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Birnberg Research Day
April 13-14, 2005

Editor-in-Chief

Matthew Fien '06

Associate Editors

Alia Koch '05 Hong Yin '05

“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 Editors **6**
Matthew Fien, Class of 2006
Alia Koch, Class of 2005
Hong Yin, Class of 2005

Letter from the Dean **7**
Ira B. Lamster, DDS, MMSc

Letter from the Academic Dean **8**
Letty Moss-Salentijn, DDS, PhD

History of the William Jarvie Society **9**
An excerpt from the *Dental Columbian*, 1933

"A Century of Progress" **10**
Matthew Fien, Class of 2006

The Birnberg Research Award **12**

Birnberg Lecturers and Research Award Recipients **13**

The 2005 Birnberg Lecturer **14**
Bruce L. Pihlstrom, DDS, MS

Birnberg Research Day Program **15**

A Message from the Jarvie President **16**
David Koslovsky, Class of 2006

2005 William Jarvie Society Membership **17**

Birnberg Day Abstracts: Pre-Doctoral Students

Identifying Virulence Factors of *Bacteriodes Forsythus* Using *In Vivo* Induced Antigen Technology (IVIAT) **20**
N.M. Ahmed, S.W. Lee

Preparation of Post-Space Barrier Using Portland Cement to Decrease the Incidence of Microleakage **21**
V. Aluf, B. Burgener, J. Chang, E. Hsu, J. Hwang, H.W. Liu, S. Menaashehoff, N. Panko, I. Shamalova, R. Turkenich, S. Wong, J. Levi

Preparation of a Post-Space Barrier Using Super EBA Cement **22**
C. Chen, C. Doan, S. Howell, K. Kim, C. Nguyen, J. Schoenberg, P. Soh, C. Tsen, J. Wu, J. Levi

Stability of Intraoral Vertical Ramus Osteotomy (IVRO) in the Treatment of Class III Skeletal Malocclusion **23**
C. Choi, S. Eisig

IgA Binding by DMBT1/SAG/gp-340 is Confined to the VXVLYXXXW Motif in its Scavenger Receptor Cysteine-Rich Domains **24**
K. Da Silva, J.M. Toon, F. Bikker, K. Nazmi, E. Veerman, A.V.N. Amerongen

Extraction and Isolation of mRNA for Surface (s-) Layer Protein of *Tannerella Forsythia* **25**
B. Goodman, S.W. Lee

RAGE-Dependent Mechanisms Modulate Neuronal Degeneration Via Inflammation in a Murine Model of Familial Amyotrophic Lateral Sclerosis **26**
C. Hocking, L. Rong, A.M. Schmidt

Student Performance After Receiving Computerized Dental Simulator (CDS) Training in Preclinical Operative Dentistry **27**
S.C. Howell, V.N. Lee, A. Urbankova, F. Hadavi, R.M. Lichtenthal, H. Graham

Immunogenicity of Periodontal Bacteria **28**
T. Huang, M. Herrera-Abreu, R. Celenti, J.Yang, P.N. Papapanou

Reconstruction of the Temporomandibular Joint with Distraction Osteogenesis and Tissue Engineered Cartilage **29**
D. Koslovsky, S. Drew, T. Polansky, C.J. Langevin, H. Israel

Novel Delivery System of Platelet-Rich Plasma (PRP) Derived Growth Factors **30**
J. Lin, M. Cozin, J. Vo, R. Tsay, R. Landesberg, H. Lu

The Effect of Anti-Resorptive Agent on Osteoblasts and Osteoclast Precursors: Rationale for the Topical Use of Bisphosphonate **31**
S. Lin, S.S. Chang, F.Y. Lee

Expression Pattern of Pax 6, A Transcription Factor Involved in Eye, Nose, and Brain Development, During Early Placode Differentiation **32**
R. Liu, A.J. Silverman

Fine Mapping of the Locus for Autosomal Recessive Hypodontia With Associated Dental Anomalies Maps to Chromosome 16q12.1 **33**
E. Michailidis, A. Kljuic, W. Ahmad, M. Bei, A.M. Christiano

S100-Stimulated SUMOylation of RAGE: A Mechanism to Trigger Activation of NF-kB **34**
J.Y. Oh, W. Kim, A.M. Schmidt

To Study a Panel of Cytokines Found In Gingival Crevicular Fluid **35**
K. Rogers, S.P. Engebretson

Periodontal Disease and the Development of CHD and Strokes Among a Clinic-Based Adult Population at NJ Dental School **36**
A. Schwartz, R. Caine Jr., J. Suh

Human Pulpal Fibroblasts Cultured in Type I Collagen Gel for Dental Pulp Tissue Engineering **37**
J. Teng, S.C. Kim, H.H. Lu, M. McAlarney, G. Hasselgren

Identification of a novel biological pathway associated with chronic periodontitis using gene expression microarray data and biological association networks: a pilot study **38**
C. Turk, S. P. Engebretson

Surface Layer Genes of Various *T. Forsythia* Strains Are Highly Conserved **39**
W. Van De Graaff, S.W. Lee

Immunohistochemical Analysis of Agrin in Human Cerebellum **40**
T. Vani, L.S. Honig

Cocaine Esterase (CocE) and Human Erythrocytes **41**
J. Wu, N. Bharat, S.X. Deng, D. Landry

Knockout of Matrix Metalloproteinase-13 in Mice Delays Wound Healing Response **42**
C. Yu, T. Zelonina, V. Lemaitre, J. D'Armiento

Interleukin profiles of gingival crevicular fluid and gingival tissue from periodontally diseased and healthy samples **43**
J.K. Yu, S. P. Engebretson

Birnberg Day Abstracts: Post-Doctoral Students

Periodontal Microbiota and Serum Antibody Responses in Type 1 Diabetes Mellitus **46**
S.J. Chang, R. Celenti, E. Lalla, S. Kaplan, P.N. Papapanou

Serum Antibodies to Periodontal Pathogens and Markers of Systemic Inflammation **47**
K. Choudhary, B.A. Dye, S.J.C. Shea, P.N. Papapanou

Tobacco Cessation and Periodontal Disease **48**
J.A. Connolly, K. Rueter, D. Albert, D. Seidman, P.N. Papapanou

Time, Nano-Technology and the Surface Micro-Topography of Titanium Implants **49**
C.L. Hsu, K. Kokosis, A.M. Chitu, P.D. Wang

Fc gamma Receptor Polymorphisms and Periodontal Status: A Case-Control Follow-Up Study **50**
D.L. Wolf, N. Westerdaal, A. M. Neiderud, K. Hinkley, G. Dahlén, J.G.J Van De Winkel, P.N. Papapanou

Abstracts Submitted After Publication **52**

Potential Role of Tih1 in the Regulation of Adipogenesis **53**
D. Liang, J. McMinn, Benjamin Tycko
Stable Expression of MXA in MCF-7 cells **55**
Xiao-Meng Zhang1, Chi-Ming Li2, and Benjamin Tykco2
Acknowledgements **56**

A Message from the Editors

The William Jarvie Society is the official student research group of the School of Dental and Oral Surgery. 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 School of Dental and Oral Surgery. The combined efforts of students and faculty from various departments of Columbia University is 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. John Grbic, Dr. Ira Lamster, Dr. Letty Moss-Salentijn, Dr. Steven Engebretson, and Dr. Martin Davis for their guidance and continued support of our goals. We also want to thank Mrs. Marlene Sanchez for her help in the preparation of this journal.

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.

Matthew Fien '06
Alia Koch '05
Hong Yin '05

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March 14, 2005

Members of the Jarvie Society,

Birnberg Student Research Day at the School of Dental and Oral Surgery (SDOS) is one of the most enjoyable events of the academic year. A research experience becomes tangible when students present their data and have the opportunity to enthusiastically discuss their work. That is the true purpose of Birnberg Day, and I am always impressed with the depth of knowledge displayed by the student researchers.

The research mission at SDOS continues to expand in terms of both the variety of funded projects active at the School and the number of research collaborations that involve our faculty. There is a new research collaboration with the Department of Dermatology at the College of Physicians and Surgeons (the new Center for Skin and Mucosal Biology), three faculty in Dermatology have been jointly appointed at SDOS, and the first research scientist has been recruited for the Center. Further, two faculty in the Department of Biomedical Engineering at the Engineering School have been jointly appointed at SDOS. A noteworthy example of the collaborative research environment at the Columbia University Medical Center is the recent national and international focus on a manuscript published in the journal Circulation that demonstrated a link between periodontal microbial pathogens and the risk for ischemic stroke. These findings were the result of study involving faculty at SDOS, the Mailman School of Public Health and the College of Physicians and Surgeons. All of these programs and projects represent research opportunities for our students.

I hope you enjoy Research Day. My congratulations to you and your mentors. With our demanding predoctoral curriculum, finding time to conduct research requires true dedication. Well done!

Sincerely yours,

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

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March 14, 2005

To the Officers and Members of the Jarvie Society,

I extend my warmest congratulations to you on the completion of another successful year. Student research is definitely alive in the School of Dental and Oral Surgery!

The abstracts in this issue reflect well on the excellent work that so many of you have done under the mentorship and guidance of dedicated faculty members of the School of Dental and Oral Surgery, the College of Physicians and Surgeons, and other divisions of Columbia University.

Research is essential for the vitality and the forward momentum of any profession. In dentistry great strides have been made in the past decades. As new researchers enter the field we hope that the present momentum will be sustained. The publication of the newest issue of the Journal of the Jarvie Society is therefore cause for celebration.

I look forward to seeing you at the poster session and hearing your presentations. Again, congratulations on a job well done!!

Sincerely,

Letty Moss-Salentijn, DDS, PhD
Associate Dean for Academic Affairs

Columbia University Medical Center

History of the William Jarvie Society*

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

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

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

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

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

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

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

*** Editor's Note: Joseph Schroff, M.D, D.D.S., was one of the first two students admitted to the dental school through the Columbia admissions process. He received the MD degree in 1920 and the Columbia DDS degree in 1922. Dr. Schroff subsequently joined the SDOS faculty, teaching Oral Surgery to generations of students until his retirement in the early 1950s.*

A Century of Progress

Every baseball fan knows the most exciting play that can occur during the game is a home run. It is the ultimate problem solver. Of course, the other thing any baseball fan knows is that while a home run can instantly increase your odds of winning, it is not the most efficient way to produce a winning record. Successful teams string together a series of hits, walks, stolen bases, and sacrifice fly balls. Teams that accumulate these various elements, which lead to run scoring, are much more successful than those that live and die by a single swing of the bat. To count on such rarities is folly. This is true in baseball as well as in most other aspects of life.

As far as scientific research is concerned, there is nothing more exciting than the transcendent breakthrough that changes the focus of entire fields of study. Unfortunately, barring some kind of accident, a scientific breakthrough of great magnitude is most likely the result of years of tedious research and experimentation. Just as baseball players must continually adjust their own strategy if they want to affect the outcome of a close game, researchers must use the discoveries and accomplishments of their predecessors to strategically produce their own results. This is the beauty of science. By creating a foundation upon which future research can be added, we take strides towards our ultimate goal of discovery. It may appear to those not involved in the entire process that science has achieved a sudden miraculous epiphany, a home run so to speak. But the reality is that these achievements are the result of years of research done by many people in various fields of study.

One of the great opportunities afforded to students at SDOS is the chance to participate in research. While many of these endeavors may appear to be mundane and fruitless on the surface, do not be fooled. Every contribution made to scientific research is of merit. As Dr. Lamster discusses in the Spring 2005 Jarvie Newsletter, “research” is too narrow a term. “Scholarly activity” is more appropriate because it more accurately defines the possibilities. While one student may elect to work in a laboratory analyzing a cellular process that accounts for dental, oral, and craniofacial disease, another student’s research may be focused on developing a new teaching tool and evaluating its effectiveness. In both cases, research has expanded our knowledge base, ultimately contributing to the success of the next generation of practitioners.

The significance of research is often understated. While William Jarvie was primarily a clinician, he certainly understood the importance of research to the practice of

dentistry. In 1905, while giving his presidential address to the New York Odontological Society, Jarvie summed up his views on research and the efforts of those people devoting their time to scientific discovery. In his own words:

> "In every country there are some men so devoted to their art or science that their entire time, talents and energies are given up to the work of original research in the direction in which they may be especially interested. These men are content if the financial result were barely sufficient to procure for themselves a most modest subsistence, being amply repaid and feeling rich indeed if they succeed in adding to the knowledge of science or accomplishing something, which may lead to the amelioration of the condition of their fellow man. To such the recognition and appreciation of the value of their efforts and the pronounced sympathy of their associates in their own profession, would tend greatly to encourage them to continue in the field of scientific research, when otherwise discouragement and discontinuance of effort would prevent the accomplishment of effects which might be the means of producing the most beneficent results upon mankind."

With that said, I would like to congratulate all those students that have participated in various research projects throughout the past year. Your efforts will not go unrewarded. As they say, Rome was not built in a day, and great achievements in science and medicine do not happen suddenly, but in reality occur over long periods of time. Every piece of data and every experiment undertaken has contributed to the furthering of knowledge and scientific discovery that is the goal of all research. Remember, the path that we have embarked upon is rooted in over eighty years of experience. During this time, members of the William Jarvie Society have made significant contributions for this noble cause. Only with a persistent effort from our students and faculty can we continue to thrive.

Matthew J. Fien, Class of 2006
Editor-in-Chief
2005 Jarvie Journal

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 SDOS 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 Research Award. Fifty-one outstanding scientists and teachers have been honored as the Birnberg Lecturer since the first Birnberg Research Award was presented in 1954.

Birnberg Lecturers and Research Award Recipients

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

**Dr. Bruce L. Pihlstrom is the 2005 Birnberg Lecturer. As Acting Director for the Division of Clinical Research and Health Promotion for the National Institute of Dental and Craniofacial Research (NIDCR), and in conformance with the NIDCR's conflict of interest policy, Dr. Pihlstrom has declined acceptance of the award.

2005 Birnberg Lecturer

Bruce L. Pihlstrom DDS, MS

Dr. Bruce L. Pihlstrom is Acting Director for the Division of Clinical Research and Health Promotion for the National Institute of Dental and Craniofacial Research. While at NIDCR, Dr. Pihlstrom is on leave of absence from his academic position at the University of Minnesota School of Dentistry where he is Professor of Periodontology and holds the Erwin M. Shaffer Endowed Chair for Periodontal Research. Dr. Pihlstrom is also an associate member of the University of Minnesota's Graduate School Faculty.

Dr. Pihlstrom received both his Bachelor's and D.D.S. degrees from the University of Minnesota. He pursued postgraduate specialty training at the University of Michigan and received his Master's degree in Periodontics in 1967. Dr. Pihlstrom is also a Diplomate of the American Board of Periodontology.

Among Dr. Pihlstrom's many contributions to dentistry is research investigating the association of periodontal disease and systemic health, genetic approaches to understanding periodontal disease and other oral conditions, and large scale multi-center clinical trials of periodontal disease therapy. Dr. Pihlstrom has also served as a member of the Editorial Review Board and Professional Advisory Board of the *Journal of Periodontolgy*, the Editorial Review Board of the *Journal of Clinical Periodontology*, the Commission on Dental Accreditation, the National Institute of Health National Review's Reserve, and as an *ad hoc* editorial reviewer (periodontics) for the *Journal of the American Dental Association*.

Birnberg Research Day Program

WEDNESDAY, APRIL 13, 2005, 2:00-5:00 P.M.
THURSDAY, APRIL 14, 2005, 12:00-2:00 P.M.

WEDNESDAY, APRIL 13, 2005

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

THURSDAY, APRIL 14, 2005

12:00-12:50 P.M.	Birnberg Day Lecture Bruce L. Pihlstrom, DDS, MS Acting Director, Clinical Research and Health Promotion NIDCR HHSC-301

"Great Opportunities and Great Expectations in Oral Health Clinical Research"

12:50-1:00 P.M.	Presentation of Awards Dentsply Award Presentation HHSC-301
1:00-2:00 P.M.	Buffet Luncheon Riverview Lounge HHSC-Fourth Floor

A Message from the Jarvie President

What a year! The William Jarvie Research Society's mission from the start has been to spark student interest in dental research. We began the 2004-5 year with a bang by hosting over 100 students and faculty members at our annual kickoff event. The overwhelming interest in student research is clearly displayed by the SDOS community as our membership total has now reached 65. To think that almost 25% of the student body is in one way or another involved in research is quite impressive.

The Jarvie Society has grown not only in size, but in dedication and motivation as well. This year alone, we have held four Lecture Lunch Series and two Birnberg Day Poster Making Seminars. In addition, our brand new website allows our members to gain access to information on Jarvie and dental research in general (www.dental.columbia.edu/jarvie). The editors of the Society have produced two outstanding Jarvie Newsletters, featuring informative articles and showcasing our membership. And of course, this year's Jarvie Journal is spectacular.

There are many individuals that helped pave the way and solidify another outstanding year for the Jarvie Society. Dr. Ira Lamster, Dr. Letty Moss-Salentijn, and Dr. Martin Davis have pledged their commitment from the onset and we thank them tremendously. Dr. Richard Abbott, in his new position as Interim Director of the Office of Research Administration, has been a true blessing to the Jarvie Society. Dr. Abbott has helped us grow and continues in helping us pursue our goals. Our faculty advisors, Dr. John Grbic and Dr. Steven Engebretson, have yet again provided the much needed guidance and support to allow Jarvie to thrive. Mrs. Marlene Sanchez has organized another successful Birnberg Day. In addition, the Society proudly recognizes Dr. Mary Beth Giacona, Dr. Shantanu Lal, and Dr. Steven Chussid (Pediatric Dentistry), Dr. Margherita Santoro (Orthodontics), Dr. Kunal Lal and Dr. Luqman Scott-Beckles (Prosthodontics), Dr. David Zegarelli and Dr. Angela Yoon (Oral Pathology), Dr. Sidney Eisig (Oral Surgery), Dr. Panos Papapanou (Periodontics), and Dr. Gunnar Hasselgren (Endodontics). They have all made the 2004-5 Lecture Lunch Series a success thanks to their wonderful contributions and effective presentations.

On behalf of the entire Jarvie Society, I would like to thank Windy Thompson, Aaron Schwartz, Scott Howell and Petar Hinic for the unequivocal dedication they have displayed as members of the executive board. Our editors, Matthew Fien, Alia Koch and Hong Yin have spent countless hours producing this year's Jarvie Journal and Newsletters and their work has been remarkable. And finally, I thank the members of this year's Jarvie Society. Without each and every one of you, it would have been impossible to achieve our goals.

To another incredible year of experience, growth and achievement – Thank you!

David A. Koslovsky
Class of 2006

2005 William Jarvie Society Membership

Officers:

President:	David Koslovsky '06
Vice President:	Windy Thompson '06
Secretary:	Aaron Schwartz '07
Treasurer:	Scott Howell '06
Webmaster:	Petar Hinic '06
Editor-in-Chief:	Matthew Fien '06
Assistant Editor:	Alia Koch '05
Assistant Editor:	Hong Yin '05

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Dr. Steven Engebretson
Dr. John T. Grbic

Staff Advisor: Mrs. Marlene Sanchez

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Rania Elbaz
Helaman Erickson
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Becky Gong
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Alex Porges
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Joseph Sanghyun
Aaron Schwartz
Neeru Singh
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Kyung (Grace) Sung
Jesse Tang
Windy Thompson
Peter Trinh
Adam Vaghari
Tina Vani
Rishi Verma
Jean Wu
Robin Yang
Hong Yin

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

Pre-Doctoral Abstracts

Identifying Virulence Factors of *Bacteriodes Forsythus* Using *in vivo* Induced Antigen Technology (IVIAT)

Nazia Ahmed[1], Seok-Woo Lee[2]
[1]*Columbia University School of Dental and Oral Surgery, NY, NY;*
[2]*Division of Periodontics, Section of Oral and Diagnostic Sciences, Columbia University School of Dental and Oral Surgery*

Background: Several hundred bacteria and their active products have been implicated in the pathogenesis of periodontal disease. Recently, *Bacteriodes forsythus*, a Gram negative non-pigmented and non-motile anaerobe, has been associated with severe and refractory periodontitis along with *A. actinomycetemcomitans* and *P.gingivalis*. Research on the pathogenesis of *B. forsythus* has been limited, with only a few virulence factors being identified and even fewer characterized. *In vivo* induced antigen technology is a novel technique. This eliminates the need to use animal models by using serum from patients who have been infected by the disease of interest. Pooled serum allows a wide variety of clinically relevant antigens to be screened using a cDNA library.

Aim: To create a cDNA library using genomic *B. forsythus*. This will be used in the future to screen periodontal patients using the new technique of IVIAT.

Methods: A cDNA library was first created using genomic DNA from *Bacteriodes forsythus*. Genomic DNA was isolated and sheared via sonication to get optimum fragments of 0.5-2 kb. After purification and blunting, the insert was ligated into prepared expression vector pET30c. Ligations were set up using varying molar ratios of vector and insert, as well as controls. Transformations were completed and plated on kanamycin plates. Colonies were incubated for 24 hours. Cells were collected and analyzed on a 2% agarose gel.

Conclusion: IVIAT has already been used to analyze *A. actinomycetemcomitans* as well as many other pathogens. Utilizing the cDNA library to screen future periodontal patients will allow identification of new virulence factors for *B. forsythus* and its role in disease.

N.M. Ahmed's Fellowship was supported by NIH T35-DE007335.

Preparation of Post-Space Barrier Using Portland Cement to Decrease the Incidence of Microleakage

Vichislav Aluf, Bruna Burgener, Jennifer Chang, Eugenia Hsu, Jungsun Hwang, Hao-Wei Liu, Sara Menaashehoff, Nancy Pancko, Irina Shamalova, Rimma Turkenich, Steven Wong, Jack Levi*

Endodontics –Senior Area of Concentration

Columbia University School of Dental and Oral Surgery, New York, NY

Background: Preparation of a post, core, and crown is often the required treatment of a tooth following root canal therapy. By preparing the post space two thirds of the root length with three to four millimeters of gutta percha filling remaining, we are often left with a weakened and disturbed apical seal. Many factors in the clinical procedure will contribute to root canal treatment failure due to microleakage. Contamination occurs when the preparation of a post space is not done under rubber dam isolation or from temporary and final restorations which do not hermetically seal the root canal. Gutta percha by itself does not closely adapt or seal against dentinal tubules. It can permit bacterial microleakage to pass through it. Portland cement has identical properties to MTA (mineral trioxide aggregate) and can be used as a post space barrier by placing 2-3mm on top of the remaining apical gutta percha filling.

Aim: The aim of this study is to show that the placement of a post space barrier proximal to the apical gutta percha will reduce the dye penetration more so than those canals that were filled with gutta percha and Columbia sealer alone. Since India ink dye penetration stimulates bacterial microleakage we can infer that if the post space barrier protects the root canal filling from dye penetration it will also be an adequate barrier to oral fluid penetration.

Methods: The study was divided into two parts, with a control and experimental group for both. The first part of the experiment utilizes 20 clear plastic central incisors filled with gutta percha and sealer by random operators. The control group consists of post space preps that end directly on the gutta percha. The experimental group has the exact same post space lengths with a 3 mm post space Portland cement barrier placed in between the end of the post-space preparation and gutta percha filling. The second part of the experiment consists of 11 human extracted molars that were accessed, cleaned, shaped, and instrumented using hand and rotary instruments. The prepared canal was then filled with gutta percha and sealer. The distal canal was used as the experimental canal with Portland cement placed in between the post and gutta percha fill. The mesial canal served as the control with post space preparation that ends directly on gutta percha filling in the root canal. India ink dye was placed in the post space for the purpose of measuring microleakage through the gutta percha filling. Induction of dye penetration was facilitated by cutting 1 mm of the apex of all teeth with a diamond bur. High speed evacuation suction tips were used until the dye penetrated the gutta percha. Length of time needed for penetration was recorded for both the control and experimental group. The human extracted teeth were made translucent by decal-stat solution, 98% ethanol, and oil of wintergreen.

Results and Conclusion: Initial results showed a 100% penetration of dye in the gutta percha controls and a negative dye penetration of 50 % in the experimental clear plastic incisors. The preparation of post space barriers filled with Portland cement does decrease the penetration of dye more so than those with a post space without barriers. This experiment illustrates the efficacy of a post space barrier in decreasing the penetration of microleakage during post space preparations

**Dr. J. Levi is the Director of Endodontics Area of Concentration*

Preparation of a Post-Space Barrier Using EBA Cement

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Background: Most root canal treated teeth require the preparation of a post. This preparation involves removing a substantial amount of root canal filling which reduces and disturbs the seal against microleakage. In most post space preparations, three millimeters of gutta percha filling is left at the apical filling. The filling is prone to leak during post space preparation due to faulty coronal restorations and from caries. Over time, the migration of microorganisms through the root canal can cause periapical inflammation. Protecting the root canal filling with a post space barrier of 2-3mm of Super-EBA (super ethoxybenzoic acid) over the gutta percha filling prior to the cementation of a post will decrease microleakage. This material has better dentinal bonding properties than gutta percha, is strong, insoluble, and can easily be removed with an ultrasonic tip if re-treatment is necessary.

Aim: The purpose of this study is to show that using Super-EBA Cement as a post space barrier in root canal therapy results in significantly less dye penetration than post space preparations with gutta percha alone.

Methods: 16 extracted human mandibular molars were prepared using the crown down technique with nickel titanium rotary files. The canals were filled with gutta percha and Columbia cement. We used the lateral condensation technique. The root canals in each tooth were prepared for a post space preparation using a Whaledent peezo bur and leaving 3 mm of gutta percha filling at the apical end. In teeth with short roots, where the crown root ratio of the post length and the thickness of barrier require leaving less than 3mm of gutta percha, the placement of the post space barrier enhanced the protection of the remaining gutta percha fill. The Super-EBA Cement was mixed with tin oxide to increase the radioopaque appearance of the radiographs. Radiographs were taken for all procedures. One canal in each tooth received a post space preparation with Super-EBA cement as a post space barrier, while the other canal, with only 3 mm of gutta percha fill at the apex, served as controls. The cement barriers were placed in direct contact with the remaining gutta percha filling. One millimeter of the tooth apex was removed to enhance the penetration of dye from the occlusal portion of the teeth. All teeth were mounted in small sheets of wax at the level of the CEJ and placed in suction devices. Next, India ink was placed at the occlusal portion while under suction for 45 minutes allowing it to penetrate through the remaining gutta percha. Only three of the sixteen allowed minimal dye penetration through the Super EBA post barrier. All controls had some dye penetration. To make the teeth translucent, the roots were decalcified in a solution of Decal-Stat, a decalcifying solution used in bone pathology studies. The teeth were then soaked in 70% ethanol, 91% isopropyl alcohol, and then methyl salicylate. Additionally, 19 plastic anterior transparent teeth, prepared with post spaces were filled with gutta percha and Columbia sealer. Half of these teeth served as controls. The other half contained the Super EBA cement as a post space barrier. India ink was placed at the occlusal end and suction was applied at the apices. A central suction drew the India ink through the filling materials, allowing the penetration to be clearly visualized.

Results and Conclusion: Recent studies have reported that gutta percha fails as a filling against microleakage because of its lack of dentinal bonding properties. The results of this study show that dye penetrated less in the plastic experimental teeth than in the controls, which showed almost complete dye penetration. The results also showed that extracted human teeth have less penetration in canals with Super EBA post space barriers. In extracted tooth control canals, 56% showed complete dye penetration to the apices and 100% showed some dye penetration. In extracted tooth experimental canals, 0% showed complete dye penetration to the apices and 19% showed some dye penetration. With this said the placement of post space barriers appears to decrease microleakage and complications from post and crown preparations.

**Dr. J. Levi is the Director of the Endodontics Area of Concentration*

Stability of Intraoral Vertical Ramus Osteotomy (IVRO) in the Treatment of Class III Skeletal Malocclusion

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Background: Mandibular prognathism is a skeletal dysplasia characterized by mandibular horizontal excess. Often, the condition is accompanied with a concurrent horizontal maxillary deficiency that exaggerates the disharmony. A clinical facial evaluation and analysis of a lateral cephalometric radiograph is essential in differentiating a true skeletal dysplasia from a dental malocclusion (pseudo-class III). Currently, two surgical procedures are most commonly used in the correction of mandibular excess: the intraoral vertical ramus osteotomy (IVRO) and the bilateral sagittal split osteotomy (BSSO). Many advocate IVRO as the preferred treatment for the correction of mandibular prognathism because of a lower incidence of inferior alveolar nerve injury, technical simplicity, the ability to reposition the condyle, and better post-operative stability. IVRO is a relatively new technique that became popular after Herbert and his colleagues refined the procedure with the use of a special saw (Stryker saw) in 1970. Its small oscillating blade and long shaft facilitated the cut and allowed the technique to evolve into a safe and relatively easy procedure. A soft tissue incision is made in the mucosa from midway up the anterior border of the ramus to the first molar, exposing the entire lateral aspect of the ramus except the condyle neck and coronoid tip. The oscillating saw is used to section the mandible from the sigmoid notch down to the inferior border no more than 5 to 7 mm anterior to the posterior border and distal to the mandibular foramen. The proximal segment is rotated laterally and stabilized while an appropriate amount of periosteum and muscle are stripped from the medial cortex down to the angle. After both sides are cut, the mandibular dentition is brought into its new position with the help of a preformed occlusal splint and stabilized in maxillomandibular fixation (MMF) for 4 to 6 weeks. The success of any surgical procedure that reshapes skeletal morphology can be judged by its postoperative stability. Surprisingly, there are very few stability studies examining the IVRO despite its extensive use.

Aim: The purpose of this study was to investigate postoperative skeletal stability associated with IVRO procedures in patients with class III skeletal malocclusion. Lateral cephalometric radiographs were analyzed to examine 1) postoperative horizontal movements of the mandible to determine extent and direction of relapse, 2) a correlation between degree of surgical movement with degree of postsurgical movement, and 3) the etiology of such observations.

Methods: The charts of 30 patients suffering from mandibular prognathism were reviewed for this retrospective study. All patients had undergone at least IVRO with pre-operative orthodontics over a three year period (2001- 2004) at Columbia University's Department of Oral & Maxillofacial Surgery. Some patients underwent a concurrent LeFort I maxillary advancement and genioplasty. Lateral cephalometric radiographs were taken preoperatively (T0), immediately postoperatively (T1), and 12 months postoperatively (T2). They were analyzed using Dolphin Imaging (version 8). Skeletal movements were evaluated based on routine landmarks and angles. Data analysis was carried out in several different ways. First, surgical change (T0 – T1) and post-surgical change (T1 – T2) were tested for significance with the paired t test ($p<.01$). Mean values for anterior and posterior movement were taken separately. Secondly, after it was determined that 2 mm of relapse in any direction was of clinical significance, the percent of cases with significant relapse was recorded. Finally, in order to examine the relationship between degree of surgical setback with degree of relapse, we divided the groups based on amount of setback (< 2mm, 2-4 mm and > 4 mm) and recorded mean relapse for each group.

Results: Chart reviews have provided data for analysis. Data analysis is currently in progress.

IgA Binding by DMBT1/SAG/gp-340 is Confined to the VXVLYXXXW Motif in its Scavenger Receptor Cysteine-Rich Domains

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Background: Salivary agglutinin (SAG) is encoded by the DMBT-1 gene (Deleted in Malignant Brain Tumors-1 gene) and is identical to gp-340 from the lungs. It is a member of the SRCR (Scavenger Receptor Cysteine Rich) superfamily, a group of immune related proteins with SRCR domains. In saliva SAG binds to bacteria and partially complexes with IgA, which may be necessary for bacterial binding. In previous studies we have shown that a 16 amino acid sequence (peptide SRCRP2; QGRVEVLYRGSWGTVC) is involved in binding to both bacteria and IgA. We have also narrowed down the minimal bacteria binding site on the SRCR domains of DMBT1 to an 11 amino acid peptide (GRVEVLYRGSW) with VEVL, and W being the critical residues in this motif.

Aim: The purpose of the present study is to determine the minimum IgA binding site on SRCP2 domain.

Methods: IgA binding was tested using ELISA's. Microplates were coated with various synthetic peptides and incubated with IgA. Bound IgA is demonstrated with HRP-conjugated antibodies and expressed as a value of absorbance.

Results: In this study, with the use overlapping peptides, we have shown that the minimal IgA binding site on SRCRP2, and thus DMBT1, is the same 11 amino acid motif required for bacteria binding (GRVEVLYRGSW). An alanine substitution scan revealed that VVLY and W are critical residues in this motif.

Conclusion: Bacteria and IgA both share the minimal binding site on the SRCR domain of DMBT1 but differ in the residues that are critical for binding in this motif (VEVLxxxxW versus VxVLYxxxW).

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Extraction and Isolation of mRNA for Surface (s-) Layer Protein of *Tannerella Forsythia*

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Background: *Tannerella forsythis* (Tf) is a pathogen indicated in periodontal disease and possibly systemic disease. The surface (s-) layer is a probable virulence factor. It has been shown to mediate hemagglutination, attachment and invasion, and is involved in abscess formation. Two proteins, a 200 kDa and a 210 kDa protein make up the s-layer virulence factor.

Aim: First, isolate the s-layer mRNA from *Tannerella f.* cultured under varying environmental conditions. Then isolate cDNA from the mRNA using the reverse transcriptase polymerase chain reaction (RT-PCR). The goal is to determine if the s-layer proteins are translated from a single transcript, or from two mRNA's. Also, to determine what environmental conditions are involved in s- layer induction and thus *Tannerella f.* virulence.

Methods: *Tannerella f.* RNA was isolated with a hot phenol extraction. The cells were harvested during logarithmic growth under anaerobic conditions. Lysozyme solution with SDS in TE buffer was used for cell lysis. RNAprotect was added to prevent RNA degradation by RNases. A chloroform and methanol mix was then used for RNA purification. The RNA was precipitated with ethanol and centrifuged to a pellet. Finally, the RNA was resuspended in formamide which is protective against degradation by RNases. Gel electrophoresis was used to confirm the successful isolation of RNA. The solution was run on 1% agarose gels and visualized with ethidium bromide. Optical density was also used to confirm the experiment. *Tannerella forsythia* mRNA was also isolated using a Qiagen RNeasy Protect Bacteria Midi Kit following the manufacturer's instructions.

Results: The results were consistently negative. Many attempts with varying protocols were used. Much was learned about the difficulty of mRNA extraction and isolation. Growth under varying environmental conditions was never attempted as the protocol for mRNA extraction and isolation could not be established in time.

Conclusion: RNA extraction and isolation is difficult. RNases are found throughout the environment and easily contaminate RNA samples resulting in degradation. Further experimentation in a clean lab dedicated to RNA research may prove successful.

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RAGE-Dependent Mechanisms Modulate Neuronal Degeneration Via Inflammation in a Murine Model of Familial Amyotrophic LateralSclerosis

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Background: RAGE, the receptor for advanced glycation end products, is a signal transduction receptor in the immunoglobulin superfamily. It consists of three domains (extracellular ligand binding domain, a short transmembrane domain, and a highly charged cytosolic domain) that are critical for signal transduction. RAGE has been implicated in a number of diseases such as diabetes, amyloidois, and amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis (ALS) is a deadly neurological disorder in which oxidative stress is believed to be a cause of motor degeneration. Enhanced oxidative stress in ALS has been hypothesized to drive generation of RAGE ligands such as AGE, thus triggering RAGE-dependent neuronal stress and microglial activation. It is the RAGE-driven pathways in ALS that initiate inflammation, tissue damage, and neuronal degeneration of ALS. By studying RAGE and its ligands within the context of ALS, we hope to learn more about the mechanisms involved in neuronal degeneration so that we may determine possible therapeutic measures for ALS.

Aim: To test the hypothesis that RAGE-linked inflammation might play a critical role in central nervous system perturbation, cell stress, and cell death in familial Amyotrophic Lateral Sclerosis (ALS).

Methods: The animal models used were single transgenic mice (SR-A-RAGE mice), which express full length human RAGE using macrophage scavenger receptor- type A promoter, ALS mice (mSOD1 G93A mice), and double transgenic mice (cross breeding SR-A-RAGE with mSOD1 G93A mice). Neuron counting: Neurons in the spinal cord lumbar segment, between the end of T13 and beginning of L4 tracts, were counted in 5 micron tissue sections taken every 200 microns of tissue and stained with cresyl violet. Pictures were captured with Axinovision software. Total cell and neuron absolute numbers are counted with KS300 software. This process is currently ongoing.

Conclusion: Since ALS is a neurodegenerative disease, we expect microglial RAGE to participate in neuron loss when compared to the transgenic mice and the wild type littermates. Double transgenic mice might show increased cell density, but decreased neuron number and neuron density.

Student Performance After Receiving Computerized Dental Simulator (CDS) Training in Preclinical Operative Dentistry

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Background: Previous study by LeBlanc et al[1], Urbankova (in review)[2], and Jasinevicius et al[3], shows that CDS training has an effect on student exam performance. This study compares the students' performance on preclinical operative dentistry exams after receiving training in two different simulation laboratory environments: 1) traditional bench-mounted typodont heads (TL) and 2) ergonomically-featured, computerized dental simulators (CDS). Individual tooth preparation analysis (versus overall exam score analysis) indicates that significant differences exist that may be due to CDS training. Therefore, this study suggests that on certain tooth preparations, CDS training may be advantageous and beneficial.

Methods and materials: Seventy-five (n=75) dental students at Columbia University SDOS were randomly assigned to two groups: 1) Control group (TL) and 2) Experimental group (CDS). Both groups received the same traditional training. In addition, the CDS Group received 8-hours of CDS training on DentSim, DenX Ltd., prior to Exam 1 and 2. Each exam consisted of two or three cavity preparations which were independently evaluated by two of the seven faculty members. Performance on the three pre-clinical operative dentistry exams, given in a traditional preclinical laboratory, was used to assess the effects of the CDS training method.

Results: On Exam 1 and Exam 2 significant differences were found between the CDS group and the TL group (Exam 1: $F=10.8$, $p=0.002$, and Exam 2: $F=8.9$, $p=0.004$). No overall performance differences were found between the two groups on Exam 3 ($F=2.56$, $p=0.11$). However, significant differences were found among Exam 3 scores when assessed at the individual tooth level. More specifically, for tooth #14MO, results showed significant differences between the CDS group and the TL group ($F=4.75$, $p=0.032$).

Conclusion: Significant differences were found between the two approaches. The effects of CDS training when implemented early in the Operative Dentistry course places the CDS group in an advantage shortly after participation in CDS training. The CDS group performed significantly better in Exam 1 and 2. As time progressed (3-4 months after CDS training), the students' overall performance leveled off in Exam 3. However, when assessed by individual tooth preparation, significant differences were noted in Exam 3. The data revealed that both groups performed almost the same on #13 MO, but on #14 MO, the CDS Group significantly outperformed the TL group. This suggests that on certain tooth preparations, CDS training appears to be advantageous and beneficial. The next question that needs to be addressed is whether CDS training has a temporary benefit or whether it possesses a distinct performance advantage throughout the year, specifically on the more "difficult" procedures. However, precise measures of what constitutes a "difficult" preparation versus an "easy" preparation are not readily available. Future research should begin to address the "tooth difficulty" question, and relate it specifically to CDS training.

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Immunogenicity of Periodontal Bacteria

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Background: Checkerboard immunoblotting is a useful method for the rapid assessment of antibody response to bacterial species in epidemiologic studies. The extent to which periodontal bacteria vary in their capacity to elicit serum IgG response is not fully appreciated.

Aim: The purpose of the present investigation was to compare the capacity of a number of subgingival species to induce serum IgG antibody responses in a large, nationally representative subject sample.

Methods: Serum samples were collected during the second phase of the National Health and Nutrition Examination Survey (NHANES III). To date, 5,780 serum samples from subjects 40 years old have been analyzed in our laboratory. The level of serum IgG responses against 19 subgingival species was determined by checkerboard immunoblotting (Sakellari et al. 1997).

Antigen preparations were produced from the following species 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.*

Quantification of titers was performed by evaluating chemiluminescent signals in an automated, computerized workstation (LumiImager, Roch-Boehringer-Mannheim).

Results: Overall, IgG responses ranged from 0 to a maximum of 92,530 µg/ml (titer to *A. actinomycetemcomitans*). Median responses, expressed in µg/ml by descending order, were as follows: *E. nodatum* (1,463), *A. actinomycetemcomitans* (1,234), *P. gingivalis* (466), *E. corrodens* (325), *P. intermedia* (320), *A. naeslundii* (271), *P. melaninogenica* (237), *S. intermedius* (202), *P. nigrescens* (201), *T. denticola* (187), *P. micros* (181), *T. forsythia* (175), *C. ochracea* (138), *S. mutans* (120), *C. rectus* (117), *S. oralis* (95), *F. nucleatum* (92), *V. parvula* (51), and *S. noxia* (42).

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.

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Reconstruction of the Temporomandibular Joint with Distraction Osteogenesis and Tissue Engineered Cartilage

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Background: Treatment of patients with severe temporomandibular joint fibrosis, osteoarthritis and/or ankylosis remains controversial, as evidenced by the numerous surgical options that have been proposed. Total joint replacement systems have emerged, but long-term success rates have not been established. Tissue engineering techniques are now being developed for the reconstruction and replacement of diseased human tissues. A recent investigation has demonstrated production of a minipig mandibular condyle using in vitro tissue engineering techniques. Distraction osteogenesis has potential to provide autogenous reconstruction of the temporomandibular joint, using an in vivo tissue engineering technique. Although there are anecdotal reports of the use of distraction osteogenesis for human temporomandibular reconstruction, there is a paucity of literature on this subject.

Aim: The purpose of this investigation is to determine the feasibility of reconstruction of the temporomandibular joint with distraction osteogenesis using a minipig model.

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 into the left temporomandibular joint to induce osteorthritic 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 was fixed to the bone and activated 1 mm/day for 20 days after a 7 day latency period. 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 osteoarthritic 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 48 kg.

Conclusion: 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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Novel Delivery System of Platelet-Rich Plasma (PRP) Derived Growth Factors

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Background: Platelet–rich plasma (PRP) contains essential growth factors that can potentially enhance wound healing and bone regeneration during oral and maxillofacial repair. Secreted by platelets, growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-B), and insulin- like growth factor (IGF) are activated in a sequence which enhances bone regeneration. The degree of bone graft healing and the clinical efficacy of PRP are dependent upon the temporal bioavailability of these factors.

Aim: Our objective is to engineer an alginate-based PRP growth factor delivery system. To control temporal release of growth factors, two types of delivery system will be designed; alginate beads and capsules.

Methods: PRP was isolated from a healthy adult volunteer. PRP-alginate beads and capsules were prepared with 1-2% alginate and 6% $CaCl_2$. PRP beads or capsules (n=4) were incubated in cultured media and collected at 0, 1, 3, 7, 14, 21 days. The levels of TGF-B; and IGF were quantified by ELISA.

Results: The concentration of TGF-B and IGF in supernatants increased with incubation time, corresponding with the measured decrease in TGF-B and IGF content in the alginate beads. Interestingly, the highest levels of TGF-B and IGF release were observed at different time points: TGF-B at 21 days and IGF at 1 day. The release of growth factors was delayed further when PRP was encapsulated in alginate capsules.

Conclusion: An effective PRP-encapsulation system which permits prolonged and continuous release of growth factors was developed. The controlled bioavailability of these growth factors may increase the long term clinical efficacy of PRP.

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The Effect of Anti-Resorptive Agent on Osteoblasts and Osteoclast Precursors: Rationale for the Topical Use of Bisphosphonate

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Background: Zoledronate, an intravenous bisphosphonate, is a potent anti-resorptive agent. There is good evidence showing that bisphosphonates inhibit differentiation and proliferation of osteoclast precursors and induce apoptosis in mature osteoclasts. Evidence also suggests that they induce osteoblast cytodifferentiation. For localized osteolytic lesions such as isolated bone tumors, periodontal osteolysis or dental implant loosening, where excessive bone resorption can occur before adequate osteogenesis during treatment, topical application of bisphosphonate seems to be a logical solution in preventing excessive bone resorption and promoting treatment success.

Aim: The purpose of this study was to determine the specific dose-range of Zoledronate which will induce apoptosis in osteoclasts while exerting minimal detrimental effects on the functions of osteoblasts.

Methods: Two cell lines were studied. The murine RAW 264.7 cell line treated with RANKL (receptor activator of NF-kappa-B ligand) readily differentiated into multinucleated osteoclasts; while the MC3T3-E1 cell line consisted of mouse embryo calvaria fibroblasts, which differentiated into osteoblasts in an osteogenic media. The two cell lines were treated with culture media containing 0µM, 2.5µM, 5µM, 10µM, 25µM or 50µM Zoledronate. Pictures of the cell cultures were taken after 3 days of Zoledronate treatment. Cell death assay using FACS flow cytometry analysis was also performed by labeling the cells with propidium iodide after 1 day Zoledronate treatment. In addition, the two cell lines were cultured on bone slices pretreated with different concentrations of Zoledronate to determine the feasibility of topical treatment.

Results: MC3T3-E1 cells treated with Zoledronate were observed to have relatively no change in survival rate compared to control, at 2.5µM and 5µM. At concentrations higher than 10µM, MC3T3-E1 cells showed increasing apoptosis in a dose dependent manner. On the contrary, RAW 264.7 cells treated with Zoledronate in the presence of RANKL showed significant rate of apoptosis even at concentrations as low as 2.5µM. FACS data indicated that while RAW 264.7 cells consistently had higher apoptotic rate than MC3T3-E1 cells, the differences increased significantly at 5µM and above. Cells cultured on bone slices also demonstrated a dose dependent apoptosis upon visual inspection.

Conclusion: Bisphosphonates are known to bind calcium containing materials such as bone, dentine, or other bone graft substitutes. When an optimal dose-range of bisphosphonate is applied, topical bisphosphonates can protect host bone and bone grafts by inhibiting osteoclastogenesis while osteoblasts are less affected. Further studies would be done to demonstrate the specific effects of bone slices pre-soaked with bisphosphonate on osteoclasts and osteoblasts.

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Expression Pattern of Pax 6: A Transcription Factor Involved in Eye, Nose, and Brain Development During Early Placode Differentiation

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Background: The chick embryo, being accessible for manipulations, is an excellent model to study the early development of the vertebrate central nervous system. Six3, a transcription factor thought to promote the formation of ectopic lens and retinal tissue, is expressed starting in presomitic stages at the most anterior portion of the neural plate, as demonstrated through in situ hybridization (P. Bovolenta et al, 1998). It is suggested that Six3 expression in the lens placodal ectoderm is controlled by Pax6 (O. Lagutin et al, 2001). Heterozygous mutation in Pax6 gene results in small eye phenotype and homozygous mutant mice have no eyes and no nasal cavities. It has been suggested by Hogan et al. (1986) that these phenotypes result from a failure of early placode differentiation, which also lead to defects in neuronal differentiation and migration in homozygous mutants. Pax6 is necessary for the normal development of eyes, nose, and forebrain.

Aim: It is the interest of the Silverman lab to investigate the neuro-differentiation and migration of gonadotropin releasing hormone (GnRH) neurons, which are the neurons responsible for sex differentiation and reproductive function. GnRH neurons migrate from the epithelium of olfactory placode to the forebrain early in development. Because GnRH neurons appear early in the nose, it is our interest to investigate any possible association of Pax6 expression and GnRH neuron differentiation and migration.

Methods: Data of double label immunocytochemistry for Pax 6 and GnRH from the time that the neurons are first seen in the placode was collected.

Conclusion: It is our conclusion that they share a similar expression localization pattern during early chick embryogenesis. For this reason, we speculate an association between the expression of Pax6 and GnRH neuron systems.

Fine Mapping of the Locus for Autosomal Recessive Hypodontia with Associated Dental Anomalies Maps to Chromosome 16q12.1

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Background: Hypodontia is a disorder of epidermal appendage development, involving the congenital absence of one or more teeth, and may include the deciduous and/or permanent dentition. Segregation analyses in several family studies have demonstrated hypodontia to be the result of mutations in a single gene, often transmitted as an autosomal dominant trait with incomplete penetrance and variable expressivity. Previously, Dr. Christiano's group studied consanguineous kindred from Pakistan with hypodontia associated with various dental anomalies, being transmitted as an autosomal recessive trait. After performing genome wide screening with a panel of 386 microsatellite markers, their results demonstrated linkage to chromosome 16q12.1, with maximum lod score of 5.6 for the marker D16S3253.

Aims: 1) Fine mapping of the linkage interval on chromosome 16.

2) Identification and analysis of additional positional candidate genes.

Methods: To refine the previously described hypodontia genetic locus, microsatellite markers covering the interval between D16S492 and D16S2620 were selected. PCR primers for these markers were designed according to available sequences. PCR reactions were performed and products were resolved on 6% non-denaturing polyacrylamide gels and visualized by ethidium bromide staining. Haplotypes were reconstructed manually assuming the minimum number of recombination events. To screen for mutations in the human *IRX 3, 5,* and *6* candidate genes, all exons and splice junctions were PCR-amplified from genomic DNA. PCR products were purified and sequenced. Search for mutations was performed by visual inspection and comparison with control sequences generated from unrelated, unaffected individuals.

Results: Clinical investigation of the affected family members demonstrated a case of hypodontia associated with dental anomalies such as enamel hypoplasia and hypocalcification, malformation, malpositioning, failure of tooth eruption, early carious lesions and attrition of erupted teeth and dentinogenesis imperfecta, leading prematurely to the edentulous state. The skin and other epidermal appendages such as hair, nails and sweat glands were normal. To refine the genetic locus, the genotypes of 18 additional microsatellite markers were used to identify new recombinations within the pedigree (n=25) and define the minimal critical linkage interval. Haplotype analysis showed critical recombination events in individuals 15 and 16 of the pedigree. These recombinations define a critical linkage interval of 9.38cM between markers D16S492 and D16S2620. The physical map between the boundary markers demonstrates a region 7.65 Mb in size containing 74 known genes. Among these, Iroquois homeobox genes (IRX) 3, 5, and 6 were considered potential candidate genes for the disease. These genes were considered because previous studies showed that mutations in other homeobox domain genes, MSX1 and PAX9, were identified for autosomal dominant hypodontia. To date, no mutations have been found in our candidate genes. Through a recent collaboration, we have been using microdissected tooth papillae from MSX1 -/- mouse and normal mouse lines to perform micro-array comparisons of these two cell populations in order to identify genes whose regulation is affected by the absence of MSX1. We hope to cross-reference these findings with our redefined linkage interval to identify more candidate genes.

Conclusion: Identification of the first gene implicated in the pathogenesis of inherited autosomal recessive hypodontia could generate a new direction of scientific investigation into the tooth bud as a model for genetic regulation of morphogenesis, particularly epithelial-mesenchymal interactions. Ultimately, discovery and modulation of this gene could provide a novel therapeutic target for the severe recessive forms as well as for the more common milder forms of this dermatologic and dental disorder.

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

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Background: The receptor for advance glycosylated end-products (RAGE) is a signal transduction receptor for at least certain S100/calgranulins, whose roles are linked to homeostatic properties, such as calcium binding in addition to activation of inflammatory signal molecule NF-kB. Activation of RAGE with these molecules such as S100A12, S100B, and glucose triggers activation of signaling cascades and generation of cytokines and proinflammatory adhesion molecules with important links to atherosclerosis initiation and progression. However, the precise molecular mechanism for the initiation of intracellular cell signaling by RAGE has remained to be elucidated. Very recently, small ubiquitin-related modifier (SUMO) has been intensively investigated for its important roles in diverse processes such as nuclear protein import, protein targeting to and formation of certain subnuclear structures, and the regulation of a variety of processes such as the inflammatory response in mammals. The process of SUMOylation results in lysine modifications of proteins by the addition of a polypeptide group to the backbone protein. SUMOylation of proteins involves at least three central steps; 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. E3 SUMO ligases then attach SUMO to the target protein.

Aim: Previous studies showed that S100-mediated activation of NF-kB is blocked by deletion of RAGE Tail domain. Therefore, we speculate that cytoplasmic tail region of RAGE has a site responsible for activation of NF-kB. Interestingly, bioinformatics analysis showed that RAGE has multiple potential SUMOylation sites, especially in its cytoplasmic tail region. We therefore have investigated the underlying molecular mechanisms for RAGE signaling with SUMOylation in smooth muscle cell (SMC) biology and explore its role in the pathogenesis of diabetic vascular complications.

Materials and Methods: Chinese hamster ovarian (CHO) cells were stably transfected with full length RAGE wild type cDNA and selected for a stable clone. Streptozotocin-treated ApoE null mice aortic vessel atherosclerotic plaque samples were sectioned and stained with anti-SUMO1, anti-Ubc9, anti-RAGE antibodies and visualized for colocalization by confocal microscopy.

Conclusion: To examine the interactions between SUMO and RAGE in the cells, CHO parent cells and RAGE-expressing cells 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 western-blotted. 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. 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 sectioned and stained with anti-RAGE antibodies and anti-SUMO1. As expected, colocalization of RAGE and SUMO1 were shown in plaque region composed of mainly (cytoplasm of) SMC. In addition, the confocal microscopy analysis with SMC culture showed that RAGE and SUMO1 are colocalized in cytoplasmic and more strongly around the nuclear region in response to S100 stimulation. Our data together with previous observations suggest that SUMO1 with Ubc9 may play an important role in subnuclear localization and signaling of RAGE upon stimulation with S100b and triggering activation of NF-kB. We are currently investigating the effects of sumoylation site mutant in the cytoplasmic tail domain of RAGE on its downstream signaling. 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 complications.

Multiplex bead array assay for the detection of cytokines in the gingival crevicular fluid of subjects with and without periodontitis

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Background: The characterization of gingival crevicular fluid **(**GCF) provides information useful in the study of periodontitis pathogenesis. However, the study of GCF is challenging because of the very small sample volumes that can be obtained and the limits of existing technologies such as enzyme-linked immunosorbent assay (ELISA). A recently developed multiplex bead array assay permits the simultaneous quantitation of multiple cytokines from small volumes of solution. We used this assay to measure a panel of cytokines from the GCF of subjects with and without chronic periodontitis.

Methods: 328 GCF samples were collected from 28 patients with untreated periodontitis and 14 volunteers without periodontitis. The Luminex 100 (Luminex Corporation, Austin, TX, USA) was used to analyze GCF for interferon-gamma (IFN), interleukin (IL) -1 alpha, IL-1 beta, IL-4, IL-6, IL-8, IL-10, IL-receptor antagonist (IL-1ra), tumor necrosis factor-alpha (TNF), and RANTES. Multiplex kits from Bio-Rad Laboratories (Hercules, CA, USA) were used according to the manufacturers guidelines. Standard curves were generated by using reference concentrations supplied by the manufacturer.

Results: TNF and IFN were below the level of detection for all samples, and IL-10 was below the level of detection in healthy subjects only. Levels of all other cytokines were detectable, with yields ranging from 83%(IL-6) to 100%(IL-1alpha).

Conclusion: Multiplex bead array technology may have substantial utility in the detection of cytokines and other biochemical markers of interest in gingival crevicular fluid.

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Periodontal Disease and the Development of CHD and Strokes Among a Clinic-Based Adult Population at NJ Dental School

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Introduction: Myocardial infarction (MI), angina pectoris, and revascularization surgery of the coronary arteries are all considered coronary heart disease events. Coronary heart disease (CHD) is the single most common cause of death in economically developed countries of the world, including the United States and Western Europe, where it is responsible for one-third of all deaths. The American Heart Association estimated that the mortality rate for CHD and strokes was 446,000 in 1998. In 2000, strokes were the third leading cause of death in the United States. Chronic Periodontitis prevalence rates may be as high as 40 percent of the adult population. Population studies in the United States demonstrate that up to 15 percent of the adult population has severe periodontitis. Current ongoing research suggests a relationship between periodontal disease, CHD, and strokes.

Objectives: The objective of this study was to determine if an association existed between chronic periodontal disease parameters and coronary heart disease or strokes among a clinic based adult population at New Jersey Dental School.

Material and Methods: Cases were adults (≥ 18 y/o) who reported having a history of a Coronary Heart Disease (CHD) event or stroke. Controls were patients without a history of a CHD event or stroke. A total of 221 patients, 70 cases and 151 controls, were used in this observational, risk-assessment, cross-sectional study. The amount of alveolar bone loss along with the number of missing teeth and the number of pulpo-periapical pathologies (PAP) were documented for each patient. Univariate, bivariate chi-square, and multivariable logistic regression controlling for statistically significant risk factors for both CHD and chronic periodontitis (smoking, hypercholesterolemia, age, and physical inactivity) were performed. Our sample size included 221 subjects, which was appropriate to achieve our desired power. Investigator bias was corrected by training sessions and calibration held prior to data collection.

Results: After controlling for confounders, the severity of alveolar bone loss (degree of periodontitis) was statistically significantly associated to the history of a CHD or stroke event, with an OR=1.50 (95% CI 1.03-2.17, p-value=0.0330) among our clinic based adult population at New Jersey Dental School.

Conclusion: Due to the high prevalence rate of periodontitis in the United States, implicating the disease as a risk factor for CHD/strokes will strengthen management and prevention guidelines for CHD/strokes. This will help reduce the mortality and morbidity rates seen with CHD/strokes, reducing the cost burden on our health care model. This area of research may have profound impact on the general population, thereby situating itself as major dental public health interest. More expensive and timely longitudinal prospective studies with larger sample sizes should continue to be encouraged, which may reveal further significant associations between periodontal disease and CHD/strokes.

Human Pulpal Fibroblasts Cultured in Type I Collagen Gel for Dental Pulp Tissue Engineering

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Significance: Annually 24 million root canal therapies (RCT) are performed in the United States. During RCT, the dental pulp is removed and the tooth is filled with gutta-percha. Limitations associated with RCT, such as risk of infection, tooth fracture, and complications due to prosthetic restorations have prompted interest in pulpal regeneration. We believe that tissue engineered pulp could take advantage of the natural healing potential of pulp and enable dentin repair.

Objective: Human pulp tissue consists of fibroblast-like cells embedded in a type I and III collagen matrix. The objective of this study was to evaluate type I collagen gel as scaffold material for pulp tissue engineering.

Methods: Human pulpal cells isolated from non-carious premolar and third molar teeth were grown in supplemented DMEM (10% FBS, NEAA and antibiotics). The cells were mixed with a DMEM-HEPES-MEM-Type I collagen solution (Invitrogen), with 2x105 cells/ml and a final collagen concentration of 2mg/ml. The collagen/cell mixture was then incubated for 2 hrs at 37°C to allow polymerization to occur. Culture medium (0.5 ml) was added to each well overnight. Subsequently, 1.0 ml of medium was added and media was exchanged every other day. Three types of gel matrix (unconfined, partially confined and confined) with pulp cells were fabricated. For unconfined gel, the culture wells were pre-coated with 2% Bovine Serum Albumin and incubated for 1 hour in 37°C to prevent gel attachment. For confined gels, Thermoplast coverslips (Fisher) were placed in the well and a 22 gauge needle was used to scratch the remaining well surface in order to create multiple sites for gel attachment. For partially confined gels, a slit was made through the center of the confined gel to create a free edge. The pulp-collagen cultures were maintained for up to 4 weeks, and growth of pulpal fibroblasts and their morphology within the collagen gel were examined as a function of gel type. Gels were imaged and gel contraction was quantified using Scion image analysis (n=5). Percent gel contraction was calculated by subtracting final gel area from the original area and dividing by the original gel area. Cell morphology was observed by light microscopy. Cell proliferation (n=5) was quantified using the Picogreen® assay (Molecular Probes). Pulpal fibroblast growth and cellular morphology were examined as a function of collagen gel type as well as culture duration (0,1,2,3,7,14,21,28 days).

Results: Pulpal fibroblasts grew readily in the collagen matrix and produced structural changes in the gel. Cells exhibited a spherical cellular morphology upon embedding in the collagen gel. By day 2, a small percentage of pulp cells began to elongate and develop orientation patterns in the loaded gels. Pulpal cells grown in the unconfined gel were found to be randomly located in the gel. Cell proliferation in confined and partially confined gels entered the plateau phase of cell growth by day 21, while that of the unconfined gels continued to increase, entering the exponential phase. These findings suggest that pulpal cells in unloaded gels are actively proliferating, while those in the confined or partially confined gels may be induced to differentiate instead of proliferating. After 14 days, gels in all groups exhibited noticeable contraction and began to detach from the well surfaces. Confined gels contracted the least at 18.97%, 23.37% for the partially confined, and 53.98% for the unconfined gels. Unconfined gels exhibited the highest rate of contraction at 3.86% versus 1.36% per day for the unconfined group. Although specific markers for pulpal stem cells have yet to be characterized, cultured pulp cells have been shown to express bone morphogenetic protein mRNA (Gu, K, Bone 1994). Studies indicate that upon wound healing, dental pulp cells have the potential to proliferate and differentiate into odontoblasts to form dentin.

Conclusion: Restricted and partially restricted gels visibly exhibited an increase in cells and orientation compared to the unrestricted gels, suggesting conditions for pulpal fibroblast growth and differentiation are favored under some degree of restriction and loading of the collagen gel. Human pulpal cells proliferated in the collagen type I matrix over time and responded to the local mechanical environment of the gel. Collagen gel may be a promising matrix for pulpal tissue engineering. Further studies will be conducted to examine the expression of specific markers and proteins during pulpal fibroblast differentiation.

Identification of a novel biological pathway associated with chronic periodontitis using gene expression microarray data and biological association networks: a pilot study

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Background: Key features of the pathogenesis of chronic periodontitis remain to be elucidated. High throughput technology such as gene expression microarray has resulted in large amounts of new information relevant to the pathogenesis of chronic periodontitis. However, the interpretation of these large amounts presents a challenge. We utilized a commercially available software suite recently developed by Stategene Software Solutions(College Station, TX) to help analyze and interpret the results of a clinical study in which microarray technology was used to identify differences in gene expression patterns of gingiva collected from periodontally diseased and healthy subjects.

Methods: Clinical measures and gingival biopsies were collected from 14 patients with untreated periodontitis and 14 volunteers without periodontitis. Standard Affymetrix GeneChip (Santa Clara, CA) protocols were used to analyze RNA extracted from biopsies using HG-U95Av2 GeneChip (Affymetrix, Santa Clara, CA) arrays. CEL files were converted to analyzable form using the GC-robust multi-chip analysis. The resulting expression data were log transformed and grouped by periodontal disease status for comparison.

Results: Significance analysis revealed several hundred differentially expressed genes. Inspection of this list led to the identification of genes of well known and lesser known function. Among genes of lesser known function, dermatopontin was chosen for further exploration. Dermatopontin was expressed at extremely low levels in diseased compared with healthy gingival tissue. A brief review of the literature revealed an association between dermatopontin and the decorin-TGF beta pathway. Using the natural language processing program Pathway Assist we created a biological association network. Based on these data we developed a hypothesis that may in part provide a biologically plausible explanation for the tissue destruction associated with chronic periodontitis.

Conclusion: We demonstrate here the use of microarray technology and bioinformatic techniques to identify a candidate biological marker for further experimental evaluation. Commercially available natural language programming software allows for rapid extraction of relevant literature from existing public databases. Biological association networks may thus be created. Interpretation of these biological networks may lead to better understanding of the pathogenesis of chronic periodontitis.

Surface Layer Genes of Various T. Forsythia Strains Are Highly Conserved

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Background: *Tannerella forsythia* (formerly *Bacteroides forsythia*) is an important periodontal pathogen with possible implications for systemic disease. Our previous studies have shown that a surface (S-) layer of *T. forsythia* is a virulence factor involved in hemagglutination, adherence and invasion activities, abscess formation in mice, and the humoral immune response in periodontal patients. It was determined that the S-layer consisted of the 200- and 210-kD glycoproteins encoded by separate genes, *tfs*A and *tfs*B, respectively. The *tfs*A (3.5 kb) and *tfs*B (4.1 kb) genes encode for 135- and 152-kD core proteins, respectively.

Aim: The purpose of this study was to compare the DNA sequences of *tfs*A and *tfs*B of nine clinical strains of *T. forsythia* to determine if there is any homology among S-layers of different *T. forsythia* strains.

Material and Methods: The *tfs*A and *tfs*B gene segments from nine strains were amplified by PCR using primers designed based on the gene sequences of *T. forsythia* strain 43037. The DNA sequences of *tfs*A and *tfs*B were directly determined from the PCR product at the Columbia University Medical Center DNA Sequencing Core Facility.

Results: The results showed that the *tfs*A and *tfs*B gene segments were successfully amplified from eight strains, except one. Further sequencing analysis indicated that these gene sequences were highly homologous, almost identical. These results suggest that the S-layer genes of various *T. forsythia* strains are highly conserved.

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Immunohistochemical Analysis of Agrin in Human Cerebellum

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Introduction: Alzheimer's disease (AD) is a major health problem, known as the principal cause of dementia in the elderly, affecting about 15 million people worldwide.[1] Although its pathogenesis is not fully understood, one issue of increasing importance is the relationship between blood vessel disease (cerebrovascular disease) and AD. Agrin is a synaptic protein found in the basal lamina of blood vessels (e.g. capillaries) in the brain.[2] It has been reported that agrin levels are higher in AD.[3] However, there is little reported research on the immuno-histochemical evaluations of agrin in the cerebellum, an affected region in neurodegenerative disease.

Aim: We undertook a pilot study to examine whether there are differences in agrin expression of human brain cerebellum affected by AD versus other neurodegenerative diseases.

Methods: An immunohistochemistry protocol was used to stain for agrin in nine samples of human cerebellar tissue from patients who had Alzheimer's disease, Parkinson's disease, and normally aged brains as controls. After the samples were cut into 8 micron sections using the Cryostrat and placed on subbed glass slides, they were stored at -20 degrees and ready for our histological analysis. First, the tissue was post-fixed in 4% paraformaldehyde in PBS and then washed with PBS. A Block solution containing Horse serum, Triton X-100 and BSA in PBS was applied. Then, a primary antibody for agrin was used to bind to the protein. Slides were left overnight at 4 degrees. PBS was used to wash the samples the next day. A secondary antibody of biotinylated anti-rabbit was applied. Slides were washed with PBS and ABC was applied which consisted of avidin and biotinylated horseradish peroxidase. Samples were washed with PBS and then DAB was applied to stain the tissue resulting in a brownish color which allowed us to visualize the protein. Lastly, slides were placed in increasing concentrations of ethanol for dehydration of excess water in the tissue and cover-slipped for microscopic visualization. We used a light microscope to analyze the slides. Blind to the samples' diagnosis of disease, we rated the intensity of staining in various layers and types of cells within the cerebellum using a scale of 0 (no staining) to 4+ (intense staining).

Results: After rating the staining intensity of the samples, we matched the specimen numbers with the recorded diagnosis for each and discovered that we had 3 with Alzheimer's Disease (AD), 4 with Parkinson's disease (PD), and 2 control brains that were normally aged. Agrin localization was greatest in the cells, both Purkinje cells and the granular cell layer. The capillaries, both in the molecular layer and white matter, were stained greatest in the AD samples, medium intensity staining in the PD samples, and the least stained in the controls. The synaptic region (molecular layer itself) was not greatly stained in either of the samples while the white matter itself was the least stained of any region in all the samples.

Discussion: This study provides some evidence that there is an increase in agrin in AD. We observed that blood vessels stained more intensely for agrin than the cells and synaptic regions, suggesting a likely relationship between cerebrovascular disease and agrin. In the future, to further examine agrin's role in AD and other neurodegenerative diseases versus controls we can increase the number of brains used in the study as well as the number of agrin isoforms tested that may be present in the brain.[4]

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Cocaine Esterase (CocE) and Human Erythrocytes

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Background: Cocaine esterase (CocE) is an enzyme that efficiently hydrolyzes an ester bond in cocaine, transforming the drug into Ecognine Methyl Ester (EME) and benzoic acid. The enzyme is from a *Rhodococcal* bacterial strain that grows in the rhizosphere soil of the cocaine producing plant *Erythroxylum coca*. In the past, attempts were made to induce mice to produce antibodies, which hydrolyze cocaine. Also, CocE has been modified, by adding soluble polymer polyethylene glycol to its surface and conjugating human bovine serum albumin, to make it more compatible with the human immune system.

Aim/Objective: The project attempts to directly insert enzyme CocE into human red blood cells (RBC). The ultimate goal is to have an immuno-compatible CocE containing RBC, which quickly hydrolyzes cocaine before cocaine reaches the receptors in the brain.

Methods: In this experiment, CocE enzyme was prepared and introduced into erythrocytes. The following steps were used to prepare the enzyme:

1. Culture bacterial strain contain the CocE enzyme:

The bacteria strain, "CocE pet-22, expressed in BL21" (CocE enzyme subcloned into pET-22b (+) and transformed into BL21-Gold (DE3) cells; Invitrogen), which had been stored at -78 °C, was thawed, then grown in yeast tryptone (2xYT) media containing 100ug/ml of ampicillin (Fisher). The initial growth took place overnight in a shaking incubator at 23 °C. The culture was then diluted 50 times with the same media and grown for 2 to 3 hours at 37 °C under vigorous shaking until the optical density reaches around 0.6 at 600nm. Then to each liter of media, 238mg of isopropyl-B-D-thiogalactopyranoside (IPTG) was added and incubated over night at 23 °C with shaking. The culture was then ready for harvest.

2. Enzyme CocE separated via osmotic shock:

The culture was centrifuged (4500rpm) at 4 °C for 30 minutes. The pellets were then suspended in about 5mL of cold 1x TES buffer (0.2M Tris-HCL, 0.5mM EDTA, and 0.5M sucrose), vortexed gently, and kept on ice for 30 minutes. Two more rounds of TES buffers (5ml/ liter each) were added, vortexed and iced. The samples were then spun down at 12,000rpm for 1 minute and the supernatant saved.

3. Enzyme CocE purified via column:

A Nickel-affinity column stored at 4 °C, packed with 10ml ProBond resin (50% slurry in 20% ethanol; Invitrogen), washed with sterile water and binding buffer was used to retrieve the CocE. The supernatant from the last step was run through the column. The column was then rinsed thoroughly with washing buffer, and the enzyme was eluted with 40 ml of elution buffer. The CocE enzyme was then concentrated using YM-10 Centriplus tubes (Amicon). The concentration of the enzyme was estimated using a BCA protein Assay (Pierce).

A test fraction of the purified CocE was added to phosphate-buffered saline (PBS) containing radioactively labeled cocaine. The mixture was incubated in a 37 °C water bath for 15 minutes. During the incubation, CocE hydrolyzed a portion of the cocaine yielding EME (unlabeled) and benzoic acid (labeled). The sample then went through an ion-exchange column (Supelco) to separate out the benzoic acid and remaining cocaine. A total of 2 ml of distilled water was used to elute the benzoic acid, followed by a total of 3 ml of 1N NaOH to elute the cocaine.

4. Enzymatic activity tested via hydrolysis of radiolabeled cocaine using scintillation counting :

After adding 5 ml of Ready Protein-scintillation cocktail (Beckman Coulter) to each, the samples containing benzoic acid and cocaine were analyzed using a scintillation counter. Percentage hydrolysis was determined from a plot of CPM readings of benzoic acid over CPM of cocaine plus benzoic acid.

Blood samples were obtained from volunteers and concentrated to a hematocrit of 85% - 95%. The erythrocytes were mixed with the enzyme containing buffer solution. The mixture was then sealed in a low molecular weight cutoff dialysis tube. The tube was placed in a low temperature lysis buffer. At this point, the CocE should enter the erythrocytes. The dialysis tube was then transferred to room temperature resealing buffer to close the erythrocytes.

Results/Conclusion: At present, enzyme CocE has been successfully purified and introduced into RBC. However, proper resealing of RBC requires further work. Variations in buffer composition and temperature are being used to modify the resealing conditions.

Knockout of Matrix Metalloproteinase-13 in Mice Delays Wound Healing Response

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Introduction: Matrix metalloproteinases (MMPs) are involved in the degradation and repair of the extra-cellular matrix in many biological processes such as inflammation, cancer, and angiogenesis. MMPs have been detected in healing wounds after skin injury, where they are believed to contribute to important events of the repair process. It is our hypothesis that MMP-13, also known as collagenase-3, is important to the process of wound healing.

Aim: Our objective is to study the role of MMP-13 in the process of wound healing utilizing mice that are knockout for MMP-13.

Methods: Male MMP-13 knock-out mice (n=6) and wild type controls (n=8) in the C57BL/6 background were stamped with a 6 mm skin biopsy punch to create a full thickness wound. The wounds were left open to heal and were traced for 6 consecutive days after injury. The area of the wound was measured and analyzed using a computer imaging program.

Results: Wound closure at day 2 was significantly delayed in the knock-out animals compared to the wild type mice ($P<0.05$). By day 3, there was no statistical difference between the two groups.

Conclusion: This study demonstrates that MMP-13 contributes to the early re-epithelialization during wound healing. Absence of MMP-13 may impair keratinocytes migration, inflammation, and collagen remodeling during the initial stages of wound healing.

Interleukin profiles of gingival crevicular fluid and gingival tissue from periodontally diseased and healthy samples

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Background: Chronic periodontitis is a multi-factorial disease characterized in part by increased levels of inflammatory cytokines in gingival tissues and gingival crevicular fluid (GCF). High throughput technologies such as multiplex bead array assay, and gene expression microarray have recently become available. In this study we tested GCF and gingival tissue gene expression levels from the same gingival site for a panel of cytokines that are believed to be involved in the pathogenesis of periodontitis.

Methods: Clinical measures, GCF, and gingival biopsies were collected from 14 patients with untreated periodontitis and 14 volunteers without periodontitis. Multiplex kits from Bio-Rad Laboratories (Hercules, CA, USA) were used according to the manufacturers guidelines to measure GCF levels of interleukins -1,-4,-6,-8,-10, and -1ra, using the Luminex 100 (Luminex Corporation, Austin, TX, USA). For the gingival tissue biopsies, standard Affymetrix GeneChip (Santa Clara, CA) protocols were used. RNA was extracted from the gingival tissue biopsies, labeled, and hybridized to HG-U95Av2 GeneChip arrays (Affymetrix, Santa Clara, CA). CEL files were generated for analysis. GeneChip data were analyzed using ArrayAssist (Ver 3.0, Strategene). The data was log transformed and correlation matrices were constructed to evaluate the relationships between tissue gene expression and GCF interleukin levels. ANOVA was used to compare healthy and diseased samples.

Results: Significant differences in cytokine levels were observed between healthy and diseased GCF and tissue samples. IL-1beta, IL-6, IL-8, and IL-10 levels were higher in diseased versus healthy GCF samples. IL-1 beta and IL-8, but not IL-6 were higher in diseased versus healthy gene expression samples. IL-4 and IL-1ra levels were not significantly different when healthy and diseased samples were compared for either GCF or tissue. Among all samples, a significant positive correlation was observed between IL-1 beta and both IL-6, and IL-8, for GCF and tissue. There were differences however, in both the direction and magnitude of these correlations when diseased and healthy samples were compared.

Conclusion: These results suggest that high-throughput methodologies may be useful for the exploration of biologically relevant cytokine networks in both GCF and gingival tissue. Future studies utilizing these methodologies are likely to suggest new biological pathways involved in periodontal disease pathogenesis.

Post-Doctoral Abstracts

Periodontal Microbiota and Serum Antibody Responses in Type 1 Diabetes Mellitus

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Objective: To investigate if the hyperglycemic state in type 1 diabetes affects the periodontal microbiota and the systemic antibody response to the bacterial challenge.

Methods: Forty-nine adult type 1 diabetic patients and 52 non-diabetic individuals, matched for gender and age, have been examined to date. All subjects received full-mouth assessments of plaque, bleeding on probing, pocket depth and attachment level at six sites per tooth. Demographic and diabetes-associated variables were collected. Eight subgingival plaque samples per subject were obtained and analyzed by whole genomic DNA probes and checkerboard hybridization with respect to 12 bacterial species. Serum IgG antibodies to the same bacteria were assessed by checkerboard immunoblotting. Bacterial counts and antibody titers were log transformed, and the subject-based mean bacterial burden was calculated for each microorganism. In addition, "infection ratios", i.e., ratios of antibody titer over the bacterial load for the homologous species were calculated for each subject.

Results: No significant differences were noted in demographic characteristics between the two groups, with the exception of family history of diabetes and insurance status. The diabetic group had long duration of disease (20.2±9.6 years), but good metabolic control (HgA1c 7.4±1.2%). No statistically significant differences were found between the two groups in any of the clinical periodontal parameters. Of the 12 bacterial species examined, only *Fusobacterium nucleatum* was significantly elevated in control subjects ($p=0.003$). Further, diabetic subjects were found to have a significantly lower IgG titer and infection ratio to *Campylobacter rectus* ($p=0.001$).

Conclusion: Our preliminary analyses revealed that type 1 diabetic patients are overall not distinguishable on the basis of subgingival infection patterns from non-diabetic subjects with similar periodontal status. Other host response differences must thus account for the well-established relationship between periodontal disease and diabetes.

Serum Antibodies to Periodontal Pathogens and Markers of Systemic Inflammation

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Aim: To examine the relationship between serum antibodies against *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* to plasma fibrinogen and serum C-reactive protein (CRP) in a nationally representative sample.

Methods: Data on 2973 participants aged >40 yrs from the second phase of the NHANES III study were used. IgG titers to *P. Gingivalis* and *A. Actinomycetemcomitans* were determined by ELISA. CRP was measured using a modified latex-enhanced assay. Fibrinogen was measured by recording clotting time in comparison with that of a standardized fibrinogen preparation in an automated coagulation analyzer. Periodontal disease was defined as presence of 1 site with attachment loss of 3mm and a concomitant pocket depth of 4mm. Three logistic regression models were constructed, adjusted for gender, race, education attainment, diabetes, and presence of other inflammatory conditions. Model 1 assumed no access to dental or periodontal data; model 2 assumed knowledge of number of teeth present but not of clinical periodontal status; model 3 included both dentate status and clinical periodontal data.

Results: High fibrinogen (>400 mg/dl) was unrelated to *P. gingivalis* and *A. actinomycetemcomitans* antibodies in any model, but was related to presence of periodontal disease in model 3 (OR 1.64, 95% CI 1.10-2.44). High CRP levels (>0.4 mg/dl) were related to high antibody levels to *P. gingivalis* in model 1 (OR 1.61, 95% CI 1.01- 2.38), model 2 (OR 1.68, 95% 1.13-2.50), and model 3 (OR 1.60, 95% CI 1.08 - 2.37). In this last model, >30% of the extent of attachment loss of 3mm conferred an OR of 1.43 (95% CI .10-1.87) for high CRP. Antibodies to *A. actinomycetemcomitans* were not associated with high CRP levels in any model.

Conclusion: High serum antibody levels to *P. gingivalis* are independently related to systemic inflammation.

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Tobacco Cessation and Periodontal Disease

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Background: Tobacco smoking has been established as a major risk factor for periodontal disease. The effect of smoking cessation on periodontal status has not been well documented.

Aim: Our study objectives are three-fold: First, to assess dentists' awareness of tobacco cessation practices and the extent to which these translate into clinical action; second, to document short-term clinical, microbiological, and serological effects of smoking cessation on periodontal disease; and third, to document whether knowledge of the detrimental effects of smoking on oral health has a positive impact on successful tobacco cessation. Our specific aims were: (1) to evaluate if Dentists ask their patients about tobacco use, to gauge their perception of cessation therapies, and to evaluate if they provide tobacco cessation therapy in their own practice; (2) to evaluate the short-term clinical, microbiological and serological effects of tobacco cessation on patients with periodontitis; and (3) to evaluate whether patients diagnosed with periodontal disease and counseled on the effect of tobacco use on oral health are more likely to successfully quit and remain tobacco free, when compared to patients who have not received such counseling.

Methods: Aim 1: A survey consisting of 16 questions is used to assess the aims stated in the above section. The survey is distributed to full, part-time, and voluntary faculty members of the School of Dental and Oral Surgery, Columbia University. Aim 2: A total of 30 patients who present with periodontal disease (> 3 teeth with > 6mm deep pockets and concomitant attachment loss of > 4mm) are recruited among the subjects attending the Smoking Cessation Clinic at Columbia University for their initial appointment. Clinical periodontal parameters (probing depth, bleeding on probing, and attachment loss) are recorded. Four subgingival plaque samples are obtained from the mesio-lingual surface of the most posterior tooth in each quadrant and analyzed with respect to multiple species by means of DNA-DNA hybridizations. A venous blood sample is obtained and processed for determination of IgG antibodies against the same bacteria by checkerboard immunoblotting. Tobacco usage is determined by CO breath test measurements. Subjects are randomized into an intervention and a control group. The intervention group receives standardized counseling on the oral effects of tobacco use. A comparison in clinical periodontal status, levels of periodontal pathogens, antibody titers, and CO levels between the two groups is performed at baseline and at 2 months follow-up. Aim 3: Cessation outcomes will be compared at 2 months.

Results: Review of the preliminary data suggests the following: (1) Over 80% of dentists ask their patients about their tobacco use all or most of the time. However, only around a third of dentists ask their patients about their use of cigars or chewing tobacco. (2) Less than 50% of dentists provide any form of tobacco cessation counseling/therapy. (3) Over 90% of dentists report having some training in tobacco cessation, approximately 50% through continuing education classes. (4) Approximately 65% of dentists report that they are not comfortable providing tobacco cessation therapy/counseling to their patients. (5) The majority of dentists reported that they are aware of the impact of tobacco on periodontal health; however, the level of awareness varied widely when knowledge on specific issues was tested. (6) Over 90% of dentists reported that they were aware of the effects of tobacco cessation on periodontal health and the outcome of periodontal therapy. (7)The majority of dentists would like to learn more about the effects of tobacco on periodontal health, providing tobacco cessation therapy, and how cessation affects periodontal health and therapy outcome. For aim 2 & 3, patient recruitment is ongoing and results are not yet available.

Time, Nano-Technology and the Surface Micro-Topography of Titanium Implants

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Background: Implant surface topography influences the proliferation, differentiation and extracellular matrix protein expressions of osteoblasts. Surface roughness of dental implant has been associated with the increased bone-to-implant contacts. Stangle et al. (2001) and Zinger et al (2003) showed that osteoblasts react differently on different types of pore geography. If successful osseointergrated implants can last for tens of years, osteoblasts must be connected with the implant surface and undergo biological remodeling processes. Osteoblasts will need to re-attach to the time-altered implant surface to maintain the osseointergrated status. At present, little is known about the detailed nano- and micro- structure of these implant surfaces both at baseline and as they become altered after a certain period of time.

Aim/Objective: The aim of our study is to provide the baseline and additional time point data of four commercial dental implants micro-surface including the SEM observation and AFM analysis of microtopography and average roughness of the surfaces.

Methods: Four different types of titanium implant micro-surfaces were examined. Two groups of implant samples were prepared, Group A were the daily clinical use implants which were provided by the manufacturer. Group B were the same samples but received cyclic-corrosion treatment (CCT) for different periods of time. Both group samples were evaluated by scanning electron microscope (SEM -Joel 5600) to register their characteristic micro-topography and further analysis by atomic force microscope (AFM) detailed micro-structure changes. Cyclic corrosion treatment will be used to simulate the aging process for these implants in the next part of the study.

Type of implant	Surface	Abbreviation	Manufacturer
Brånemark® smooth surface	Machined	Machined	NobelBiocare AB, Göteborg Sweden
Brånemark® TiUnite	Oxdized	TiUnite	NobelBiocare AB, Göteborg Sweden
ITI® SLA	Blasted and Etched	SLA	Straumann Ltd, Waldenburg Swizerland
3i Osseotite®	Dual acid etched	Osseotite	3i (Implant Innovation Inc), FL,USA

Results: SEM:

Machined surface: Characterized by a clearly visible, well-defined unidirectional structure, which is the result of machining. Small cavities, protrusions and torn metal particles, typical for machined metal surfaces were observed.

TiUnite surface: Viewing from direct top of the rough surface, a uniformly porous surface composed of small craters with holes at the center (Donut shape) is observed. The lateral view consisted of an irregularly rough structure with protrusions and troughs (Volcano shape).

SLA surface: Characterized with indentations or cavities of 2 different sizes. A much finer microstructure due to the effect of acid-etching with cavities of typically 0.5 to a few µm diameter is superimposed onto the rougher structure with average size of 20–90 µm resulted from preparation and particle size of the aluminum oxide sand-blasting.

Osseotite surface: Characterized by a “crystallographic” appearance and a very fine cavity like microstructure with dimensions well below the micro range.

AFM imaging demonstrated the Ra and Rrms levels of different implant surfaces.

Conclusion: Different surface characteristics of commercial available dental implants were investigated under SEM and AFM. Further studies on the surface alterations after implant aging will be presented in the later part of the study.

Fc gamma (γ) Receptor Polymorphisms and Periodontal Status: A Case-Control Follow-Up Study

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Background:
Fc γ receptors are molecules found on the surfaces of leukocytes. They serve as a link between the humoral and cellular immune system. Binding of an antibody-antigen complex to the Fc γ receptor triggers phagocytosis, antibody dependent cytotoxicity, release of inflammatory mediators, and enhancement of antigen presentation. There are three classes of Fc γ Receptors- Fc γ Receptor I, II, and III. Particular polymorphisms in Fc γ RII and RIII are associated with impaired immune function and are viewed as risk factor for certain diseases (ex. SLE, systemic lupus erythematosus). Individuals may possess either arginine(R) or histidine(H) in position 131 of Fc γ receptor IIa. This variation impacts the affinity of an IgG molecule for the Fc γ receptor with the arginine polymorphism having a lower affinity. Similarly, there are two allotypes of the Fc γ receptor IIIb termed neutrophil antigen 1 and 2 (NA1 and NA2). Cells bearing the NA2 receptor have less phagocytic ability than those with the NA1 receptor. Studies in the periodontal literature have found associations between Fc γ receptor polymorphisms and periodontal disease.

Aim: The goals of this case-control study were (i) to examine the relationship between Fc γ receptor polymorphisms and clinical periodontal status, subgingival microbiota, and serum IgG antibodies to periodontal bacteria, and (ii) to longitudinally assess whether these polymorphisms are related to long term clinical, microbiologic, or serologic status.

Methods: 132 periodontitis patients ('cases') were age and gender matched with 73 periodontally 'intact' controls, i.e., subjects with no/minimal loss of periodontal tissue support (age range 24-77 yrs). Full-mouth assessments of plaque, bleeding on probing, pocket depth, and attachment level were performed at six sites per tooth. Fourteen sub-gingival plaque samples were obtained and analyzed by species-specific whole genomic DNA probes and checkerboard hybridization with respect to 19 bacterial species. Serum IgG antibodies to periodontal microbiota were assessed by checkerboard immunoblotting. Polymorphisms in the Fc γ Receptor IIa (131H/R) and IIIb (NA1/NA2) were assessed by PCR. Cases were treated with oral hygiene instruction, extraction of non-salvageable teeth, scaling with root planning, and access periodontal surgery when appropriate. Eighty-nine cases were followed-up at four and 30 months at which points clinical, microbiologic, and immunologic data were collected. The chi-square test was used to detect differences in the distribution of the polymorphisms among cases and controls. Associations between the polymorphisms and clinical parameters, sub-gingival microbiota, or systemic antibodies were analyzed by ANOVA or the Kruskall-Wallis test, as appropriate.

Results: There were no skewed distributions of any of the studied polymorphisms among cases and controls. Overall, no statistically significant associations were found between any polymorphism and clinical periodontal status, subgingival bacterial profiles, or systemic antibodies to periodontal microbiota in either the cross-sectional or the longitudinal analysis.

Conclusion: Contrary to earlier studies, the present data failed to demonstrate a significant relationship between the Fc γ Receptor IIa (131H/R) or IIIb (NA1/NA2) polymorphism and periodontal status, assessed by clinical, microbiologic, or serologic means.

ABSTRACTS SUBMITTED AFTER PUBLICATION

Potential Role of Tih1 in the Regulation of Adipogenesis

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Background:
Obesity is an increasingly common condition in the United States today. Several recent studies indicate that a diet high in fat predisposes certain ethnic populations to obesity and Type 2 diabetes. This suggests that the obesity pandemic has a genetic as well as an environmental component, which has spurred numerous investigations focused on finding candidate obesity genes. Preliminary studies from our laboratory indicate that Tih1-/- mice administered a 45% kcal high fat diet for 8 wks gain significantly more weight and body fat as compared to wild-type mice. In addition, we observed an approximate 50% suppression of TIH mRNA expression in the perigonadal fat pads of wild-type mice fasted for 24h as compared to mice fed a standard chow diet ad libitum. We therefore hypothesized that Tih1 may be a candidate obesity gene that downregulates adipogenesis of mice in the fed state to maintain body adiposity within a narrow physiological range.

Aim 1:
To determine the level of Tih1 expression in differentiating preadipocytes.

Study 1 Methods:
3T3-L1 preadipocytes were subjected to a standard adipocyte differentiation protocol and assayed every two days for murine Tih1 expression over a 30-day time course. Briefly, cells were grown to confluence in four 6-well plates in DMEM with L-glutamine supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S) antibiotic (DM)(Day 0). Cells were allowed to grow an additional two days postconfluence at which time (Day 2) medium was changed to fresh DM supplemented with 1 mM dexamethasone (dex), 1 mg/mL insulin and 0.5 mM isobutylmethylxanthine (IBMX) for four days. Two wells not treated with hormones for the duration of the experiment served as controls. Beginning on Day 7, the medium was changed to DM supplemented with 1 mg/mL insulin for two days, and on Day 9, medium was switched back to DM with no hormones for the remainder of the experiment. Approximately every two days, two wells of cells were rinsed with phosphate-buffered saline (PBS), trypsinized, washed in DM and stored in Trizol at -80*C. After 30 days, RNA was isolated for Northern blot analysis.

Results and preliminary conclusions:
Cells began to visibly differentiate into adipocytes after Day 8. Hybridization of the resulting Northern blot with a murine Tih probe labeled with P32 revealed a steady decline in TIH mRNA expression over the 30-day time course after normalization to a G3DPH loading control (Pearson $r = -0.8084$, $p = 0.0015$). This finding further supports the inhibitory role of TIH1 in adipocyte differentiation and prompted an observational study using mouse embryonic fibroblasts (MEFs) isolated from Tih1-/- mice.

Aim 2:
To determine the rate of adipocyte differentiation in a TIH1-deficient mouse embryonic fibroblasts (MEFs). Based on the results of Study 1, we hypothesized that adipocyte differentiation in Tih1-deficient MEFs might progress more rapidly and profusely than in wild-type cells given that Tih1-/- mice show increased accumulation of adipose tissue (i.e. greater adipocyte differentiation) than in wild-type mice. However, lacking a wild-type control at the time of this experiment, this trial was strictly observational and not a direct test of this hypothesis.

Study 2 Methods:
MEFs were harvested from Tih1-deficient mice, plated into two 6-well plates, and subjected to the standard adipocyte differentiation protocol as described above, which has been used previously to differentiate MEFs. Cells were observed microscopically each day for signs of differentiation.

Results and preliminary conclusions.
After 14 days, cells showed no sign of differentiation, suggesting that either 1) Tih1 deficiency prevents adipocyte differentiation, contrary to our hypothesis; 2) MEFs require a longer exposure to the differentiation medium containing hormones than 3T3-L1 cells; 3) MEFs may require additional induction with an insulin sensitizer such as troglitizone. Future experiments will examine each of these possibilities and will compare differentiating Tih1-deficient cells to wild-type MEFs, which will serve as a control.

Overall conclusions:
In vivo and in vitro studies suggest that TIH1 has an important role in the regulation of adipose stores, specifically in the inhibition of fat accumulation. However, additional in vitro studies as described above will be necessary to compare the in vitro Tih1-deficiency system with our in vivo observations.

Stable Expression of MXA in MCF-7 cells

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Down syndrome (DS) is caused by chromosomal abnormalities, such as non-disjunction, mosaicism, and translocation. Regardless of the type of abnormality, all patients with DS have an extra copy of chromosome 21. This genetic aberration accounts for many clinical characteristics, including a decreased susceptibility to solid tumors. To identify candidate genes that are differentially expressed in DS patients, previously, scientists in our lab have used oligonucleotide microarrays to analyze global gene expression profiling in control and +21 fibroblasts. The result showed that several genes on chromosome 21 (HC21) are over-expressed. Among them, MXA gene expression had a marked non-linear increase. Western Blot was done and suggested an over expression of MXA in trisomy fibroblasts. Since methylation of DNA CpG in promoter region is a common mechanism to inactivate tumor suppressor gene, scientists in our lab carried out a Southern blot to analyze CpG-methylation in MXA promoter. Results showed that the MXA promoter is methylated in MCF7 breast carcinoma cells. MXA gene acts as a signaling target for interferon. Induction by interferon results in MXA over-expression and thus excessive production of its protein p78/MXA, a large GTPase protein. To test if interferon or induced MXA over-expression can account for the anti-tumor activity, we used retroviral gene transfer technology to infect MCF7 cells with gene constructs of MXA, MXA/âˆ†CED, and MXA/âˆ†LEU respectively. Cells were allowed to grow with or without interferon, and their growth is evaluated after 4 days. Results have shown that all these cells show reduction in growth in the presence of interferon, suggesting a global inhibitory effect of interferon on cancer cell growth. The specific effects of MXA over expression in DS patients need to be investigated further in our studies.

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