the Irving Center outpatient unit the next morning for a 24 hour follow-up blood draw. Blood will be analyzed for endotoxin, TNF- α , IL-6, TAT, PAI-1, fibrinogen, and C-reactive protein for comparison with pre-treatment values.

Results: We hypothesize that circulating endotoxin, inflammatory cytokines, and coagulation products will rise acutely in the hours following the full-mouth debridement, in a manner similar to that observed during experimental endotoxemia. To date we have completed 7 of the 8 scheduled subjects.

Conclusions: This experimental human study is designed to determine whether there is a direct link between chronic periodontitis and clinically relevant hemostatic and inflammatory variables. These data should provide insight into the mechanisms of the periodontitis-systemic disease relationship.

An Overview of Muscular Dystrophy: Clinical Manifestations and Treatment Modalities

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Background: Muscular dystrophies consist of a group of genetic diseases characterized by progressive weakness and degeneration of skeletal muscles. There are several forms of muscular dystrophy, each differing in terms of pattern of inheritance, age of onset, rate of progression and distribution of weakness. The three most common forms of muscular dystrophy are Duchenne, Myotonic, and Facioscapulohumeral. Patients with muscular dystrophy present with wasting, weakness and progressive atrophy of skeletal muscles. As the muscle fibers undergo cellular degeneration, they are replaced with fibrous connective tissue. Patients often will have difficulty with mobility, possessing a clumsy, unsteady gait, eventually some may lose the ability to walk. As there is no cure, treatment consists of respiratory and physical therapy for support in order to sustain the quality of life. In some cases, orthopedic surgery may be attempted. The prognosis of muscular dystrophy patients varies according to the advancement of the disease. Mild cases progress very slowly, affording the patient a relatively normal lifespan while severe cases succumb rapidly to functional disabilities.

Objective: The purpose of this presentation is to demonstrate the clinical diagnosis and treatment planning of patient with Muscular Dystrophy, which involves multi-disciplinary treatment, especially in the areas of pediatrics, orthodontics, physical therapy, anesthesiology, oral and maxillofacial surgery, and speech therapy.

Materials and Methods: A clinical case is presented, including full diagnostic records, diagnosis, medical considerations, treatment objectives, and computer generated treatment simulations to elucidate the complexity of the disease.

Discussion: In order to improve the quality of life and to provide satisfactory treatment to the patients, several treatment options are planned and discussed. These range from supportive treatment without intervention to orthodontic treatment and extensive oral and maxillofacial surgery.

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