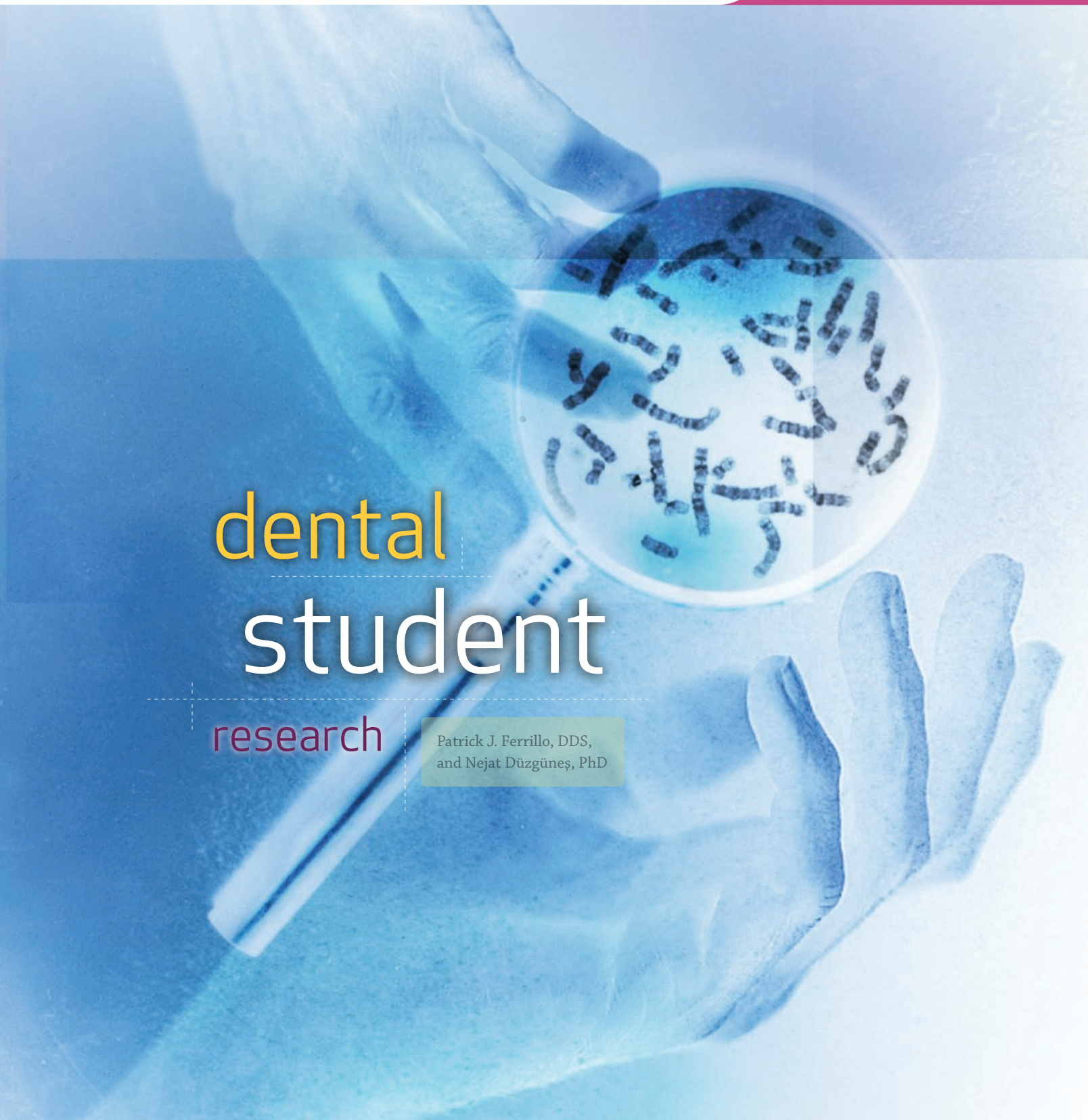
OF THE CALIFORNIA DENTAL ASSOCIATION

Journal

DECEMBER 2009



dental student research

Patrick J. Ferrillo, DDS,
and Nejat Düzgüneş, PhD

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DEPARTMENTS

FEATURES

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Rogers Hornsby and the Gecko

KERRY K. CARNEY, DDS

What could Rogers Hornsby and the Geico gecko have in common? They are both icons of industries that share an unusual commonality: antitrust law exemption.

In 1922, Rogers Hornsby played second base for the St. Louis Cardinals and had one of his best years ever. In that year, he became the only player in history to hit more than 40 home runs and bat over .400 in the same season. That was the first year that he won baseball's triple crown: batting average (.401), home runs (42) and runs batted in (152). While he was busy on the field, Major League Baseball was busy in the courtroom.

In 1922, the Supreme Court ruled in the Federal Baseball Club of Baltimore, Inc. v. National Baseball Clubs case. The issue was whether baseball was an interstate or an intrastate event. The suit had been brought by the federal league claiming that it was hindered in its ability to sign players as a result of antitrust violations by the National and American leagues. Justice Oliver Wendell Holmes wrote in the decision that though the teams traveled from state to state that the essential business occurred within the state. At the time, "there was no revenue sharing, no radio or television and no national sponsors or licensing deals."[1] Interstate commerce is subject to federal regulation; intrastate commerce falls under state regulation. As a result of this judicial ruling, MLB enjoys an exemption from antitrust laws unique in its breadth and duration.

Now how about that gecko? Before 1944, insurance had been defined as a simple contract of indemnity and was subject to state regulation. In 1944, that changed when the United States Supreme Court affirmed a lower court's decision that "the business of insurance did indeed affect interstate commerce and thus was subject to congressional authority pursuant to the Commerce Clause of the United States Constitution."[2]

Everyone likes to be the beneficiary of regulation, but no one likes to be regulated.

Everyone likes to be the beneficiary of regulation, but no one likes to be regulated. The ruling on the United States v. South-Eastern Underwriters Association, 322 U.S. 533 (1944) set the insurance industry in motion. It lobbied Congress and succeeded in convincing Pat McCarran, a senator from Nevada, and Homer Ferguson, a senator from Michigan, to sponsor a bill that would exempt the insurance industry from some federal antitrust laws. Unlike the baseball exemption that came from the judicial branch, the insurance exemption was the result of legislation.

The McCarran-Ferguson Bill was introduced within two weeks of the South-Eastern Underwriters decision. Versions of the bill passed in the Senate and the House without any hearings and little debate. When the conference committee considered the versions, the following phrase was inserted, "insurers are exempt from federal antitrust scrutiny so long as they are 'regulated by state law.'" Judicial interpretation of that phrase has transformed a temporary moratorium on federal scrutiny and prosecution into a permanent antitrust exemption. "Courts have interpreted this phrase to require only that state regulators have jurisdiction over particular conduct, regardless of whether that authority is ever exercised … As a result, anticompetitive conduct may escape both regulatory oversight and antitrust scrutiny."[3]

The McCarran-Ferguson Act of 1945 was supposed to be a stopgap to allow the insurance industry to get its house in order before it came under federal antitrust laws. This was so clearly articulated in the Congressional Record that President Roosevelt provided the following press release after signing the bill, "After a moratorium period, the antitrust laws … will be applicable in full force and effect to the business of insurance."[3]

During the past 64 years, the insurance industry has forestalled attempts to repeal or modify McCarran-Ferguson. During the aftermath of Hurricane Katrina, bill S 618 was introduced in an attempt to allow the Federal Trade Commission regulatory control and jurisdiction over areas of the business of insurance.

This bill failed in 2007. The Insurance Industry Competition Act of 2009, HR 1583, was introduced in March. The two bills are virtually identical. The ADA's stance on the bill is clear, "The fact is that dentists, their patients, and the public health are all victims of McCarran-Ferguson's negative impact on robust competition among insurance companies, and all would benefit from its repeal."[2]

The argument for maintaining the insurance exemption springs from the notion that the pooling of historical and projected loss experience helps set rates more accurately and thereby benefits consumers. It is also proposed that smaller companies may compete more effectively based on pooled information than they could based on their own limited experience. Finally, opponents of lifting the antitrust exemptions for the business of insurance argue the status quo. If it has operated well for the past 64 years, why change now?

The proponents of eliminating the insurance exemption point out there are other mechanisms that allow competitive exchanges of information by industries that do not enjoy the same exemption as the insurance industry. They also remind us that the McCarran-Ferguson Act was proposed and passed in 1945 for the benefit of the insurance industry not for the benefit of consumers. Specifically, the ADA points to the potential for unfair and biased industry calculations for usual, customary, and reasonable fees that may result in economic disadvantages for consumers.[4] The ADA concluded, "the limited antitrust exemption enjoyed by the insurance industry distorts markets to the serious detriment of both consumers and businesses."[2]

"The fact is that dentists, their patients, and the public health are all victims of McCarran-Ferguson's negative impact on robust competition among insurance companies, and all would benefit from its repeal."

In California, we have another factor to consider. In the spirit of transparency of purpose and potential conflict, there is our relationship with TDIC. The for-profit subsidiary of our association is in the insurance business. The insurance business has been, in large part, responsible for the stability we have enjoyed in our dues. We have a disincentive to invite a more onerous regulatory environment for that business.

Things are seldom as simple as they initially seem. So the next time you see the gecko advertising insurance, imagine him being tagged out at second base by Hornsby. Take a moment to wonder about their anomalous antitrust law exemptions and what that might have to do with dentistry. ■■■■

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Address comments, letters, and questions to the editor to kerry.carney@cda.org.

Letter

Kudos for 'Pipeline' Commentary, Project

I wanted to let you know that I read your article on the "Pipeline" project and really enjoyed it. When I graduated from USC in 2006, I was one of four Hispanics in our class. We were encouraged by our peers to volunteer with the Pipeline project and this is one example of why I really enjoyed USC's program — it exposed me to many mentors and community clinic environments such as the Union Rescue Mission (downtown LA's Skid Row), Children's Dental Clinic, Ayuda, Mobile Clinic, and St. John's Community Clinic.

I don't know what the Pipeline's current statistics show but, for me, I believe it worked. These programs had a tremendous impact on my desire to work with nonprofits and I now work with Northeast Valley Health Corporation, a nonprofit community clinic in Sun Valley. I feel very fulfilled and my patients are such nice people.

Thanks for your time.

RACHEL MISMAS, DDS
Los Angeles

Deborah Zemke

Private Ethics

BY DAVID W. CHAMBERS, PHD

Most moral transgressions are committed by people who consider themselves to be basically ethical. I am not talking about burglaries and auto theft — the folks who do that know they are in the wrong. Annually, they cost America a little less than $16 billion dollars. The kind of private practice I have in mind is wardrobing. A wardrober selects some nice clothes to purchase for an event, wears them, and then returns them as not fitting properly. Wardrobing adds more to the bill the rest of us pay for clothes each year than the combined cost of burglaries and car thefts.

Consider employee theft and fraud in the workplace. The most recent estimate is $600 billion dollars, enough to make almost all dental care free in America.

CONTINUES ON 847

AIM LED by Burton Medical

→

With 10 times the life and using 55 percent less energy the AIM LED examination light by Burton Medical is an efficient alternative to your normal exam light. The AIM LED examination light has a Y-shaped design, which minimizes the obstruction of light into the surgical field. This design also provides a larger and adjustable light pattern that does not cast shadows.

The AIM LED comes in four models to meet your installation needs. For more information go to burtonmedical.com

New DVD Series Now Available to Dentists

Promoting good oral health just got more entertaining.

"Toothflix," a new patient education series on DVD, features 3-D animation and clinical footage that dentists can show in their treatment rooms, waiting rooms, or elsewhere in the community to encourage regular dental visits and good oral health practices.

There are 23 segments, each about five minutes in length, on topics ranging from good eating habits and oral care to periodontal disease and implant dentistry. The series also includes four child-friendly animated films.

"As advocates for America's oral health, the ADA takes pride in providing dentists with the tools they need to enhance communication with patients," said Kathleen O'Loughlin, DMD, MPH, American Dental Association executive director.

The cost for "Toothflix" is $699 for the complete series or $349 for a topic-specific package; $1,049 for non-ADA members or $524 for a topic-specific package. Introductory discounts are available.

For more information, go to ada.org/goto/Toothflix or call 800-947-4746.

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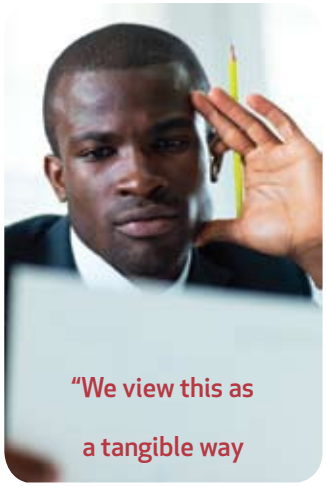

"We view this as a tangible way of rewarding and expressing appreciation to our dedicated peer reviewers."

MICHAEL G. NEWMAN, DDS

U.S. Dental Publication First to Offer C.E. Units to Reviewers

Elsevier announced recently that editors of the *Journal of Evidence-Based Dental Practice* will start offering continuing education units to its peer reviewers and recognized experts.

"We view this as a tangible way of rewarding and expressing appreciation to our dedicated peer reviewers," said Michael G. Newman, DDS, professor emeritus at the University of California, Los Angeles, School of Dentistry, and editor-in-chief of *JEBDP*, of the new policy that makes the publication the first dental journal in the country to offer continuing education units to reviewers.

"Granting CEUs also recognizes the unique role played by reviewers for *JEBDP* in developing the authoritative content included in our journal," Newman said.

Reviewers can claim up to 15 continuing education units annually for their work in developing and evaluating content for that publication, according to a press release. Credits will be awarded by the Continuing Dental Education Program at the UCLA School of Dentistry.

"We think this approach will help further expand our lineup of top-notch dental reviewers," said Bruce A. Dye, DDS, MPH, senior associate editor of *JEBDP* and a dental epidemiology officer at the U.S. Centers for Disease Control and Prevention, National Center for Health Statistics, in a previous interview.

"Elsevier, like all scientific publishers, relies on expert reviewers to uphold the quality and validity of individual articles as well as the overall integrity of the journals we publish," said Glen P. Campbell, executive vice president, GMR Journals Publishing in Elsevier's Health Sciences Division, in a press release. "With 300,000 reviewers producing a million reviews a year, we are deeply appreciative of the contributions our reviewers make to Elsevier journals on a daily basis."

ADA Supports Tobacco-Free Schools

The American Dental Association, which recently adopted a resolution supporting tobacco-free school laws and policies to prevent smoking and addiction in children, is now encouraging its state dental societies and members to partner with school officials, parents, students, and community members to create and foster campuses that are tobacco-free.

At the ADA's governance meeting, delegates voted to support laws or policies incorporating the "Guidelines for School Health Programs to Prevention Tobacco Use and Addiction," which was developed by the U.S. Centers for Disease Control and Prevention, in collaboration with tobacco-use prevention experts in the nation. According to a press release, the guidelines include tobacco-use prevention and education in kindergarten through 12th grade, and support for cessation efforts among students and school staff.

A tobacco-free school can help prevent and reduce tobacco use in the young and help them avoid cancer and heart disease, tobacco-related diseases. An estimated 438,000 people in the United States die every year from smoking or second-hand smoke exposure, and an additional 8.6 million suffer from serious illnesses stemming from their smoking habit, according to the CDC.

If Americans' smoking habits continue, it is projected that 5 million people under the age of 18 will die early of tobacco-related diseases.

Going Green

The Eco-Dentistry Association announced its GreenDOC dental office certification program, the first international standard for an eco-friendly dental practice.

The "GreenDOC Checklist: Standards for Green Dental Offices" provides eco-friendly initiatives in eight implementation categories ranging from location and waste reduction to patient care. The certification program also includes action plans, worksheets, a how-to guide, and a product guide to help EDA members achieve the program's rigorous benchmarks through a simple-to-complete process, according to a press release.

"This is a critical development for dentistry," said Fred Pockrass, DDS, cofounder of the Eco-Dentistry Association, in a press release. "By defining what constitutes a green dental office through the GreenDOC program of the EDA, we're doing for dentistry what the U.S. Green Building Council did for building with their LEED certification program for building construction: setting rigorous, consistent, and attainable standards for the greening of our industry."

Prior to the GreenDOC program, dentists who wanted to lighten their environmental impact used recycled paper or changed to more efficient light bulbs. Dentists also could tap into local Green Business Certification Programs, but they are not consistent across the country and do not always include dentistry-specific standards.

"The GreenDOC program is the only certification with specific measures related to dental processes, procedures, and administration," said Susan Beck, EDA director. "This is a tremendous benefit for dentists who want to go as 'dark green' as possible; and for patients, who now have an easily recognizable seal that verifies their dentists' claims to practicing in an environmentally friendly way."

TRUST, CONTINUED FROM 845

Insurance fraud, bogus inflation of reported claims is a hefty $25 billion that goes on the bottom line of what we pay and makes insurance companies prickly. We need to find some way to finance health care in this country and make the coverage more balanced. One place to look might be the income tax. The gap between what the IRS estimates is legitimately owed and what is paid is $350 billion each year. There cannot be that many crooks in this country: surely some of the damage must be caused by a group that includes us.

It is more than just the money involved in immoral behavior that we have chosen to accept as the normal wear and tear on society: It is the "no shame, no guilt" attitude we now live with. Plea bargaining is used to negotiate the level of guilt individuals and society find mutually convenient. Companies that pollute or sell defective and dangerous products sign consent decrees with cash settlements but no admission of wrongdoing. Many legal settlements contain a provision that the facts of the matter cannot be discovered, even in the event of repeat offenses. We want to use our own definitions of cheating.

Dan Ariely, Duke University professor of behavioral economics, who studies our little dishonesties, would summarize it this way: Most people cheat; we cheat just a little bit, but often; we are much more likely to cheat if cash is not involved; and we do not believe we are cheaters. One of Ariely's experiments involved placing a six-pack of Coke and a plate with six $1 dollar bills in the refrigerators in the common rooms used by students at MIT. The Cokes were gone in a few days, but the money remained untouched.

The nub:

1. There is something wrong with the Golden Rule if it is taken to mean we get to decide what is right as long as we allow others to cheat too.
2. It may be easier to change the system than the people in it, which, after all, includes us.
3. Nobody who benefits significantly from the conspiracy to get just a little extra should be counted on as an ally in trying to reform the system.

David W. Chambers, PhD, is professor of dental education, Arthur A. Dugoni School of Dentistry, San Francisco, and editor of the Journal of the American College of Dentists.

"In any moment of decision, the best thing you can do is the right thing. The worst thing you can do is nothing."

THEODORE ROOSEVELT

UPCOMING MEETINGS

2010

April 11–17	United States Dental Tennis Association, Amelia Island Plantation, Fla., dentaltennis.org.
April 26–28	National Oral Health Conference, St. Louis, Mo., nationaloralhealthconference.com.
May 13–16	*CDA Presents The Art and Science of Dentistry*, Anaheim, 800-CDA-SMILE (232-7645), cda.org.
Sept. 9–11	*CDA Presents The Art and Science of Dentistry*, San Francisco, 800-CDA-SMILE (232-7645), cda.org.
Nov. 7–13	United States Dental Tennis Association, Grand Wailea, Hawaii, dentaltennis.org.

To have an event included on this list of nonprofit association continuing education meetings, please send the information to Upcoming Meetings, CDA Journal, 1201 K St., 16th Floor, Sacramento, CA 95814 or fax the information to 916-554-5962.

Study Results: Listerine Antiseptic Destroys Potentially Harmful Germs

A new clinical study of people with mild to moderate gingivitis showed that Listerine Antiseptic reduces the amount of microorganisms that pass through the mouth to the bloodstream.

"The findings from this study serve as compelling evidence to further the theory that plaque and gingivitis germs that migrate from the mouth to the bloodstream may contribute to broader health problems such as diabetes and heart disease," said Daniel H. Fine, DMD, chair of the Department of Oral Biology at the University of Medicine and Dentistry of New Jersey and lead investigator of the study, which was funded by Johnson & Johnson Consumer & Personal Products Worldwide, Division of Johnson & Johnson.

"While additional research in this area is necessary, this study undoubtedly proves that Listerine Antiseptic kills the germs in your mouth that cause plaque and gingivitis before they have a chance to travel to the bloodstream," said Fine.

The randomized, controlled, crossover study conducted at the University of Medicine and Dentistry of New Jersey found that participants using Listerine Antiseptic as directed experienced a reduction in aerobic and anaerobic bacteria in the blood stream (67.3 percent and 70.3 percent, respectively), according to a press release. Twenty-two individuals with a confirmed diagnosis of mild to moderate gingivitis participated in the study. Each patient had blood drawn to establish baseline bacteremia levels. Subjects took three bites of an apple to induce bacteremia. Blood was drawn about two minutes after the first bite to determine the bacteremia level.

The subjects then were provided with an assigned mouthrinse (control or Listerine Antiseptic), a toothbrush, commercial fluoride toothpaste, and a diary to log their brushing and rinsing habits. Patients were instructed to rinse for 30 seconds, twice daily for two weeks with 20 mL of their assigned mouth rinse. Subjects returned on Day 15 to the clinic to have a new sample taken to determine the level of bacteremia in their blood post-treatment. Following a wash-out period, the study protocol was repeated with participants using the alternate mouthrinse.

Groundbreaking Education Project Launched

The American Dental Education Association and the American Dental Association launched its education project that gave remote access to select live-patient continuing education courses held last month at the ADA Annual Session in Hawaii.

The program, developed by the ADA and ADEA, and sponsored by Henry Schein Dental, gave predoctoral and postdoctoral dental students and faculty in North America password-protected access to six, live-patient courses each year in the ADA's "Education in the Round" learning format. This interactive format gave students the opportunity to pose real-time questions to the presenters at the 2009 annual Session. Similar courses will be featured at the 2010 and 2011 sessions.

"The ADA is excited to work with ADEA on this unique, state-of-the-art opportunity," said Kathleen O'Loughlin, DMD, MPH, executive director of the ADA. "This collaboration enables dental students — our future ADA members — to participate in high-quality education on relevant topics and clinical procedures that may not be easily available due to geographical or financial barriers. Effective collaborations such as this leverage our individual association strengths to create greater value for our current and future members."

Richard W. Valachovic, DMD, MPH, ADEA executive director, agreed. "The ADA's Education in the Round program provides an innovative live learning opportunity many ADEA members would otherwise miss. We are excited to offer this unique educational experience for the first time."

"This collaboration enables dental students — our future ADA members — to participate in high-quality education on relevant topics and clinical procedures that may not be easily available due to geographical or financial barriers."

KATHLEEN O'LOUGHLIN, DMD, MPH
ADA EXECUTIVE DIRECTOR

In previous years, the ADA offered 20 Education in the Round courses during which more than 2,750 dentists had the opportunity to explore topics ranging from esthetic crown lengthening and root coverage grafting to endodontic treatment and esthetic restorations. In postcourse evaluations, attendees of these courses rated the EIR learning environment and effectiveness of teaching methods higher than the average for standard lectures.

This year's ground-breaking addition to the program raised the bar. "The live Web feed allowed a student to not only watch the procedure, but to get involved by asking the presenters questions in real time," said Steve Carstensen, DDS, 2009 program chair. "We were excited to provide them with this annual session exclusive experience, another example of 150 years of continuous improvement of our meeting."

While the live courses offered in the Education in the Round learning format are ADA Continuing Education Recognition Program-approved opportunities for dental professionals, the live Web feed does not qualify for educational credit for dental students.

Honors

The Academy of General Dentistry awarded nearly two dozen dentists from California who were presented fellowship and mastership awards this past summer in Baltimore.

A fellowship award is earned after completing 500 hours of continuing dental education, passing a comprehensive written exam, and five years of continuous membership in the AGD. This year's awardees and CDA members are: **Muna Almoayad, DDS, FAGD,** Madera; **Bruce Bosler, DDS, FAGD,** Vacaville; **Justin T. Chapman, DDS, FAGD,** Merced; **Karen A. Giannotti, DDS, FAGD,** Fremont; **Cheryl D. Goldasich, DDS, FAGD,** Torrance; **Sandhya Hegde, DDS, FAGD,** Bonita; **Katherine A. Kucera, DDS, FAGD,** Ceres; **Sireesha Penumetcha, DDS, FAGD,** Elk Grove; **Reed T. Puelicher, DDS, FAGD,** Sacramento; **Maryam Saleh, DDS, FAGD,** Roseville; **John K. Tong, DDS, FAGD,** Cupertino; **Christopher F. Wong, DDS, FAGD,** Fresno; and **Inwoo Yi, DDS, FAGD,** La Cañada Flintridge.

To earn the mastership designation, these awardees took 1,100 hours of continuing dental education in the 16 disciplines of dentistry, including 400 hours dedicated to practicing hands-on skills, and techniques. This year's recipients and CDA members are **Craig R. Brandon, DDS, MAGD,** San Diego; **Aria Irvani, DDS, MAGD,** Foothill Ranch; and **Lilian Ong, DDS, MAGD,** West Covina.

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Implant Failure: Attempting Procedures Beyond Skill Level

TAIBA SOLAIMAN

Once a quarter, the *Journal* features a TDIC risk management case study, which provides analysis and practical advice on a variety of issues related to liability risks.

Authored by TDIC risk management analysts, each article presents a case overview and real-life outcome, and reviews learning points and tips everyone can apply to their practice.

A general dentist's treatment failure leads to lawsuit and loss of friendship.

In January 2000, Jeff DeMagio presented to Dr. Francis, a general dentist and family friend, to evaluate tooth No. 8 after it was traumatized in a weekend skiing accident. After reviewing the radiographs, Dr. Francis determined the prognosis of tooth No. 8 was hopeless and recommended extraction and replacement with an implant. He discussed the treatment plan with the patient. It consisted of using a flipper immediately following the extraction and restoring the tooth with a Maryland bridge, spanning teeth Nos. 7-9, during the healing phase. Once the implant was integrated and restored, Dr. Francis would place crowns on teeth Nos. 7 and 9.

He informed the patient that due to the root being ankylosed, the extraction required removal of some of the underlying bony structure and osseous grafting. Additionally, he explained the risks, benefits, and alternatives of the treatment. Mr. DeMagio accepted the treatment plan and signed an informed consent form. Dr. Francis proceeded to take impressions to fabricate the flipper. Mr. DeMagio appointed to return for an extraction and grafting two days later.

Following the extraction, Dr. Francis added osseous grafting material to the surgical site and delivered the flipper. He reviewed postoperative instructions and prescribed antibiotics and Vicodin for pain management. Mr. DeMagio returned to the office two weeks later for suture removal and bridge preparation. The site was healing within normal limits. He reappointed in two weeks for the bridge delivery. Dr. Francis recommended assessing the area for an implant in four to six months.

In June, Mr. DeMagio presented to Dr. Francis for a routine recall appointment. During this appointment, Dr. Francis noted gingival recession in the area of tooth No. 8 and added composite filling to the bridge for esthetic purposes. He also noted bone resorption in the area of tooth No. 8; however, he did not consider the level of bone loss to be a contraindication for implant placement. Dr. Francis instructed the patient to return in two months for re-evaluation.

A month later, Mr. DeMagio returned to the office complaining of discomfort and pain. After performing an evaluation and taking radiographs, Dr. Francis noticed bone loss around tooth No. 9. He explained to the patient the tooth was not salvageable and recommended replacing it with an additional implant. Mr. DeMagio agreed to the change in his treatment plan.

A month later, Mr. DeMagio reappointed to extract tooth No. 9, osseous grafting and implant placement in the area of teeth Nos. 8 and 9. The procedure appeared successful. Dr. Francis reviewed postoperative instructions, prescribed antibiotics, and pain medication. Two weeks later, Mr. DeMagio returned for suture removal. Healing appeared to be within normal limits and he did not report any complications or complaints.

During a routine follow-up visit in September, Dr. Francis noted localized infection at the implant sites and prescribed erythromycin. The patient was reappointed in two weeks for reevaluation. A week later, Mr. DeMagio presented without an appointment complaining of discomfort and redness under his lip, near the implants. Dr. Francis diagnosed a fungal infection and prescribed an antifungal ointment. A week later, Mr. DeMagio returned to the office complaining of continued discomfort and constant headaches. Dr. Francis noted that the redness still existed. He decided to surgically expose the area and noticed more bone resorption. He decided to remove the implants allowing the area to heal.

During the next four years, Dr. Francis attempted to repeat the implant placement in the area of teeth Nos. 8 and 9 two additional times, reusing the original implants both times. All attempts were unsuccessful.

He was upset when Dr. Francis recommended removing the implants; however, due to their friendship, he refrained from expressing his concerns.

Due to repeated implant failures and the prolonged period of treatment, Mr. DeMagio went to a new general dentist, who referred him to an oral and maxillofacial surgeon. After examining Mr. DeMagio, the OMS concluded his implants were failing. Additionally, due to the severe bone loss, teeth Nos. 7 and 10 needed to be extracted. Feeling angry and betrayed, Mr. DeMagio contacted Dr. Francis's office for a copy of his records.

Dr. Francis received a letter from Mr. DeMagio's attorney stating he was suing for repeated implant failure, prolonged period of treatment, lack of proper referral for a second opinion, and negligence. He was also seeking compensation for past and future hospital and medical expenses, future loss of earnings, and attorney fees. Dr. Francis contacted TDIC who assigned a claims representative to handle the case through litigation.

During Discovery

During his deposition, Dr. Francis admitted he reused the original implants twice. Dr. Francis claimed he cleaned the implants through a "passivation" process, which made them perfectly "fine" to reuse. Dr. Francis explained this process cleans the implant adding an oxide layer to prevent corrosion and promote integration. The defense attorney contacted multiple oral and maxillofacial surgeons regarding the passivation process. While each was familiar with the process, none had heard of this procedure as an acceptable protocol for implant reuse. Manufacturers used the "passivation" process during implant fabrication.

Mr. DeMagio claimed that following the second implant placement, he experienced inflammation, burning sensation, and discomfort similar to what he experienced after the initial attempt. He was upset when Dr. Francis recommended removing the implants; however, due to their friendship, he refrained from expressing his concerns, assuming Dr. Francis knew what he was doing. Dr. Francis also stated he assumed his friend would tell him if he had any treatment concerns.

The plaintiff's attorney asked Dr. Francis how he handled patients with persistent infections. Dr. Francis stated he handled them on a case-by-case basis using his clinical judgment and taking patient's preferences into consideration.

When questioned about his failure to refer, Dr. Francis stated he did what he thought was proper. The patient was comfortable with his clinical assessment and did not ask for a referral. Dr. Francis assumed Mr. DeMagio was happy although he appeared to be frustrated with the length of the treatment.

At the conclusion of the discovery process, Dr. Francis' defense attorney urged him to settle the case. He believed the lack of expert support, the reuse of implants, the failed treatment and the pain and suffering Mr. DeMagio experienced would compel a jury to decide Dr. Francis performed treatment beyond his skill level resulting in substandard care. The plaintiff's original demand was more than $500,000. The case settled for an undisclosed amount.

Learning Points

Tunnel Vision

It is easy to become fixated on one particular area of treatment and not pay attention to the entire treatment plan. Tunnel vision can result in repeating the same procedure to achieve the anticipated treatment outcome. Dr. Francis was responsive to Mr. DeMagio's complaints; however, he was so focused on the implant procedures he forgot about the patient's overall care.

The original treatment plan included crowning tooth No.7, however, no documentation existed to support whether or not Dr. Francis addressed it. The patient went through three failed implant attempts in five years. Dr. Francis should have referred to a specialist after the initial implant failure and together they could have worked toward a resolution. When unforeseen treatment outcomes occur, the dentist must assess the situation for the best possible patient care. If a procedure continues to fail, the dentist should evaluate his or her skill level regarding the procedure and refer to a specialist rather than redoing a procedure multiple times.

Treating Friends

Treat all patients equally regardless of friendship. Although at times the urge is to "bend the rules" for a friend, it should never affect sound clinical judgment. For example, the dentist may recommend treatment that is convenient or inexpensive instead of referring to a specialist. Alternatively, a friend may be concerned over offending the dentist if treatment recommendations are questioned. Friends should fill out the same forms as other patients: data, health history, treatment plan, and specific procedure consents.

Referrals are required when a patient's treatment needs are beyond the skill level or training and experience of the treating dentist.

Mr. DeMagio did not voice his concerns to Dr. Francis because of the friendship. His lack of communication led Dr. Francis to believe Mr. DeMagio was content with his dental treatment. Dr. Francis admitted because they were friends, he assumed Mr. DeMagio would have voiced his concerns. Dr. Francis should have addressed treatment complications when they began. This could have led to open communication resulting in a specialist referral and a positive result.

Skill Level

Referrals are required when a patient's treatment needs are beyond the skill level or training and experience of the treating dentist. A referral can take place anytime, including after an initial exam or evaluation, when a patient's advanced condition is noted, or during the course of ongoing treatment.

Deciding whether to attempt completion or to refer a difficult case can be the difference between a happy patient and a litigious situation. Know your limitations and competency level. Referring to colleagues who are trained to handle difficult cases demonstrates professionalism and genuine care for the patient's well-being. Treating a patient beyond your skill level or training could expose you to allegations of negligence. General dentists performing specialty treatments are held to the specialist's standard of care. Ensure you are able to perform the diagnosed treatment and are comfortable doing so prior to beginning treatment.

In this case, Dr. Francis's failure to recognize when treatment progressed beyond his skill level contributed to the patient's decision to pursue legal action. As a general dentist, he is licensed to perform grafting and implant placement. However, specialists commonly perform these procedures. The grafting procedure proceeded without incident; however, the implant procedure did not. Instead of continuing to re-treat the area, Dr. Francis should have referred to a specialist for assistance. The general rule is that if performing a specialty procedure, the generalist should be able to predict the potential and therefore prepared for complications (i.e., bone loss or infection), timely recognize the complications to minimize injury, and treat or refer the patient to a specialist for treatment of the complication. ■■■■

Taiba Solaiman is a risk management analyst with TDIC.

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ARTHUR A. DUGONI SCHOOL OF DENTISTRY

Our school is built on the three tenets of education, service, and research. Dugoni provides an environment where students interested in research are nurtured. Much as the school is oriented toward educating clinicians and serving patients and the community, we also contribute to the advancement of knowledge in both the clinical and biomedical sciences.

Gene Delivery to Oral Cancer Cells by Nonviral Vectors: Why Some Cells Are Resistant to Transfection

CASSIDY LAVORINI-DOYLE, DDS; SENAIT GEBREMEDHIN, BS; KRYSTYNA KONOPKA, PHD, MD; AND NEJAT DÜZGÜNEŞ, PHD

ABSTRACT The use of cationic lipid-DNA complexes (lipoplexes) for gene therapy of oral squamous cell carcinoma may be limited by the resistance of some cell types to transfection. Using fluorescence microscopy, fluorometry and luciferase luminescence, the authors examined whether the variability arises from the intracellular fate of lipoplexes. In transfection-resistant cells, the efficacy-limiting step appeared to be lipoplex processing beyond binding and internalization, possibly including DNA escape into the cytoplasm and transport into the nucleus.

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Oral squamous cell carcinoma, OSCC, is the most common form of oral cancer and develops from the stratified squamous epithelium lining of the mouth and pharynx.[1] Since OSCC constitutes about 90 percent of oral malignancies, its diagnosis and treatment is at the root of the oral cancer problem. Cancers of the lips, tongue, floor of the mouth, palate, gingival, alveolar mucosa, and oropharynx constitute about 30,000 new cases a year.[1]

Oral and pharyngeal cancers cause about 10,000 deaths every year. The five-year relative survival rate in the United States was 53 percent in the period 1974-1976; this increased to only 56 percent in the period 1992-1997.[1] Traditional cancer therapies, including surgery, radiotherapy, and chemotherapy

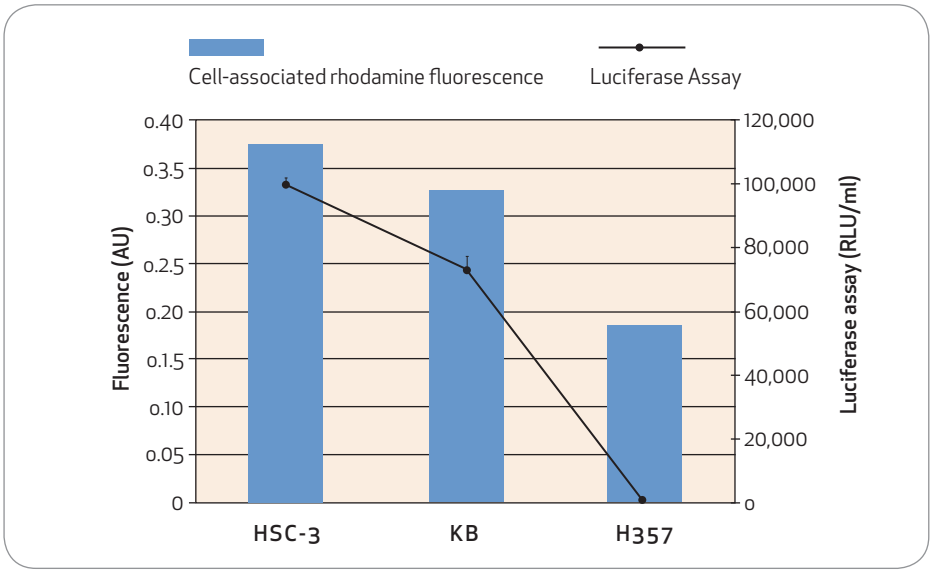

FIGURE 1. Lipoplex uptake vs. luciferase expression.

have not improved the survival time of patients with SCC of the head and neck.

Gene therapy is a field of experimental therapeutics involving the delivery of genetic material into cells to correct an existing abnormality or to confer a new function.[2-6] With gene therapy, defective or missing genes can be replaced, or genes that can convert inert prodrugs into active drugs can be introduced into diseased cells. Cancer cells that survive by expressing a mutant p53 protein may be treated with the gene for wild-type p53, thereby inducing apoptosis.

"Suicide" gene therapy involves the introduction of genes encoding enzymes that are able to convert prodrugs into cytotoxic drugs, and includes the delivery of the HSV thymidine kinase (HSV-tk) gene followed by ganciclovir administration and the delivery of the cytosine deaminase gene followed by treatment with 5-fluorocytosine.[6]

One of the major problems in gene therapy is the ability to deliver a sufficient amount of genes to target cells. The two main gene carrier systems that are being used are viral vectors and nonviral delivery systems. Viral vectors usually have a high efficiency of gene delivery, but the present use has shown serious problems with immunogenicity, toxicity, risk of introducing tumorigenic mutations, and generating active viral particles through recombination.[5,7] Because of these concerns, a primary objective is to develop nonviral delivery systems that are efficient, nontoxic, and with relatively high specificity toward the target cell.

Synthetic cationic lipids and the complexes they form with DNA ("lipoplexes") are among the most widely used nonviral vectors. A significant disadvantage of lipoplexes is their lower transfection efficiency, compared to viral vectors. Recent data from the authors' laboratory, have shown, however, that some lipoplexes are considerably more efficient than adenoviral vectors in gene delivery to oral cancer cells (unpublished data).

Among the strategies employed to enhance lipoplex-mediated transgene expression in mammalian cells are 1) the attachment of targeting ligands to promote cell binding and receptor-mediated endocytosis; 2) the use of synthetic membrane-active peptides to induce the destabilization of endosomes and release of the genetic material into the cytoplasm; 3) the use of cationic polypeptides to condense DNA, thereby enhancing serum resistance and reducing the size of the complexes; 4) the association of albumin with cationic liposomes to confer serum resistance and enhance gene transfer; and 5) the complexation or covelent attachment of nuclear localization peptides to mediate the nuclear entry of the plasmid.[4]

One of the current problems in applying these methods to enhance the transfection efficiency of lipoplexes is the variability in efficiency from one OSCC cell type to the next. To develop a widely applicable gene therapeutic, it is important to understand the mechanisms by which transfection occurs and investigate the different stages in transfection where the different cell types show variation. The authors have reported previously on the transfection efficacy of the cationic liposomal reagent Metafectene in both human and murine OSCC cells.[8,9] The authors have investigated the uptake of Metafectene lipoplexes by HSC-3, KB and H357 cells that differ widely in transfection activity, using fluorescent liposomes prepared in collaboration with the manufacturer of the liposomes.

Materials and Methods

Cells

Human KB cells derived from epidermoid carcinoma of the pharynx (the KB cell line, originally thought to be derived from an epidermal carcinoma of the mouth, was subsequently found to have been established via HeLa cell contamination), and HSC-3 and H357 cells derived from SCCs of the tongue were maintained in 5 percent CO_2 at 37 degrees Celsius in DME medium supplemented with 10 percent fetal bovine serum, 4 mM L-glutamine, 100 µ/ml penicillin and 100 µg/ml streptomycin (all obtained from the University of California, San Francisco, Cell Culture Facility, in San Francisco).

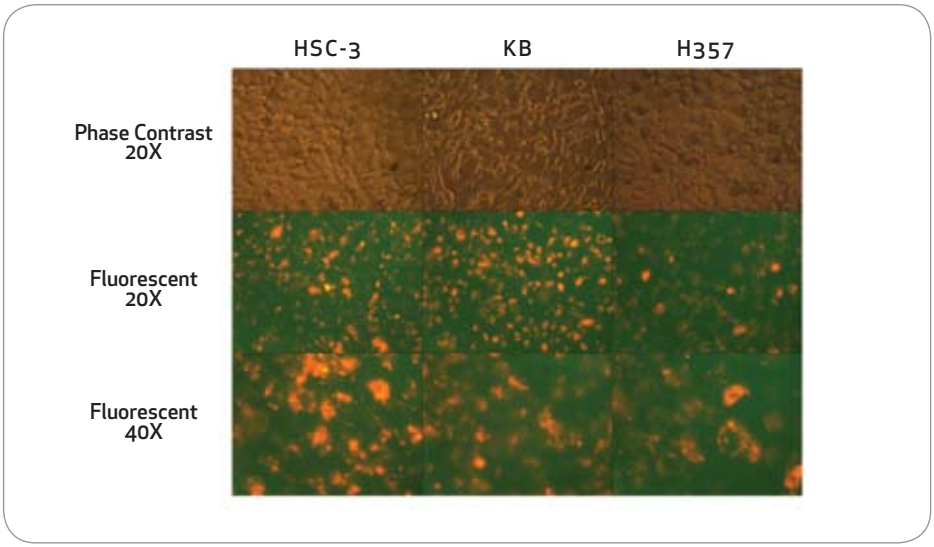

FIGURE 2. Fluorescence and phase contrast microscopy of HSC-3, KB, and H357 cells viewed 48 hours after the onset of transfection. Fluorescence images were acquired with 20x and 40x objectives, and the phase-contrast images correspond to the 20x fluorescence fields.

Plasmid

The plasmid pCMV.Luc (VR 1216) encoding luciferase under the control of cytomegalovirus (CMV) promoter was a gift of Dr. Philip Felgner.

Transfection Reagent

Fluo-Metafectene, a polycationic liposomal transfection reagent, containing polyamino-lipid, dioleoyl phosphatidylethanolamine and 2 mole percent 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl), was synthesized in collaboration with Biontex GmbH (Planegg/Martinsried, Germany).

Transfection Conditions

Cells were seeded in 48-well culture plates the day before transfection at a density of $2x10^5$ cells/well and used at approximately 85 percent confluence. One microgram of pCMV.Luc was complexed with 2 µl/well of Fluo-Metafectene.

Transfection Activity

Cells were washed with phosphate-buffered saline (PBS) 48 hours after transfection, solubilized with Cell Culture Lysis Reagent (Promega, Madison, Wis.), and centrifuged to discard debris. Luciferase expression was assayed using the Luciferase Assay System (Promega) and a Turner Designs TD-20/20 (Sunnyvale, Calif.) luminometer. Luciferase activity was expressed as relative light units (RLU)/ml cell lysate.

Quantification of Fluorescence

Rhodamine fluorescence in cell lysates was measured using a Perkin-Elmer LS 50B Luminescence Spectrometer and then plotted against a calibration curve using different amounts of Fluo-Metafectene.

Fluorescence Microscopy

The cells were plated in 2- or 4-well Lab-Tek coverslip chambers. Following incubation with lipoplexes for four or 48 hours, the medium was removed to eliminate any unbound lipoplexes, and phosphate-buffered saline (PBS) was added to the chambers. The cells were observed under a Nikon Diaphot epi-fluorescence microscope, equipped with a Q-Imaging digital camera connected to an Apple G3 computer.

Results

The levels of luciferase activity in HSC-3, KB and H357 cells following transfection with Fluo-Metafectene are shown in **FIGURE 1**. While HSC-3 and KB cells expressed relatively high levels of luciferase, H357 cells displayed very low transfection activity.

The uptake of lipoplexes by the cells was measured by fluorometry (**FIGURE 1**). The Fluo-Metafectene µl equivalents that were bound to or internalized by HSC-3 and KB cells was 0.375, 0.325, respectively, while H357 cells took up lipoplexes to a considerably lower extent (0.185).

Fluorescence microscopy of HSC-3 and KB cells indicated a high level of cell-associated rhodamine fluorescence (**FIGURE 2**). Nevertheless, substantial cell-associated fluorescence was observed in H357 cells. The lipoplex particles were attached to the cell surface and also internalized in endocytotic vesicles.

Discussion

Successful gene delivery via lipoplexes requires several conditions to be met: 1) condensation of the DNA into the lipoplex structure and its protection from degradation by intracellular and extracellular nucleases, 2) adhesion of the DNA-lipid complex to the cell membrane, 3) lipoplex internalization via endocytosis, 4) destabilization of the endocytotic or endosomal membrane by the lipoplex, 5) escape of the DNA from the endocytotic compartment, 6) transport of DNA into the nucleus, 7) dissociation of the DNA from the cationic lipid component, and 8) successful gene expression.[4,5]

Resistance to transfection in a particular cell line may be the result of a block in any of these processes. Widespread applicability of gene therapy in the treatment of OSCC will require the ability to deliver therapeutic genes effectively in many, if not all, types of oral cancer cells. In this study, the authors focused on one transfection-resistant cell line (H357) and two readily transfectable cell lines,

and examined the uptake and intracellular fate of lipoplexes carrying the gene encoding luciferase as a reporter gene.

Cell-associated fluorescence in H357 cells was lower than in HSC-3 and KB cells, although luciferase activity in these cells was only 0.15 percent that in HSC-3 cells. Fluorescent lipoplexes localized in endocytotic vesicles. Lipoplex uptake by H357 cells was about half as much as that in HSC-3 cells, but this did not translate into significant luciferase expression.

These observations indicate that the uptake and endocytosis of lipoplexes by OSCC cells do not necessarily translate into gene delivery and expression. Thus, the efficacy-limiting steps in gene transfer in transfection-resistant OSCC cell lines involve lipoplex processing beyond endocytosis. These additional steps include destabilization of the endosomal membrane, escape of the DNA into the cytoplasm, and the transport of DNA into the nucleus.

Low transfection activity (reporter gene levels) or efficiency (percentage of cells transfected) obtained with a nonviral vector does not necessarily mean that eventual gene therapy applications will be ineffective. For example, although less than 5 percent of H357 cells were transfected with the cationic reagent Fugene, 80 percent cytotoxicity could be achieved with the use of the HSV-tk/ganciclovir suicide gene therapy system, possibly via the bysander effect.[10]

To obtain more precise information on the intracellular localization of lipoplexes in different OSCC cells, the authors currently are using confocal laser-scanning microscopy, together with fluorescent markers for lysosomes and nuclei. It will be of interest to examine the intracellular fate of fluorescently labeled DNA, in addition to the cationic liposomes. ■■■■

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LOMA LINDA UNIVERSITY

The goal of student research at Loma Linda University School of Dentistry is to help raise and maintain the quality of dental research and education by encouraging participation of dental, dental hygiene, graduate students, and dental school faculty in research to improve oral health care.

In addition, research study encourages critical thinking skills and fosters a better understanding of the scientific literature essential to the practice of evidence-based dentistry.

Digital Multimedia Instruction Enhances Teaching Oral and Maxillofacial Suturing

J.M. WEAVER, BS; MEI LU, DDS, MS, PHD; K.L. MCCLOSKEY, BS; E.S. HERNDON, BS; AND W. TANAKA, DDS

ABSTRACT OBJECTIVE: To develop digital multimedia instruction on intraoral suturing. **METHODS:** A DVD was developed to describe instruments, materials, and techniques. Two groups of dental students were asked to close an incision in a simulated model. One used written materials only and another used additional DVD. The performance was evaluated using 10 grading criteria. **RESULT:** Students who used the DVD performed better than students who did not. **CONCLUSION:** This DVD could be used widely in teaching dental students.

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The search for innovative teaching methods that allows information, skills, and concepts to be communicated more clearly and efficiently is one that seems to be rising in importance at all types of educational institutions around the world.[1] In the realm of dental education, there is a particularly interesting juxtaposition of "book" or basic science learning and practical or clinical procedure-based learning.[2] Of course the two are eventually used together so that the practitioner can deliver treatment to a patient based on researched and proven scientific principles.

As the dentist's training progresses, there is a progression from learning the theory of basic science concepts to the application and learning of clinical procedures based on those previously

learned principles.[3] These two themes in education demand different learning styles and thus the student may respond better to varied methods of teaching.

A classic example would be instruction of a clinical procedure by means of textbook or lecture alone as tools for initial learning. Without completely discounting the value of these teaching methods, however, to the novice clinician, additional resources that demonstrate clinical procedures in a visual and audio form is a crucial part of an efficient and innovative teaching approach. Nowhere is there a more perfect example than in the area of teaching suturing techniques and placement.

The aim of this study was to develop an educational resource that provides a more comprehensive instruction on basic intraoral suturing techniques and to test its effectiveness as a teaching tool.

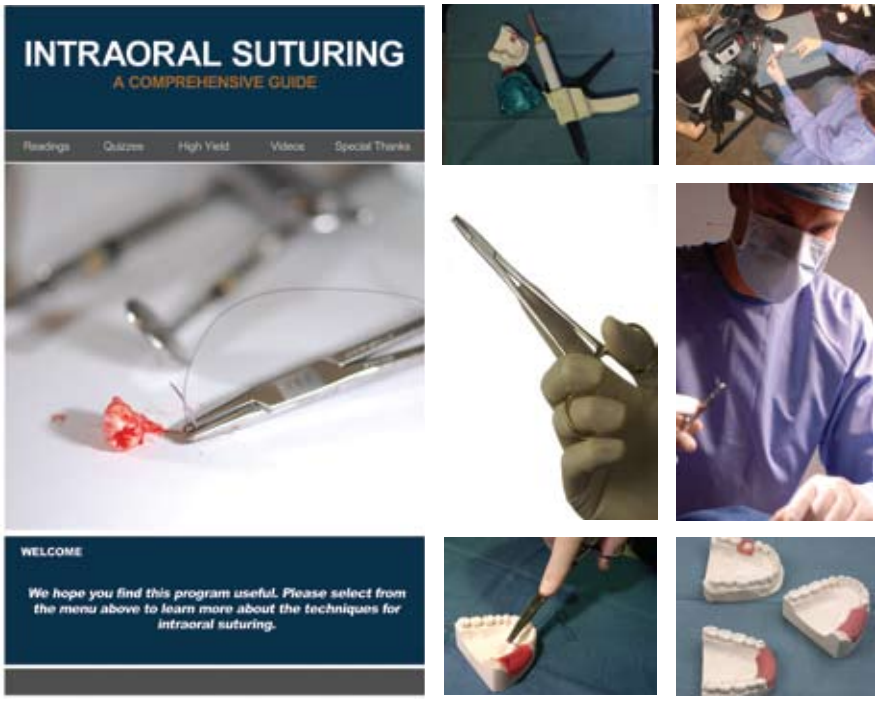

FIGURE 1. Intraoral suturing technique in DVD.

Methods and Materials

Fabricating the Model in the Laboratory

Impressions of typodont arches were made and stone models were poured with white improved stone. Selected teeth were ground off the stone models to simulate missing teeth, and PVS (polyvinylsiloxane) material was added to simulate gingival tissue. This resulted in casts of the mandibular arch with various edentulous areas covered in simulated gingival tissue.

Creating the DVD

Using the fabricated models, incisions were made in the PVS gingiva and video sequences were filmed of various suturing techniques. Suturing techniques filmed included 1) instrument tie surgeon's knot, 2) simple interrupted, 3) figure 8, 4) continuous locking, and 5) continuous nonlocking. Principles of suturing outlined in the videos were taken from the textbook *Contemporary Oral and Maxillofacial Surgery*.[4] Suturing techniques in the DVD were supervised by attending faculty who served as tutors for students. Videos were filmed in a laboratory setting. Audio segments were recorded separately and synchronized to fit with video.

Photos of instruments and materials commonly used in suturing were included, along with concise literature reviews outlining the different techniques and materials used when suturing. The literature reviews, photos, and video sequences were organized using html and css programming languages and then published onto a DVD (**FIGURE 1**).

Evaluation of the Product

To evaluate the effect of the DVD program, 12 students who met the following criteria were randomly selected. They had completed all lectures offered in the Loma Linda School of Dentistry curriculum on the subject of suturing, but they didn't perform sutures on patients. Once selected, the students were randomly split into two groups of six students each. One group was asked to prepare using only written materials from previous classes. Another group used the written materials and, in addition, was given the DVD as a teaching tool. Both groups were given clinical scenarios on the model (**FIGURE 1, BOTTOM RIGHT**) before suturing and were asked to perform two suturing techniques, the simple interrupted and continuous locking sutures.

Upon completion, sutures were determined to be either clinically acceptable or not clinically acceptable. The student's suturing performance was evaluated based on 10 grading criteria by oral surgery attending faculty who served as tutors for students (**TABLE 1**).

Results

The two groups of students demonstrated different levels of competence when placing sutures. All students of the group with the additional DVD achieved clinically acceptable sutures in both simple interrupted sutures and continuous locking sutures, while only four students in the non-DVD group were able to complete a clinically accept-

TABLE 1

Grading Criteria for Suturing Techniques

1.	Suture placed from posterior to anterior
2.	Sutures placed from unattached to attached gingiva
3.	Sutures placed 2-3 mm from edges of the wound
4.	Appropriate spacing between sutures
5.	Correct knot tying (2-1-1)
6.	Adequate suture tension
7.	Knot position to the facial
8.	Correct hand position when using the instruments
9.	2-3 mm suture tail lengths
10.	Needle placed in the safe position

able simple interrupted suture, and none of those students were able to finish the continuous locking suture (FIGURE 2).

In a simple interrupted suture, all students of the group with additional DVD met nine of 10 grading criteria while the majority of students of the group without a DVD did not meet nine of the 10 grading criteria (FIGURE 3). In continuous locking sutures, the majority of students of the group with additional DVD met nine of the 10 grading criteria while only a few students of the group without DVD met nine of the 10 grading criteria (FIGURE 4).

The last grading criteria — putting the needle in a safe position — was omitted by all students of both groups in both suture techniques.

Discussion

Sutures are an essential prerequisite for optimal healing following procedures, such as flap manipulation during periodontal therapy, hard- and soft-tissue regeneration, excision of pathologic tissue, extraction sites, and or caring for oral-antral fistulas.[4] Sutures function to reapproximate tissue, giving a fresh wound sufficient strength to withstand normal stresses placed on the tissue during the initial stages of healing. A correct suturing technique, using the appropriate thread type, diameter, and tension, allows for optimum healing by primary intention.[5]

Incorrectly placed sutures may not only delay healing but actually result in more damage to the tissue. If the edges of a wound are not closely approximated and supported by sutures, blood clots may form between the bone and flap which delays the healing of a wound.[5]

A proper suturing technique is an important skill for any surgeon. To maintain a high standard of care, the dental student or practicing dentist must be familiar with and be able to use acceptable suturing techniques. There are many suturing techniques that are commonly utilized in surgical procedures in the oral cavity. Among them, simple interrupted, continuous locking or nonlocking, and figure 8 are the most common suturing techniques, which were selected in this study DVD as a teaching tool.

Suturing techniques in the DVD were supervised by attending faculty. By working closely with attending faculty who serve as tutors for students, the common mistakes from a novice student were able to be addressed in the DVD. This helped guide the design of the DVD so that it could deliver information in a clear and concise manner to novice dental students.

This study DVD contains photos of instruments, materials, and concise literature reviews outlining the different techniques used in intraoral sutures. The authors' result after testing this teaching tool on third-year dental students demonstrated that this DVD is full of didactic knowledge and useful for intraoral suturing.

FIGURE 2. Clinical scenario.

In both the simple interrupted and continuous locking scenarios, the students who used the DVD, in addition to the textbook and lecture materials, performed the procedure more correctly with better adherence to the 10 basic criteria chosen to evaluate each student's performance (FIGURES 3 AND 4). The 10 criteria tested the student's aptitude for each of the various principles that are important to correct suture technique. Each was chosen to allow the grading to be clear-cut and objective.

The dental student's curriculum demands they master many tactile and procedural based skills in addition to didactic-based learning. The dental student, on a daily basis, switches back and forth between these two very different learning styles.[3] The DVD was created from fabricated models in the laboratory that allowed clinical application to be integrated into videos. With this practical teaching tool, dental students are able to do some preclinical review between textbook and clinical procedures on a patient.

By utilizing technology in teaching other than traditional textbook, practical concepts, as well as didactic concepts, can be delivered and meshed so that the novice clinician feels more confident and attains a higher level of initial clinical performance.[6] Also, dental students may go months with-

out using their suturing skills. The benefit of this DVD may not only increase initial learning but also serve as an aid when reviewing a clinical technique. This allows the student to deliver the best possible treatment to the patient and do it in a highly confident manner. Therefore, this study DVD in the dental school setting will benefit the students, teachers, and patients.

In addition, this DVD also applies to general practice dentists who may need to train staff in assisting with suture placement. This resource could make the training more efficient by decreasing the learning time and allowing the practitioner and assistant to work as a good team. This benefits the patient and the efficiency of the office.

Conclusion

Students who used the additional DVD were more likely to complete clinically acceptable sutures than the students who used textbooks and lecture notes only. This study DVD benefits in initial learning and also facilitates a long-term understanding of the basic suture principles. This educational resource could be used widely in training dental students in a school setting.

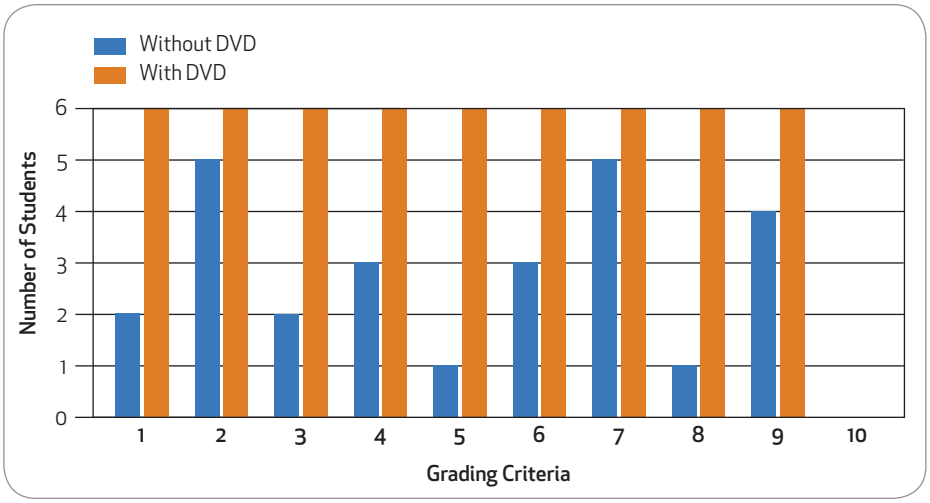

FIGURE 3. Grading of student performance on the simple interrupted suture.

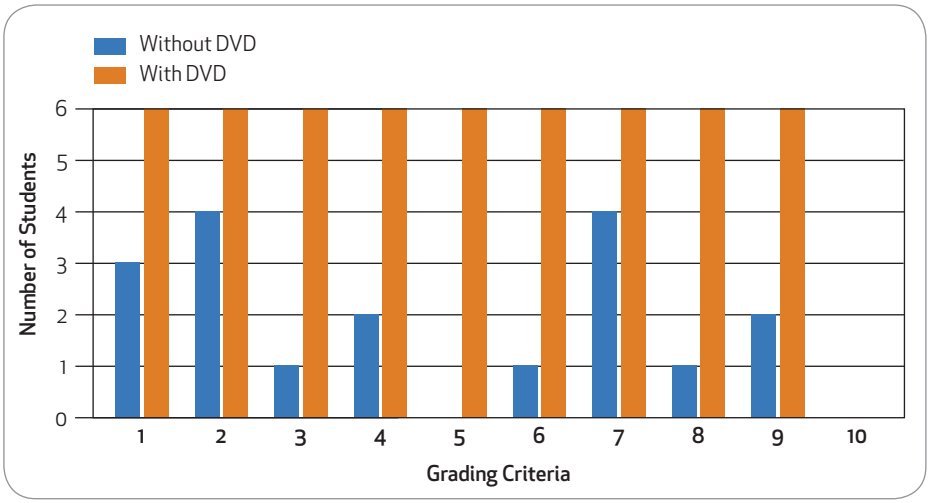

FIGURE 4. Grading of student performance on the continuous locking suture.

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UNIVERSITY OF CALIFORNIA, LOS ANGELES

Successful mentorship creates astute clinicians who are best positioned to advance themselves, their profession, their home institutions, and the wider community through evidence-based practice and therapeutic innovation. Research mentoring should be based upon a foundation of institution values that are shared by students and faculty, supported by appropriate resources, measured through quantitative and qualitative metrics, and rewarded when success is achieved.

Research participation offers an opportunity to work one on one with a professional who is interested in the student and the topic of study. Hands-on research involvement is an experiential, collaborative experience that engages the student and faculty member in an exploration of a subject area, similar to clinical dental education but unlike a traditional didactic learning experience. Research experience is associated with a higher likelihood of acceptance to advanced training programs. Why choose a research experience? It is exciting, fun, rewarding, and informative of a variety of nontraditional dental careers.

Dental Enamel: Genes Define Biomechanics

RICK J. RAUTH, DDS, MS; KAREN S. POTTER, DDS; AMANDA Y.-W. NGAN, DDS, MS; DEEMA M. SAAD, BS; RANA MEHR, BS; VIVIAN Q. LUONG, BS; VERNA L. SCHUETTER; VETEA G. MIKLUS, DDS, MS; PEIPEI CHANG, DDS; MICHAEL L. PAINE, BDS, PHD; RODRIGO S. LACRUZ, PHD; MALCOLM L. SNEAD, DDS, PHD; AND SHANE N. WHITE, BDENTSC, MS, MA, PHD

ABSTRACT Regulated gene expression assembles an extracellular proteinaceous matrix to control biomineralization and the resultant biomechanical function of tooth enamel. The importance of the dominant enamel matrix protein, amelogenin (Amel); a minor transiently expressed protein, dentin sialoprotein (Dsp); an electrogenic sodium bicarbonate cotransporter (NBCe1); the timely removal of the proteinaceous matrix by a serine protease, Kallikrein-4 (Klk4); and the late-stage expression of Amelotin (Amtn) on enamel biomechanical function were demonstrated and measured using mouse models.

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Shane N. White, BDENTSC, MS, MA, PHD, is a faculty member and student research mentor at the University of California, Los Angeles, School of Dentistry.

Tooth enamel is a composite bioceramic composed largely of carbonated hydroxyapatite, Hap, and very small amounts of protein remnants. Human enamel rarely undergoes catastrophic mechanical failure despite a lifetime of repeated masticatory, parafunctional, and occasional impact loading in a hostile wet environment. In fact, tooth enamel is the most durable of all tissues surviving for millennia as long as it is not exposed to carious acid attack. Mature enamel reflects the unique molecular and cellular activities that are owed to its formation during development. The gene activity and protein expression profiles of ectodermal-derived ameloblasts produce a protective mineralized enamel that protects soft dentin from the ravages of wear and erosion in a functional tooth (**FIGURE 1**).

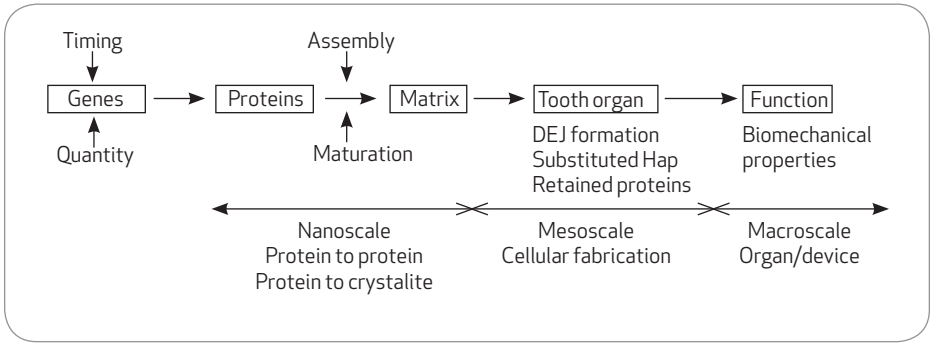

FIGURE 1. The hierarchy of tooth formation from genes to functioning teeth

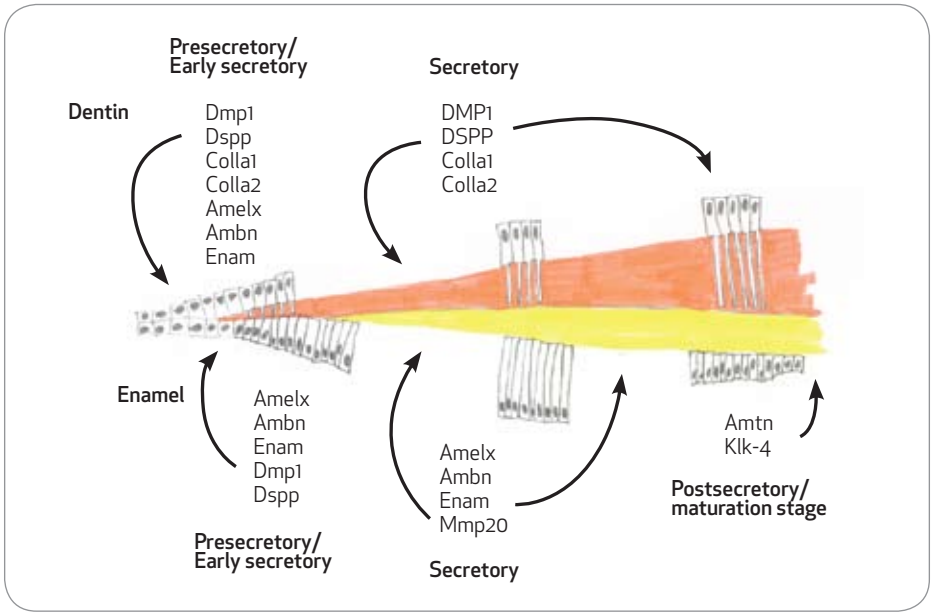

FIGURE 2. Gene expression through enamel and dentin formation. Schematic of proteins secreted into the dentin matrix by odontoblasts and the enamel matrix by ameloblasts at the various stages of formation. With the exception of collagen, odontoblasts, and ameloblasts, expression profiles for the secreted proteins is very similar in the very early secretory stages. During the secretory stages both odontoblasts and ameloblasts gene expression profiles are entirely distinct as the enamel matures, amelotin, and kallikrein-4 are upregulated.

The dentinoenamel junction, DEJ, durably unites dissimilar hard brittle enamel and tough flexible dentin. In contrast to artificial bonds between restorations and dentin, the DEJ rarely fails, except when it is affected by inherited disorders.

Ameloblasts and odontoblasts are lined up face-to-face, or basement membrane-to-basement membrane, in the developing tooth bud. After a series of interactions, the odontoblasts migrate away from the DEJ toward the future pulp, whereas the ameloblasts migrate outward toward eventual tooth surface (**FIGURE 2**). Through programmed gene expression, the migratory ameloblasts leave a trail of secreted proteins in their wake. The selection of expressed genes, their timing during development and their relative abundance is under genetic control. This mixture of proteins undergoes self-assembly to form an enamel extracellular organic matrix, or scaffold, that controls the initiation, rate of growth, and habit of the inorganic carbonated hydroxyapatite crystallites that form tooth enamel.[1,2] During maturation, almost all of the organic protein matrix is broken down and removed, to be almost completely replaced by inorganic crystallites. Hence, despite an embryonic origin in protein, mature enamel is a hard, wear-resistant, and surprisingly tough composite-ceramic biomaterial.

Enamel has a hierarchical organization (**FIGURE 3**). At the nanoscale to macromolecular scale, proteins interact to form a matrix that guide the habit of the individual crystallites to produce long, thin crystallites. Each ameloblast secretes a cylinder-like volume of matrix in which individual crystallites are bundled to form a rod (prism). Organized continuities, and discontinuities, among the matrix cylinders secreted by adjacent ameloblasts create a complex 3-D continuum through interrod crystallites.[3] At the microscale or cellular scale, the matrix is defined by the secretory products or zones of influence of individual ameloblasts as they migrate from the DEJ to the outer surface, dance, and weave a complex 3-D web of enamel rods and interrod. At the macroscale or organ level, the tooth is defined by the biomechanical function of its structural components.

Purpose

The purpose of this article is to review selected advances, highlighting dental student research contributions, in the understanding of the genetic, molecular, and structural aspects of enamel biology. Through examples, a model of enamel formation is presented that relates gene expression to the assembly of an extracellular protein matrix that in turn controls biomineralization, structural hierarchy, and biomechanical function of tooth enamel.

Experimental Strategy

The authors' strategy has been to introduce known mutations to enamel genes, using transgenic (gain of function) and gene knock-in (gene engineering) ani-

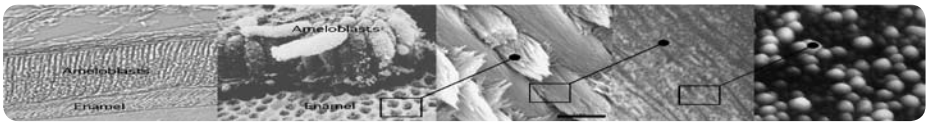

FIGURE 3. Enamel formation. From left to right: A layer of columnar ameloblasts lay down their protein matrix, which becomes mineralized to form enamel. Each individual ameloblast produces a cylindrical matrix field that becomes mineralized as a rod within a "honeycomb"-like continuum of interrod; the complex migratory vectors of ameloblasts weave rods into a complex fibrous network. Each rod is composed of multitudes of individual crystallites organized by amelogenin nanospheres; amelogenin proteins spontaneously form nanospheres in physiologic conditions.

mal approaches that serve to selectively perturb normal enamel development and structure so that a localizable and quantifiable functional defect can be measured and related to a specific genetic change.

The authors have proposed a long-term paradigm in which these defects will eventually be shown to mirror, or closely resemble, naturally occurring defects in humans that result from genetic defects, such as, amelogenesis imperfecta and acquired human enamel defects, such as fluorosis.[1,4,5] Unlike prior studies of enamel defects performed on a few isolated individuals of unknown genetic etiology, or other studies using general toxicity to create defects, the studies described herein are genetic in their origins and thus are repeatable because the single genetic cause is known and consistent. Additionally, the data from the authors' studies will be useful in providing a mechanistic understanding of enamel that will permit the future engineering of replacement enamels.[6]

Mice and Men

Mouse models have several major advantages. The mouse genome is known and is not unduly dissimilar from our own. Mice also mature quickly, in a matter of weeks. They are small and relatively easy to house and maintain safely. The authors have defined the structural and biomechanical differences between murine and human incisor enamel.[7] These differences are a reflection of evolutionary pressures from differences in biomechanical function.

The authors have defined the biomechanical zones of immature, mature, and degrading enamel in the mouse incisor so

as to permit reproducible results. Because murine incisors are rather small, the authors used a variety of micro and nanomechanical tests, as well as an array of imaging techniques to measure the effects of known genetic changes on structure and function.[3]

The Continuously Erupting Mouse Incisor

Because rodent incisors erupt continuously, the whole life cycle of a tooth from stem cells, through maturation, to wear and dissolution can conveniently be found and studied in a single mouse incisor. For enamel and dentin, a midzone was located where data points could be predictably measured. Both dentin and enamel incisal zones had decreased hardness, attributable to oral dissolution. Likewise, apical areas displayed immature and incomplete mineralization, with dentin maturing markedly more slowly than enamel. Knowledge of these zones and the rate of tooth eruption facilitate the use of the continuously erupting mouse incisor as an experimental model to study the impact of both genetic and environmental factors on tooth formation and function.

Amelogenin Self-Assembles Into an Organized Scaffold

Amelogenin (Amel) is the dominant protein in the developing enamel scaffold matrix.[8] Amelogenin proteins self-assemble into nanospheres that guide the mineralization of long thin Hap crystallites in an ordered subparallel arrangement, that is the crystallites are almost parallel, but diverging or converging slightly. Two defined domains (A and B) within amelogenin appear essential for this self-assembly according to in vitro model systems. Transgenic animals were used to test the hypothesis that these self-assembly domains operated in vivo.[9]

Transgenic animals bearing either a domain-A-deleted or domain-B-deleted amelogenin transgene expressed the altered amelogenin exclusively in ameloblasts. This altered amelogenin participated in the formation an organic enamel extracellular matrix and, in turn, in enamel mineralization. At the nanoscale level, the forming matrix adjacent to the secretory face of the ameloblast showed alteration in the size of the amelogenin nanospheres for both transgenic animal lines and the resultant enamel exhibited inferior mechanical properties.[10]

Amelogenin Isoforms and Function

In mice and humans, alternative splicing creates a dozen or more amelogenin isoforms of different lengths, but their potential functions and/or purpose of this complexity remains unknown. A knock-in genetic approach was used to engineer enamel so that it would be produced from a single amelogenin protein isoform. This knock-in approach reduced amelogenin protein isoform complexity by one order of magnitude, resulting in enamel fabricated with only the most common M180 amelogenin protein. This resulted in an enamel that was significantly harder, or more wear resistant, but also significantly less tough, or less fracture resistant, than wild-type enamel.[11]

Microstructural organization was indistinguishable between wild-type and transgenic enamel and dentin. Thus, despite a marked reduction in the enamel matrix protein complexity, these substantial design changes produced an engineered enamel with unaltered architecture and acceptable material properties. This finding has profound implications for the future fabrication of replacement enamels. The trade-off, or balance between the opposing mechanical properties of hardness or wear resistance and toughness or fracture resistance provided insights to the importance of the subtle organization in packing of hydroxyapatite crystallites to optimize species-specific biomechanical functions.

Dentin Sialophosphpoprotein, the DEJ, and Enamel Hardness

Rather surprisingly a dentin gene, dentin sialophosphphoprotein (Dspp), is transiently expressed in early-stage, secretory ameloblasts at the time of DEJ formation. This is consistent with Dspp having a role in producing specialized first-formed harder enamel adjacent to the DEJ.[12] The expression of "odontoblast" proteins such as DSPP and DMP1, at the time the DEJ is formed, appears to be products of both odontoblasts and ameloblasts. This is well-described in the literature; however, expression of these products (DSPP and DMP1) is short-lived in ameloblasts but continues in odontoblasts until dentine formation is complete.[13,14] Crack diffusion by branching and dissipation within this specialized first-formed enamel close to the DEJ prevents catastrophic interfacial damage and gross tooth failure.[15] Once Dspp is secreted, it is subjected to proteolytic cleavage that results in three distinct proteins referred to as dentin sialoprotein (Dsp), dentin phosphoprotein (Dpp), and a recently described protein resident between these two termed dentin glycoprotein.[16]

The authors' purpose was to investigate the contribution of Dsp and Dpp to enamel function. Transgenic mice were engineered to overexpress either Dsp or Dpp throughout the duration enamel formation instead of just very transiently in the first-formed enamel at the DEJ.[17] Dsp and Dpp contributions to enamel formation greatly differed. The inclusion of Dsp in bulk enamel significantly and uniformly increased enamel hardness by approximately 20 percent, whereas the inclusion of Dpp softened and weakened bulk enamel.[18] This result was consistent with the supposed role of Dsp in the biomechanical function of the DEJ and resulted in an enamel that was biomechanically superior to wild-type enamel being engineered.

Ion Transport: NBCe1 and Enamel Mineralization

After the formation of a competent proteinaceous scaffold to guide mineralization, the ameloblasts secrete the mineral component of enamel in an environment conducive to crystallite precipition. Electrogenic sodium bicarbonate cotransporters (e.g., NBCe1), are found widely in the renal proximal tubule, pancreas, eye, heart, brain, and in developing teeth. Some patients with certain inborn kidney diseases also have enamel abnormalities.

In ameloblasts, NBCe1 helps to maintain the pH buffering system required during mineralization of hydroxyapatite. Protons are released during enamel mineralization when apatite crystals grow from precursor forms of phosphate. The release of protons during hydroxyapatite formation requires a pH buffering system to prevent acidosis and enamel demineralization. By comparing the dentition of NBCe1-null animals to their wild-type littermates, the authors demonstrated that the NBCe1-null mutants produced enamel that was too soft to even measure, and dentin that was significantly softened.[19]

Dentin may have been less affected than enamel because it is simply less mineralized than enamel or because other ion transport mechanisms may exist. Heterozygous NBCe1 mice produced enamel and dentin of comparable hardness to their wild-type control littermates suggesting that a single copy of NBCe1 may adequately fulfill the biological task or that other ion transporters may be able to compensate in part.

Kallikrein-4 and Removal of the Amelogenin Scaffold

Mature functional enamel contains only very minimal amounts of protein. These minimal remnants may act as a plasticizing agent, allowing some slippage of enamel rods over one another to relieve stresses and provide some

toughening.[3,20] However, almost all of the enamel scaffold must be removed before mineralization can be completed. Amelogenin, the dominant enamel protein is a known substrate of the Kallikrein-4 (Klk4) proteolytic enzyme in vitro. Kallikrein-4 is a secreted serine protease found primarily in prostate and enamel. Amelogenin is secreted by ameloblasts through early and midenamel formation.

In contrast, Klk4 is normally secreted from these same ameloblasts late in enamel formation. Transgenic mice overexpressing Klk4 in a spatiotemporal pattern simultaneous to endogenous amelogenin expression displayed a soft hypomineralized enamel.[21-23] Hence, disruptions to the normal expression pattern of kallikrein-4 in the developing tooth organ clearly impacted the function of the enamel matrix, enamel mineralization, and the integrity of the dentin-enamel junction through premature removal of amelogenin before mineralization has sufficiently matured. Timing is everything.

Ameloblast Maturation, Senescence, and Amelotin

Amelotin (Amtn) is a recently identified enamel matrix protein with expression apparently limited to late-stage enamel formation. The outer layer of last-formed enamel is believed to be softer than bulk enamel and to lack its regular organization, either by design or by default. To date, the role of amelotin in amelogenesis remains unclear. Amelotin-overexpressing animals were generated, in which amelotin gene expression was extended throughout enamel formation.[24]

The spatiotemporal distribution of the other enamel matrix proteins was not significantly affected, suggesting that the overexpression of amelotin does not exert any inhibitory effect on them. However, the resultant enamel was extremely soft and of a more amorphous structure, consistent with amelotin's likely role in producing the final thin layer of softer outer surface enamel. The mechanism by which amelotin acts remains unknown, but it may play a function in diverse activities such as biomineralization, Ca++ transport, pH balance, and/or cellular differentiation/dedifferentiation.

Clinical Impact

Understanding the genetic and molecular events that regulate the formation of enamel will result in improvements in the prevention, diagnosis, and treatment of heritable and acquired diseases of enamel, including caries, as well as insights that allow the engineering of replacement enamels for therapeutic interventions. ■■■■

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Continuing Education Courses

Listed are C.E. courses offered by California's dental schools, local dental societies, ethnic dental societies and specialty organizations, from January through June 2011. For more information, please contact the course provider.

TOPIC	DATE	LECTURER(S)	LOCATION	COST	UNITS
ALPHA OMEGA DENTAL FRATERNITY — LOS ANGELES CHAPTER					**310-398-9626**
Botox Application for TMD	Feb. 23	David Dana, DDS	Los Angeles	$85	3
Access to Care	April 27	Alan Felsenfeld, DDS	Los Angeles	$85	3
ARTHUR A. DUGONI SCHOOL OF DENTISTRY					**415-929-6486**
The Essentials of Aesthetics	Jan. 29	Howard Chi, DMD, MA; Maritza Mende, DMD	Stockton	$395	7
Infection Control and the California Dental Practice Act	Feb. 25	Eve Cuny, BA, MS; Bruce Peltier, PhD, MBA	San Francisco	$125	4
Atraumatic Extraction, Ridge Preservation and Crown Lengthening Study Club	Feb. 25, 26; March 25, 26	Gretchen Bruce, DDS, MBA; William Lundergan, DDS, MA; Frank Martinez, DDS; Anders Nattestad, DDS, PhD	San Francisco	$2,195	28
CALIFORNIA ASSOCIATION OF ORAL AND MAXILLOFACIAL SURGEONS					**916-783-1332**
CALAOMS 2011 January Anesthesia Meeting	Jan. 15-16	O. Ross Beirne, DMD, PhD; Jacob Haiavy, DDS, MD, FACS	Monterey	TBD	9
CALAOMS 11th Annual Meeting	May 21-22	Jason B. Cope, DDS, PhD; Alan L. Felsenfeld, DDS	Rancho Palos Verdes	TBD	TBD
CALIFORNIA DENTAL HYGIENISTS' ASSOCIATION					**818-500-8217**
Systemic Perio and Osteoporosis/Osteopenia: Clinical Implications in Periodontal Therapy	May 13	Joan Otomo-Corgel, DDS	Anaheim	$130	5
CALIFORNIA DENTAL SOCIETY OF ANESTHESIOLOGY					**626-287-1185**
Peri-Anesthetic Complications in the Dental Office	March 30-31	Robert C. Bosack, DDS	Irvine	$350	8
CALIFORNIA SOCIETY OF PEDIATRIC DENTISTRY					**831-625-2773**
CE Online	Continuous	Various	cspd.org	Varies	Varies
CSPD/WSPD 36th Annual Meeting	April 7-10	Multiple	San Francisco	$500 Reg Fee	16

TOPIC	DATE	LECTURER(S)	LOCATION	COST	UNITS
CONTRA COSTA DENTAL SOCIETY					**925-932-8662**
Pediatric Dentistry: Are We Having Fun Yet?	Feb. 11	Marvin Berman, DDS	Walnut Creek	$195	7
California Dental Practice Act and Infection Control	March 4	LaDonna Drury-Klein, RDA	Concord	$80	4
Virtues of Profitable Dentistry	April 8	Howard Farran, DDS	Walnut Creek	$195	7
FRESNO-MADERA DENTAL FOUNDATION					**559-224-8747**
Local Anesthesia Update	Jan. 7	Alan Budenz, MS, DDS, MBA	Fresno	$140 Member/ $170 Non-Member/ $90 Auxiliary	7
Update in Periodontics	Feb. 4	Gary Armitage, DDS	Fresno	$140 Member/ $170 Non-Member/ $90 Auxiliary	7
OSHA, Infection Control and Dental Law	March 11	William Carpenter, DDS; Bruce Peltier, PhD, MBA	Fresno	$190 Member/ $220 Non-Member/ $105 Auxiliary	7
Restorative Update 2011	April 15	Parag Kachalia, DDS	Fresno	$140 Member/ $170 Non-Member/ $90 Auxiliary	7
Glass Ionamers – Direct Restorative Science Behind the Product	May 6	Joe Oxman	Fresno	$140 Member/ $170 Non-Member/ $90 Auxiliary	7
Management of the Extraction Site	June 3	Bach Le, DDS, MD, FICD	Fresno	$140 Member/ $170 Non-Member/ $90 Auxiliary	7
FRESNO-MADERA DENTAL SOCIETY					**559-438-7284**
Dental Practice Act, Infection Control, OSHA and HIPAA Updates	Jan. 21	Stanley Surabian, DDS, JD; Leslie Canham, CDA, RDA	Fresno	$150 Member/ $100 Staff/$75 RDA and Hygiene Students	8
HERMAN OSTROW SCHOOL OF DENTISTRY OF USC CONTINUES ON NEXT PAGE					**213-821-2127**
Implant CPR! Successful Management of Prosthetic Implant Complications (Module I)	Jan. 21	Harel Simon, DMD	Los Angeles	$275 Dentist/ $175 Auxiliary	7
Implant CPR! Successful Management of Prosthetic Implant Complications (Modules I and II)	Jan. 21-22	Harel Simon, DMD; Faculty	Los Angeles	$1,570 Dentist/ $995 Auxiliary	14
Implant CPR! Successful Management of Prosthetic Implant Complications (Modules I and II)	Jan. 21-22	Harel Simon, DMD; Faculty	Los Angeles	$1,450 Dentist/ $955 Auxiliary	14
Implant CPR! Successful Management of Prosthetic Implant Complications (Module II)	Jan. 22	Harel Simon, DMD; Faculty	Los Angeles	$1,345 Dentist/ $895 Auxiliary	7
USC Periodontal and Implant Symposium: Hands-On Cadaver Workshop I – Maxillary Sinus Augmentation	Jan. 27	Homayoun Zadeh, DDS, PhD; Pascal Valentini, DDS	Los Angeles	$1,795	7

TOPIC	DATE	LECTURER(S)	LOCATION	COST	UNITS
HERMAN OSTROW SCHOOL OF DENTISTRY OF USC CONTINUES ON NEXT PAGE					213-821-2127
The USC 36th Annual International Periodontal and Implant Symposium	Jan. 28-29	Homayoun Zadeh, DDS, PhD; International Speakers	Los Angeles	$495 Dentist/ $325 Auxiliary	14
The USC 36th Annual International Periodontal and Implant Symposium: Dental Hygiene Forum	Jan. 29	Homayoun Zadeh, DDS, PhD; International Speakers	Los Angeles	$155	7
USC Periodontal and Implant Symposium: Hands-On and Cadaver Workshop II — Alveolar Ridge Augmentation	Jan. 30	Homayoun Zadeh, DDS, PhD; Sascha Jovanovic, DDS, MS	Los Angeles	$1,795	7
Mastering Molar Endodontics	Feb. 4-5	Ilan Rotstein, DDS; Faculty	Los Angeles	$1,485	14
Oral Surgery for the General Practitioner	Feb. 5	Bach Le, DDS, MD, FICD; Faculty	Los Angeles	$285 Dentist/ $185 Auxiliary	7
Porcelain Veneers: Optimizing Results Using Supra-Gingival Principles and Understanding Adhesion and Occlusion	Feb. 11	Jose-Luis Ruiz, DDS, FAGD; Edward Lynch, PhD, Lond, MA, BDentSc, TCD	Los Angeles	$215 Dentist/ $145 Auxiliary	7
Complications Associated With Implant Treatment (Las Vegas)	Feb. 12	Bach Le, DDS, MD, FICD; Baldwin Marchack, DDS, MBA	Las Vegas, NV	$345 Dentist/ $225 Auxiliary	7
Emerging Diseases, Infection Control and California Dental Practice Act	Feb. 12	Joyce Galligan, RN, DDS; Gerald Vale, DDS, JD	Los Angeles	$190 Dentist/ $145 Auxiliary	6
Basic Protocols in Implant Surgery and Restoration	Feb. 24-27	Homayoun Zadeh, DDS, PhD; Faculty	Los Angeles	$2,695 Dentist/ $1,195 Auxiliary	22
Chronic Orofacial, Orodental and Headache Pains for the Dentist	Feb. 25-26	Glenn Clark, DDS, MS; Faculty	Los Angeles	$495 Dentist/ $315 Auxiliary	14
USC Ruth Ragland 25th Dental Hygiene Symposium	March 5	Diane Melrose, RDH, BS; National Speakers	Los Angeles	$185	7
Applied Hypnosis: Treat Pain, TMD and Other Dental Conditions	March 5-6	Peter Stone, DDS; Ronald Kaminishi, DDS	Los Angeles	$595	14
Implant Therapy in the Esthetic Zone	March 11-13	Homayoun Zadeh, DDS, PhD; Faculty	Los Angeles	$1,995 Dentist/ $995 Auxiliary	20
Esthetic Full-Mouth Implant Reconstruction: From Treatment Planning to Fixed Restoration (Module I)	March 18	Harel Simon, DMD	Los Angeles	$275 Dentist/ $175 Auxiliary	7
Interdisciplinary Dentistry to Promote Success in Clinical Practice	March 18	Ilan Rotstein, DDS; Faculty	Los Angeles	$75 Delta Dental Dentist/ $215 Non-Delta Dental Dentist	7
Esthetic Full-Mouth Implant Reconstruction: From Treatment Planning to Fixed Restoration (Module I, II, and III)	March 18-20	Harel Simon, DMD	Los Angeles	$1,945 Dentist/ $1,595 Auxiliary	21
Esthetic Full-Mouth Implant Reconstruction: From Treatment Planning to Fixed Restoration (Module II)	March 19	Harel Simon, DMD	Los Angeles	$275 Dentist/ $175 Auxiliary	7
Esthetic Full-Mouth Implant Reconstruction: From Treatment Planning to Fixed Restoration (Module III)	March 20	Harel Simon, DMD; Faculty	Los Angeles	$1,795	7

TOPIC	DATE	LECTURER(S)	LOCATION	COST	UNITS
HERMAN OSTROW SCHOOL OF DENTISTRY OF USC CONTINUED					213-821-2127
Mastering Bone Grafting for Esthetic Implant Site Development — Lecture and Hands-On Workshop (Module I)	March 25	Bach Le, DDS, MD, FICD; Faculty	Los Angeles	$1,195 Dentist/ $595 Auxiliary	7
Mastering Bone Grafting for Esthetic Implant Site Development — Cadaver Workshop (Module II)	March 26	Bach Le, DDS, MD, FICD; Faculty	Los Angeles	$1,765 Dentist/ $995 Auxiliary	7
Obstructive Sleep Apnea, Snoring and Dental Advancement	April 1-2	Glenn Clark, DDS, MS; Faculty	Los Angeles	$495 Dentist/ $315 Auxiliary	14
Advanced Implant Restoration	April 1-3	Homayoun Zadeh, DDS, PhD; Faculty	Los Angeles	$1,995 Dentist/ $995 Auxiliary	20
Esthetic Periodontal Surgery for the General Practitioner (Module I)	April 8	Ziv Simon, DMD, MSc	Los Angeles	$295 Dentist/ $175 Auxiliary	7
Esthetic Periodontal Surgery for the General Practitioner: A Hands-On Course (Module I and II)	April 8-10	Ziv Simon, DMD, MSc	Los Angeles	$1,795	21
New Approaches for Antimicrobial Treatment of Periodontal Disease (Las Vegas)	April 9	Jorgen Slots, DDS, DMD, PhD, MS, MBA	Las Vegas, NV	$345 Dentist/ $225 Auxiliary	7
Digital Clinical Photography: All You Need to Know! (Part I and Lecture)	April 15	Abdi Sameni, DDS; Gary Harmatz, DDS	Los Angeles	$245	7
Fundamentals of Restorative Implant Dentistry for the General Dentist (Part I)	April 15	Baldwin Marchack, DDS, MBA	Los Angeles	$245	7
Fundamentals of Restorative Implant Dentistry for the General Dentist (Part I and II)	April 15-16	Baldwin Marchack, DDS, MBA	Los Angeles	$995	14
Digital Clinical Photography: All You Need To Know! (Part II and Hands-On)	April 16	Abdi Sameni, DDS; Gary Harmatz, DDS	Los Angeles	$895	7
Common Oral Lesions: Soft and Hard Tissue Disease	May 6	Parish Sedghizadeh, DDS, MS; Faculty	Los Angeles	$225 Dentist/ $145 Auxiliary	7
Physical Evaluation	May 16	Stanley Malamed, DDS; Ken Reed, DMD	Los Angeles	$275 Dentist/ $175 Auxiliary	7
Emergency Medicine	May 17	Stanley Malamed, DDS; Ken Reed, DMD	Los Angeles	$275 Dentist/ $175 Auxiliary	7
Monitoring and Clinical Emergency Medicine	May 18	Stanley Malamed, DDS; Ken Reed, DMD	Los Angeles	$375 Dentist/ $215 Auxiliary	7
Atraumatic Extraction and Minimally Invasive Implant Site Development (Module IA)	May 21	Bach Le, DDS, MD, FICD; Faculty	Los Angeles	$295 Dentist/ $185 Auxiliary	5
Atraumatic Extraction and Minimally Invasive Implant Site Development (Modules IA and IB)	May 21	Bach Le, DDS, MD, FICD; Faculty	Los Angeles	$995 Dentist/ $695 Auxiliary	8
Endodontics From A to Z: Hands-On Workshop for the General Practitioner	June 3-5, 17-19	Ilan Rotstein, DDS; Faculty	Los Angeles	$2,945	42
Implant Therapy in the Compromised Sites — Cadaver Workshop	June 10-12	Homayoun Zadeh, DDS, PhD; Faculty	Los Angeles	$2,995 Dentist/ $1,595 Auxiliary	26
Temporomandibular Disorders, Arthrocentesis and Botox/Trigger Point Injections	June 24-25	Glenn Clark, DDS, MS; Faculty	Los Angeles	$495 Dentist/ $315 Auxiliary	25

TOPIC	DATE	LECTURER(S)	LOCATION	COST	UNITS
HUMBOLDT-DEL NORTE DENTAL SOCIETY					**707-443-7476**
Implant Options for Edentulous Patients	Jan. 28	Eugene LaBarre, DMD, MS	Arcata	$135 Member/ $100 Auxiliary	6
Treatment Planning and Behavior Modification for the Pediatric Patient	March 25	Ignatius Nate Gerodias, DDS	Arcata	$135 Member	6
Risk Management 101: The Fundamental Concepts	March 31	Carla Christensen	TBD	TBD	2
KERN COUNTY DENTAL SOCIETY					**661-327-2666**
Infection Control, Dental Practice Act, OSHA Compliance	Jan. 21	Marcella Oster, RDA	Bakersfield	$200 Member/ $300 Non-Member/ $75 Auxiliary	6
Occlusion for Dummies	Feb. 25	Donald Reid, DDS	Bakersfield	$200 Member/ $300 Non-Member/ $75 Auxiliary	6
Cone Beam CT in Your Practice	March 25	Gurminder Sidhu, BDS, DDS, MS	Bakersfield	$200 Member/ $300 Non-Member/ $75 Auxiliary	6
Antimicrobial Treatment of Periodontal Disease	April 29	Jorgen Slots, DDS, DMD, PhD, MS, MBA	Bakersfield	$200 Member/ $300 Non-Member/ $75 Auxiliary	6
LOMA LINDA UNIVERSITY SCHOOL OF DENTISTRY **CONTINUES ON NEXT PAGE**					**909-558-4685**
An Interdisciplinary Approach to the Cleft Repair and Care	Jan. 30	Alan Herford, DDS, MD; Anna Chen, DDS, MS, PhD; Bonnie Nelson, DDS	Loma Linda	$195 Dentist/ $135 Auxiliary	8
Ponic Design for Ridge Development	Feb. 10	Dennis Smith, DDS, MS	Loma Linda	$20	1
Track 1 Implant: Techniques for Sinus Augmentation	Feb. 10	Aladdin Al-Ardah, DDS	Loma Linda	$20	1
Track 1 Implant: 3D Model and Computer Guided Dental Implant Surgery	Feb. 10	Yshuji Yoshino, DDS	Loma Linda	$20	1
Track 1 Implant: Is the Platform Switch a More Predictable Abutment Connection?	Feb. 10	Yun-Chi Wang, DDS	Loma Linda	$20	1
Track 1 Implant: Management of Complications in Implant Dentistry	Feb. 10	John Won, DDS	Loma Linda	$20	1
Track 1 Implant: Comprehensive Implant Treatment Planning and Sequencing Workshop	Feb. 10	Montry Suprono, DDS	Loma Linda	$20	1
Track 1 Implant: The Role of Connective Tissue Grafts in Immediate Implant Placement in the Esthetic Zone	Feb. 10	Juan Mesquida, DDS	Loma Linda	$20	1
Track 1 Implant: Vertical Ridge Augmentation Prior to Implant Placement	Feb. 10	Jaime L. Lozada, DMD	Loma Linda	$20	1
Track 2 Periodontics: Implant Treatment Planning; Principles and Guidelines	Feb. 10	Wesam Salha, DDS	Loma Linda	$20	1
Track 2 Periodontics: Diabetes and Periodontal Disease	Feb. 10	Elham Javadi, DDS	Loma Linda	$20	1

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LOMA LINDA UNIVERSITY SCHOOL OF DENTISTRY CONTINUES ON NEXT PAGE					909-558-4685
Track 2 Periodontics: Interrelationship Between Periodontics and Restorative Dentistry: The Basics	Feb. 10	Adrian Mobilia, DDS	Loma Linda	$20	1
Track 2 Periodontics: Socket Preservation, What You Need to Know	Feb. 10	Mohammad Hassan, DDS, MS	Loma Linda	$20	1
Track 2 Periodontics: The Perio-Systemic Connection	Feb. 10	Craig Ririe, DDS, MS	Loma Linda	$20	1
Track 2 Periodontics: Implant Complications: Prevention and Management	Feb. 10	Chun-Xiao Sun, DDS, MS	Loma Linda	$20	1
Track 3 Operative/Restorative: Ceramic Bonding Issues	Feb. 10	Michael Meharry, DDS, MS	Loma Linda	$20	1
Track 3 Operative/Restorative: Overview of Ceramic Restorative Materials	Feb. 10	Ronald Forde, DDS, MS	Loma Linda	$20	1
Track 3 Operative/Restorative: Who Caries?	Feb. 10	Brian Novy, DDS	Loma Linda	$20	1
Track 3 Operative/Restorative: RPD Alternatives	Feb. 10	Mark Estey, DDS	Loma Linda	$20	1
Track 3 Operative/Restorative: Post and Core Materials and Procedures	Feb. 10	Nadim Baba, DDS	Loma Linda	$20	1
Track 3 Operative/Restorative: Treatment Plan Considerations for Worn Dentition	Feb. 10	Robert Walter, DDS	Loma Linda	$20	1
Track 4 Dental Hygiene: Win the Battle Against Biofilm: Leverage the Power of Ultrasonics	Feb. 10	Karen Hays, RDH, BS	Loma Linda	$80	4
Track 5 Miscellaneous: Denture Treatment Issues	Feb. 10	Madelyn Fletcher, DDS	Loma Linda	$20	1
Track 5 Miscellaneous: Preparing to Sell: Maximize the Value of Your Practice	Feb. 10	Bette Robin, DDS, JD	Loma Linda	Free	0
Track 5 Miscellaneous: Minor Equipment Repair	Feb. 10	Stan Lillard	Loma Linda	Free	0
Track 5 Miscellaneous: Tooth Whitening Overview: Clinical and Research Perspectives	Feb. 10	Sean S. Lee, DDS	Loma Linda	$40	2
Track 5 Miscellaneous: Extrusion Cases	Feb. 10	Frederick Berry, DDS	Loma Linda	$40	2
Track 6 ODRP: Applying the Pareto Principle to TMD Care – An 80% Solution	Feb. 10	Harold Avila, DDS, MS	Loma Linda	$40	2
Track 6 ODRP: Panographic Radiology in a Cone Beam World	Feb. 10	Dwight Rice, DDS	Loma Linda	$40	2
Track 6 ODRP: What in the World is That? — A Review of Common Mucosal and Radiographic Lesions	Feb. 10	Lane Thomsen, DDS, MS	Loma Linda	$40	2
Track 10 Endodontics: Creative Off Label Methods for Handling Common Endodontic Complexities	Feb. 10	C. John Munce, DDS, MS	Loma Linda	$20	1

TOPIC	DATE	LECTURER(S)	LOCATION	COST	UNITS
LOMA LINDA UNIVERSITY SCHOOL OF DENTISTRY CONTINUES ON NEXT PAGE					909-558-4685
Track 7 Prosthodontics: All-On-4? An Overview	Feb. 11	Amir Khatami, DDS	Loma Linda	$20	1
Track 7 Prosthodontics: Endodontic Treatment or Implant, Where Do You Draw the Line?	Feb. 11	Mehdad Fay, DDS	Loma Linda	$20	1
Track 7 Prosthodontics: Occlusal Analysis: Look Before You Leap!	Feb. 11	Myron Winer, DDS	Loma Linda	$20	1
Track 7 Prosthodontics: Mandibular Implant Overdenture – Standard of Care?	Feb. 11	Fernando Munguia, DDS	Loma Linda	$20	1
Track 8 Operative/Restorative: Esthetic Communication Issues and Procedures	Feb. 11	Richard Young, DDS	Loma Linda	$40	2
Track 8 Operative/Restorative: Esthetics in Operative Dentistry Methods and Materials	Feb. 11	Carlos Chavez, DDS	Loma Linda	$30	1.5
Track 9 Oral and Maxillofacial Surgery: Surgically Assisted Rapid Palatal Expansion (SARPE)	Feb. 11	Carlos M. Moretta, DDS	Loma Linda	$20	1
Track 9 Oral and Maxillofacial Surgery: Odontogenic Infections and Management	Feb. 11	Chan M. Park, DDS, MD	Loma Linda	$20	1
Track 9 Oral and Maxillofacial Surgery: Bone Grafting Options for Implant Placement	Feb. 11	Young Jun, DDS, MD	Loma Linda	$20	1
Track 9 Oral and Maxillofacial Surgery: Soft Tissue Manipulation Around Implants in the Aesthetic Zone	Feb. 11	Jeffrey A. Elo, DDS, MS	Loma Linda	$20	1
Track 10 Endodontics: Local Anesthesia for Non-Surgical Endodontic Therapy	Feb. 11	Kurt Marcks, DDS	Loma Linda	$20	1
Track 10 Endodontics: Root Canal Therapy: Keys to Long Term Success	Feb. 11	John Pratte, DDS	Loma Linda	$20	1
Track 11 Pediatrics: Ectodermal Dysplasia	Feb. 11	Meghanne Kruizenga, DDS	Loma Linda	$10	0.5
Track 11 Pediatrics: Case Presentation 1	Feb. 11	Monserrat Jorden, DDS	Loma Linda	$10	0.5
Track 11 Pediatrics: Case Presentation 2	Feb. 11	Laura McCormack, DDS	Loma Linda	$10	0.5
Track 11 Pediatrics: Case Presentation 3	Feb. 11	Noha Abdel-Salam, DDS	Loma Linda	$10	0.5
Track 11 Pediatrics: Cancer and Prosthesis	Feb. 11	Samah Omar, DDS	Loma Linda	$20	1
Track 11 Pediatrics: Child Abuse	Feb. 11	Wesley Okumura, DDS	Loma Linda	$20	1
Track 11 Pediatrics: Review of Behavior Management Techniques	Feb. 11	Bonnie Nelson, DDS, MS	Loma Linda	$20	1
Track 12 Miscellaneous: Overdentures and Overpartials; Over Teeth and Over Implants	Feb. 11	Judy Strutz, DDS	Loma Linda	$40	2
Track 12 Miscellaneous: How Many Root Canals Are Too Much? Differential Diagnosis for Odontogenic vs. Non-Odontogenic	Feb. 11	Susan Roche, DDS, MS, MA; Robert Handysides, DDS	Loma Linda	$40	2
Interdisciplinary Orthodontic Treatment	Feb. 11	Vincent O. Kokich Jr., DDS, MSD	Loma Linda	$160 Dentist/ $110 Auxiliary	7
Medically Compromised	Feb. 11	Heidi Christensen, DDS; Karen Well, MD	Loma Linda	$150 Dentist/ $95 Auxiliary	6

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LOMA LINDA UNIVERSITY SCHOOL OF DENTISTRY CONTINUED					909-558-4685
31st Anesthesia Symposium	Feb. 13	Larry Trapp, DDS, MS; Barry Krall, DDS	Loma Linda	$195 Dentist/ $135 Auxiliary	8
My Patient Wants to Quit Smoking — What Do I Need to Know?	Feb. 13	Lindsay Ferry, MD, MPH; Lane Thomsen, DDS; Hyma Gogenini, PharmD; et al.	Loma Linda	$160 Dentist/ $110 Auxiliary	7
Oral Surgery Symposium	March 6	Alan Herford, DDS, MD	Loma Linda	$195 Dentist/ $135 Auxiliary	7
The Annual Implant Dentistry Study Club LLUSD and AAID MaxiCourse	March 10–Dec. 16	Jaime L. Lozada, DMD; Mathew Kattadiyil, DDS, MDS, MS	Loma Linda	$13,500	300
Infection Control and California Dental Practice Act	March 13	W. Eugene Rathbun, DDS; Bette Robin, DDS, JD; Nancy Andrews, BS, RDH	Loma Linda	$160 Dentist/ $110 Auxiliary	7
4th Annual Periodontic Symposium	April 3	Craig Ririe, DDS; Dennis Smith, DDS	Loma Linda	$195 Dentist/ $110 Auxiliary	8
Esthetic Symposium	April 10	James Dunn, DDS; Michael DiTolla, DDS; et al	Loma Linda	$195 Dentist/ $110 Auxiliary	8
Medical Emergencies	April 17	Steven Filler, DDS	Loma Linda	$160 Dentist/ $110 Auxiliary	7

TOPIC	DATE	LECTURER(S)	LOCATION	COST	UNITS
MARIN COUNTY DENTAL SOCIETY					**415-472-7974**
BLS/CPR Recertification Class	Jan. 27	TBD	San Rafael	$60 Member/ $120 Non-Member	3.5
General Membership Meeting	Feb. 15	Charles McNeill, DDS	Mill Valley	$45 Member/ $90 Non-Member	2
BLS/CPR Recertification	Feb. 24; March 31; April 28; May 26	TBD	Mill Valley	$60 Member/ $120 Non-Member	3.5
General Membership Meeting	March 15	David C. Hatcher, DDS, MSc	Mill Valley	$45 Member/ $90 Non-Member	2
General Membership Meeting	May 17	Steve Tiret, CPA	Mill Valley	$45 Member/ $90 Non-Member	2
MID-PENINSULA DENTAL SOCIETY					**650-328-2242**
Infection Control/Dental Practice Act	Jan. 21	Carolyn Mortenson; Staci Pruitt	Palo Alto	$100	6
Sleep Symposium	April 29	World Renowned Speakers/ Dental Health Foundation Fundraiser	Palo Alto	$250	7
Emergency Medicine and Sedation	May 20	Stanley Malamed, DDS	Palo Alto	$250	7
MONTEREY BAY DENTAL SOCIETY					**831-658-0168**
Diagnosis and Treatment Planning for TMD	April 1	Terry Tanaka, DDS	Monterey	$280 Member/ $130 Auxiliary	7
California Dental Practice Act and Infection Control	April 15	Art Curley, JD; Eve Cuny, RDA, MS	Monterey	$140 Member/ $60 Auxiliary	4
Clinical Tips and Material Recommendations Based on the Most Recent Clinical Research	May 20	Rella Christensen, PhD	Monterey	$280 Member/ $130 Auxiliary	6
Recipes for Predictable Anterior Esthetics	June 17	Gerard Chiche, DDS	Monterey	$280 Member/ $130 Auxiliary	7
NORTHERN CALIFORNIA DENTAL SOCIETY					**530-527-6764**
Nutrition for the Dental Patient and "Mental Health, What Dental Professionals Should Know"	Jan. 14	Tieraona Low Dog, MD	Red Bluff	$125 Member/ $225 Non-Member/ $55 Auxiliary	6
Presenting Dental Findings and Treatment Option	Feb. 3; March 3	John Van der Werff, DDS	Chico; Redding	$45 Member/ $90 Non-Member/ $30 Auxiliary	2
Six Steps to a Paperless Practice	Feb. 18	Lorne Lavine, DMD	Red Bluff	$125 Member/ $225 Non-Member/ $55 Auxiliary	7
CDPA, OSHA Refresher and Infection Control	March 18	LaDonna Drury-Klein RDA, CDA, BS	Red Bluff	$125 Member/ $225 Non-Member/ $55 Auxiliary	6
Clinical Jewels You Can Count On	April 15	Patrick Roetzer, DDS, FICD	Red Bluff	$125 Member/ $225 Non-Member/ $55 Auxiliary	6
Effective Communication and Enrollment Skills "Think Outside the Mouth" Treatment Planning	May 20	Karen Davis, RDH, BSDH, RDHMP	Red Bluff	$125 Member/ $225 Non-Member/ $55 Auxiliary	6

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ORANGE COUNTY DENTAL SOCIETY					**714-634-8944**
Infection Control/CDPA	Jan. 11	Leslie Canham, RDA, CDA	Irvine	$69	4
BLS	Jan. 19	Helen McCracken, RDH, MS	Orange	$69	3
You've Got It – Now Flaunt It: Marketing Your Brand	Feb. 8	Stewart Gandolph, MBA	Irvine	$69	2.5
A Bridge to Paperless: Using Technology to Improve Efficiency	March 8	Baldwin W. Marshack, DDS	Irvine	$69	2.5
Stuck On You: Profitable Adhesive Dentistry	April 12	Brian LeSage, DDS	Irvine	$69	2.5
PACIFIC COAST SOCIETY FOR PROSTHODONTICS					**360-459-4400**
Annual Meeting and Scientific Session	June 22-25	Multiple Speakers	Pasadena	$695	16
PUNJABI DENTAL SOCIETY					**866-422-5573**
Infection Control, Risk Management and California Dental Practice Act	Jan. 23	Luis R. Dominicis, DDS; Nancy Andrews, RDA; Gail Harris, RN, MS	Montebello	$79	7
California Dental Practice Act, Infection Control and Risk Management	Feb. 13	Luis R. Dominicis, DDS; Gail Harris, RN, MS; Rodney Stine, BA, MA	San Jose	$99	7
Advancing Your Vision In Restorative Dentistry	March 27	Lou Graham, DDS	Montebello	$79	7
Hands-On Contemporary Esthetics and Restorative Dentistry	April 24	Paresh Shah, MS, DMD	Diamond Bar	$149	7
The Art of Aesthetics and Occlusion	May 22	Todd C. Snyder, DDS	San Jose	$99	7
Oral Surgery Made Easy for General Dentists	June 26	Anil P. Punjabi, DDS	Montebello	$79	7
SACRAMENTO DISTRICT DENTAL SOCIETY **CONTINUES ON NEXT PAGE**					**916-446-1227**
CPR Basic Life Support (BLS) Renewal Course	Jan. 8, April 2	SDDS Instructors	Sacramento	$55 Member	4
Shift Happens: Incorporating New Protocols Into Practice	Jan. 11	Kristy Menage Bernie, RDH, BS, RYT	Sacramento	$57 Member	2
2011 Labor Law Update - HR Audio Conference	Jan. 13	California Employers Association	Sacramento	$35 Member	1
SDDS 31st Annual Mid-Winter Convention	Feb. 3-4	Visit sdds.org for speakers	Sacramento	Visit sdds.org for pricing	Various
Removable Partial Dentures: Clinical Considerations	March 4	Alan Carr, DMD, MS	Sacramento	$187 Member	5
Skin Cancer — Diagnosis and Treatment	March 8	Barbara Burrall, MD	Sacramento	$57 Member	2
Build Your Own Employee Handbook Workshop	March 18	Mari Bradford; California Employers Association	Sacramento	$69 Member	4
The Numbers of Your Practice: The Good, The Bad, Avoiding the Ugly	March 24	John Urrutia, CPA	Sacramento	$69	0
Crown Lengthening for the General Practitioner — Hands-On Course	April 8	Timothy Hempton, DDS	Sacramento	Call SDDS for cost	5

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SACRAMENTO DISTRICT DENTAL SOCIETY CONTINUED					916-446-1227
Turn It On and Off: What's New In Local Anesthesia	April 12	Alan Budenz, MS, DDS, MBA	Sacramento	$57 Member	2
Top 10 SDDS Hotline Questions—HR Audio Conference	April 19	California Employers Association	Sacramento	$35 Member	1
Practice Management: Straight Talk About Balancing It All (People, Systems, Results)	April 21	Gayle Suarez, Dental Management Solutions	Sacramento	$69 Member	2
Infant and Early Childhood Oral Care	May 10	Jeffrey Wood, DDS	Sacramento	$57 Member	2
2nd Annual Right In Your Own Backyard	May 14	SDDS Members	Sacramento	$119 Member	4
California Dental Practice Act and Infection Control — Licensure Renewal Course	May 20	LaDonna Drury-Klein, RDA, CDA, BS	Sacramento	$125 Member	4
CPR — Basic Life Support (BLS) Full Course	June 25	SDDS Instructors	Sacramento	$70 Member	5
SAN FERNANDO VALLEY DENTAL SOCIETY					818-884-7395
Hot Topics in Esthetics, Dental Ceramics and Restorative Dentistry	Jan. 12	Ed McClaren, DDS	Van Nuys	$175 Member/ $300 Non-Member/ $90 Auxiliary/$75 Retired	7
How to Diagnose and Manage Common Oral Pathologies	Feb. 9	Diana Messadi, DDS	Van Nuys	$175 Member/ $300 Non-Member	7
CA Dental Practice Act and Infection Control	March 9	Nancy Andrews, RDH	Van Nuys	$175 Member/ $300 Non-Member/ $90 Auxiliary/$75 Retired	7
The Wonderful World of Prosthodontics	May 11	Mark Exler, DDS, FACP	Van Nuys	$175 Member/ $300 Non-Member	7
Pharmacologic Management of the Surgical Patient	June 22	John Yagiela, DDS	Van Nuys	$175 Member/ $300 Non-Member/ $90 Auxiliary/$75 Retired	7
SAN FRANCISCO DENTAL SOCIETY					415-928-7337
CPR for Healthcare Providers and Renewal Only	Jan. 26, Feb. 23, March 30, April 27, June 29	Adrian Curry, EMT	San Francisco	$67	4
The Role of Gingival Biotypes in Restorative and Implant Dentistry	Jan. 27	Richard T. Kao, DDS, PhD	San Francisco	$69	2
California Dental Practice Act (CDPA)	Feb. 25; May 20	Marcella Oster, RDA	San Francisco	$60 Member/ $90 Non-Member	2
OSHA Bloodborne Pathogen/Infection Control and Hazardous Communication Refresher	Feb. 25; May 20	Marcella Oster, RDA	San Francisco	$97 Member/ $145 Non-Member	4
Predicting Employee Success	April 2	Sally McKenzie, CMC	San Francisco	$95	3
Caries Management by Risk Assessment — The Caries Balance	May 5	John D. B. Featherstone, MSc, PhD	San Francisco	$69	2
CPR BLS Certification for Healthcare Providers and Basic Course	May 21	Adrian Curry, EMT	San Francisco	$97 Member/ $140 Non-Member	4
Restoring the Edentulous Mandible	June 9	Gaurav Setia, DDS	San Francisco	$69	2

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SAN GABRIEL VALLEY DENTAL SOCIETY					**626-285-1174**
CA Law and Infection Control	Jan. 18	Leslie Canham, CDA, RDA	Alhambra	$65 Member/ $100 Non-Member	4
Comprehensive Esthetic Dentistry Update	Feb. 15	Avishai Sadan, DMD	Alhambra	$65 Member/ $100 Non-Member	3
Mental Health and Well-Being for the Dental Professional	March 15	Jessica S. Mosich, PhD	Alhambra	$65 Member/ $100 Non-Member	3
Treatment of Endodontically Restored Teeth	April 19	Nadim Baba, DDS, MSD, FACP	Alhambra	$65 Member/ $100 Non-Member	3
SAN JOAQUIN DENTAL SOCIETY					**209-951-1311**
Implant Complications and Management	Feb. 24	Michael Jacobs, DDS	Stockton	TBD	3
The Virtues of Profitable Dentistry	March 24	Howard Farran, DDS	Lodi	TBD	7
California Dental Practice Act and Infection Control	April 21	Ladonna Drury-Klein, CDA, RDA, BS	Stockton	TBD	4
The Virtues of Profitable Dentistry	April 24	Howard Farran, DDS	Lodi	TBD	7
Evidence Based Dentistry and Practice Based Research Networks	May 19	Paul Benjamin, DMD, MAGD, FACD	Murphys	TBD	3
SAN MATEO COUNTY DENTAL SOCIETY					**650-637-1121**
New Professionals Forum	Jan. 6, March 3, April 7, May 12	TBD	Redwood City	$10 Member/ $25 Non-Member	0
AHA CPR – BLS Renewal Course	Jan. 18, March 15, April 19, May 17, Sept. 27, Nov. 15	Stephen R. John, DDS	Redwood City	$45 Member/ $60 Non-Member	4
7 Things Every Dentist Must Know About Data Security and HITECH ACT	Jan. 27	Lorne Lavine, DMD	Foster City	$45 Member/ $55 Non-Member	3
AHA CPR - BLS Renewal Course	Feb. 21, April 11, May 9, June 13	Richard A. Fagin, DDS	Redwood City	$45 Member/ $60 Non-Member	4
Dental Caries: Advances in Detection and Disease Management	Feb. 24	Karen Hays, RDH, BS	Foster City	$45 Member/ $55 Non-Member	3
Occlusion/Supra-gingival Dentistry	March 24	Jose-Luiz Ruiz, DDS, FAGD	Foster City	$45 Member/ $55 Non-Member	3
Introduction to Sleep Apnea Treatment for the General Dentist	April 21	Steve Keller, DMD	Foster City	$45 Member/ $55 Non-Member	3
Dental Office Regulatory Compliance Training	April 29, June 24	Julian Goduci, CHMM	Redwood City	$120 Member/ $150 Non-Member	8
Practice Management	May 26	Debbie Castagna; Virginia Moore	Foster City	$45 Member/ $55 Non-Member	3

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SANTA BARBARA-VENTURA COUNTY DENTAL SOCIETY					**805-656-3166**
A Systematic Approach to Bonded Porcelain and Dental Implants	Feb. 11	Mohamadali Reshad, DDS, MSc	Oxnard	$185	7
Practice Management 101: Creating An Unforgettable Practice	March 25	William Van Dyke, DDS	Goleta	$185	6
Infection Control and Dental Practice Act	April 22	Noel Kelsch, RDH; Jason Wood	Oxnard	$150	4
Evolution and Management of Oralfacial Pain	June 10	Steven Graff-Radford, DDS	Thousand Oaks	$185	7
SANTA CLARA COUNTY DENTAL SOCIETY					**408-289-1480**
Management of Pediatric Trauma	Feb. 10	Ann Greenwell, DMD	Campbell	$35 Non-Member	2
Cone Beam CT Scans	March 10	Sotirios Tetradis, DDS, PhD	Campbell	$35 Non-Member	2
CAD/CAM Dentistry	April 14	Dino Javaheri, DDS	Campbell	$35 Non-Member	2
TBA	May 12	Terry Donovan, DDS	Campbell	$35 Non-Member	2
SOUTHERN CALIFORNIA OROFACIAL ACADEMY					**626-287-1185**
Implant Placement, Grafting, Membranes, Sinus Lift Techniques, Use of Osteotomes	March 11-13	Frank L. Pavel, DMD; Graham L. Simpson, DDS	San Diego	$500	10
TRI-COUNTY DENTAL SOCIETY					**909-370-2112**
Reconstructive Dentistry	Feb. 24	Tony Daher, DDS	Colton	$40	2
Soft Tissue Grafting and Socket and Bone Grafting	April 7	Armen Mardirossian, DDS; Gregg Filippelli, DDS	Colton	$40	2
TULARE-KINGS DENTAL SOCIETY					**559-625-9333**
Got OSHA? 6 Steps to Office Safety	March 10	Leslie Canham, RDA, CDA Speaker's Bureau	Visalia	TBD	2
Course on Full Mouth Reconstruction Using Dental Implants	April 8	Robert Bell, DDS	Visalia	TBD	3
Practicing Periodontics: From the Center to the Edge	April 8	John Kwan, DDS	Visalia	TBD	4
UNIVERSITY OF CALIFORNIA LOS ANGELES SCHOOL OF DENTISTRY **CONTINUES ON NEXT PAGE**					**310-206-8388**
RDAEF Expanded Duties Module III	Starts Jan. 15-16	Richard G. Stevenson, DDS; Joseph Cooney, BDS, MS	Los Angeles	$5,995 RDA/ $3,495 RDAEF	104
Sleep Medicine Mini-Residency	Starts Feb. 11-12	Dennis R. Bailey, DDS; Robert L. Merrill, DDS, MS	Los Angeles	$5,995	40
California Dental Practice Act and Infection Control	Feb. 26	Andy Wong, DDS	Los Angeles	$135 Dentist/ $95 Auxiliary	4
Advanced Anterior Esthetics	March 4-6, April 15-17	Jeff Morley, DDS	Los Angeles	$5,995	46
Pediatric Dentistry for the G.P. – An Update	March 5	Kumar Shah, BDS	Los Angeles	$198	7

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UNIVERSITY OF CALIFORNIA LOS ANGELES SCHOOL OF DENTISTRY CONTINUED					310-206-8388
Hypnosis and Its Application to Dentistry	March 5-6	Don M. Goodman, PhD, CCHt; Ken Dubner, CHHt	Los Angeles	$495	14
Evidence-Based Dentistry for the Clinician	March 12	Francesco Chiappelli, PhD; Janet Bauer, DDS, MS	Los Angeles	$198	7
RDA Required Course – Infection Control	March 12	Cara Batson, RDA; Charlene Flowers-Taylor, RDA	Los Angeles	$250	8
Removable Partial Denture Course	March 12	Ting-Ling Chang, BDS	Los Angeles	$198	7
Dental Ethics for a Changing Profession	March 19	Gary Herman, DDS	Los Angeles	$198	7
Re-Certification in Pediatric Oral Sedation	March 19	John A. Yagiela, DDS, PhD; Cristine Quinn, DDS, MS	Los Angeles	$295	8
RDA Required Course – Pit and Fissure Sealants	March 26-27	Cara Batson, RDA; Charlene Flowers-Taylor, RDA	Los Angeles	$575	16
Find Your First Job	April 2	Michael Okuji, DDS	Los Angeles	$150 Dentist	7
UCLA Endodontic Continuum	April 7-10, April 28-May 1	Bernice Ko, DDS	Los Angeles	$3,995	58
RDA Required Course — Coronal Polishing	April 9	Cara Batson, RDA; Charlene Flowers-Taylor, RDA	Los Angeles	$325	8
Advanced Implant Therapy	April 25-29	Sascha A. Jovanovic, DDS, MS; Henry H. Takei, DDS, MS	Los Angeles	$3,995	40
Moderate Sedation with Multiple Oral and Parenteral Agents	April 14-17, May 19-22	John A. Yagiela, DDS, PhD; Roger J. Wendel, DMD	Vancouver, WA	$11,500	80
UCLA Implants A to Z 2011	Starts April 16	George Perri, DDS; Sascha A. Jovanovic, DDS, MS	Los Angeles	$3,995	56
Preventing and/or Resolving Patient Dissatisfaction	April 30	Jeffrey Goldstein, MBA, PhD; Ronald Mito, DDS, FDS	Los Angeles	$198	7
RDAEF Expanded Duties Module I	April 30-May 1	Richard G. Stevenson, DDS; Joseph Cooney, BDS, MS	Los Angeles	$3,995	104
The Integration of Technology — Building a Better Practice	May 7	Todd R. Schoenbaum, DDS	Los Angeles	$145	4
Dental Photography Workshop and Digital Presentations for Esthetic Treatment Planning	June 11	Brian P. LeSage, DDS	Los Angeles	$395	7
California Dental Practice Act and Infection Control	June 25	Andy Wong, DDS	Los Angeles	$135 Dentist; $95 Auxiliary	4

TOPIC	DATE	LECTURER(S)	LOCATION	COST	UNITS
UNIVERSITY OF CALIFORNIA SAN FRANCISCO SCHOOL OF DENTISTRY					**415-476-1101**
115th Scientific Session	Jan. 14-15	Various	San Francisco	$300	15
Implementing Occlusion Into Everyday Dentistry	Jan. 28	Jose-Luis Ruiz, DDS	San Francisco	$225	7
Clinicopathological Correlations	Feb. 5	M. Anthony Pogrel, DDS, MD; Richard Jordan, DDS, MSc, PhD	Hawaii	$225	4
18th International Symposium in OMFS	Feb. 7-11	Various	Hawaii	$995	20
17th Annual Island Dental Colloquium	Feb. 21-25	Christine Peters, DMD; Ove Peters, DMD, MS, PhD; Peter Loomer, BSc, DDS, PhD, MRCD	Maui, Hawaii	$695	20
17th Annual UCSF/UOP Island Dental Colloquium	Feb. 21-25	Various	Maui, Hawaii	$695	20
Pediatric Restorative Dentistry	March 4	David Rothman, DDS	San Francisco	$225	7
Veneers Made Easy— Workshop	March 5	Daniel Mendoza, DDS	San Francisco	TBD	7
Oral Surgery for the General Practitioner Part I	March 11	M. Anthony Pogrel, DDS, MD; Mehran Hossaini, DMD	San Francisco	$225	7
Medical Emergencies	March 12	Richard Smith, DDS	San Francisco	$225	7
UCSF Endodontic Research Day	March 18	Various	San Francisco	$250	7
Advanced Periodontal Instrumentation	April 1-2	Ana Pattison, RDH, MS	San Francisco	TBD	14
Oral Surgery for the General Practitioner Part II	April 8	M. Anthony Pogrel, DDS, MD; Mehran Hossaini, DMD	San Francisco	$225	7
Digital Photography - Workshop	April 9	Mark Dellinges, DDS	San Francisco	TBD	7
Fixed Prosthodontics	April 15	Terry Donovan, DDS	San Francisco	$225	7
WESTERN LOS ANGELES DENTAL SOCIETY					**310-349-2199**
Periodontics	Feb. 8	Ziv Simon, DMD, MSc	Culver City	$75 ADA Dentist/$120 Non-ADA Dentist/$60 Non-Dentist	3
Prosthodontics	March 8	Mamaly Reshad, DDS	Culver City	$75 ADA Dentist/$120 Non-ADA Dentist/$60 Non-Dentist	3
Dental Management for the Cancer Patient	April 12	Eric Sung, DDS	Culver City	$75 ADA Dentist/$120 Non-ADA Dentist/$60 Non-Dentist	3

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

About 15 to 20 fellows are supported each year by funds from three federal T32 Training Grants, the UCSF Clinical and Translational Research Institute and from our four School of Dentistry academic departments. An application and review process has been in place through the more than 30 years of this program. Students who are interested are encouraged to discuss a possible project with one of a list of potential mentors. They prepare a proposal and these are reviewed in April of the year in which they seek a fellowship.

Successful candidates are supported on a fractional NIH-level predoctoral stipend for three months and are provided also with modest research support and travel funds. During that summer quarter, the fellows conduct their project and also participate in seminars, a journal club, and sessions with faculty leaders where they discuss their projects. The opportunity to mingle with other students doing research and with faculty members is highly valued by these student fellows.

Modulation of EGFR by Oral Squamous Cell Carcinoma Cell Lines

DONGMIN DANG, MD; STEPHEN SADLER; AND DANIEL M. RAMOS, DDS, PHD

ABSTRACT Oral cancer is the sixth most frequent cancer worldwide.[1] Prognosis for these patients remains poor. Recently, the epidermal growth factor receptor has been targeted as an adjunct to radiotherapy and surgery with limited success. The authors now present data suggesting that the αvβ6 integrin, which is a marker for aggressive oral cancer, may regulate epidermal growth factor receptor expression. The authors suggest perhaps targeting both αvβ6 and EGFR may provide additional benefits.

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Oral cancers are a heterogeneous group of neoplasms. Despite recent advances in treatment, (surgery, radiation, and chemotherapy) prognosis for oral SCC has not improved significantly in more than 60 years. One-third of these patients develop local and/or regional tumor recurrence following surgery, which suggests a lack of understanding the mechanism underlying this disease. One characteristic of invasive oral squamous cell carcinoma is the process of epithelial to mesenchymal transition, EMT. It is during this process that tumor cells are released from the primary tumor and assume a less differentiated, more mesenchymal phenotype.

A variety of molecules have been associated with this process including TGFβ1, EGFR, and αvβ6. Others such as E-cadherin and keratin have been more traditionally associated with the epithelial phenotype. αvβ6 integrin is an epithelial specific adhesion receptor. αvβ6

is not typically expressed in oral keratinocytes. However, upon wounding or neoplastic transformation αvβ6 is highly expressed.[2-4] αvβ6, an adhesion receptor for fibronectin and tenascin-C, also activates TGFβ1 by binding to LAP (latency associated peptide)/TGFβ complex.

The authors recently demonstrated that expression of the full-length β6 subunit is required for maintenance of the mesenchymal phenotype and that removing the C-terminal 11 amino acids restores the epithelial phenotype.[5]

EGFRs are small proteins that are found on the surface of all cells. EGFR binds exclusively to small growth factor proteins circulating in the blood. The binding action between EGFR and growth factors stimulates biological processes within the cell to promote growth of a cell in a strictly controlled manner. The EGFR-Ras-RAF-MAPK signaling cascade is an important pathway in cancer development, and recent reports show that EGFR and its downstream signaling molecules are mutated in a number of cancers.[6] However, in many cancer cells, EGFR is either abundantly overexpressed or the EGFR biological processes that normally stimulate cell growth are constantly active, leading to the uncontrolled and excessive growth of the cancer cell. EGFR is a transmembrane protein one of the ErbB family of receptors. This receptor has intrinsic tyrosine kinase activity and regulates cell growth in response to binding of ligands like epidermal growth factor, EGF, or transforming growth factor α.[6]

The activated receptor recruits signaling complexes and activates the RAS-RAF-MAPK pathway. This pathway, along with other signaling pathways of EGFR, is a potent regulator of tumor cell growth invasion, angiogenesis, and metastasis. Recently, several mutations of EGFR have been found in head and neck tumors.[7] The association of EGFR with integrins has not been made. The authors have evidence that the expression of the integrin β6 regulates EGFR expression and this association may be used when designing future drug strategies.

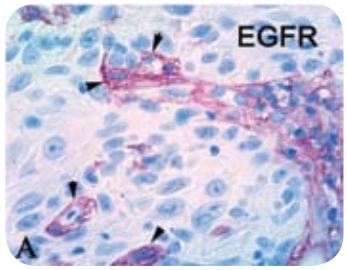

FIGURE 1A.

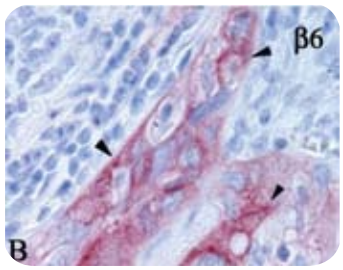

FIGURE 1B.

FIGURE 1. Immunohistochemical localization of EGFR and αvβ6 in oral squamous cell carcinoma. Biopsy specimens were evaluated and compared for the expression of EGFR **(A)** and αvβ6 **(B)**. 5 µM thin sections were selected from formalin-fixed, paraffin-embedded blocks and prepared for immunohistochemistry using antigen retrieval. Antibodies to EGFR (Millipore) and β6 (4B5) were used at 1:50. Both EGFR **(A)** and β6 **(B)** staining was concentrated to the leading edge of the tumor fingers. Light staining throughout the tumor could also be seen. Note complete absence of staining from the connective tissue.

Materials and Methods

Cell Culture

The SCC9 cell line (derived from a tongue lesion) was obtained from Dr. James Reinwald (Brigham and Woman's Hospital, Harvard School of Medicine) and has been described elsewhere.[9] SCC9β6 cells were generated in the authors' laboratory through retroviral transduction with the full-length β6 cDNA.[8] SCC9 cells were also transfected with the blank vector as described to generate the SCC9SN cell line.[9] The SCC9 and the SCC9SN gave exactly the same results, and for the sake of conciseness, the authors will only show results for the SCC9SN cell line. cDNAs for kinase-dead Fyn (KDFyn) and constitutively active Fyn (CAFyn) were a generous gift of Dr. H. Kawakatsu (University of California, San Francisco). The KDFyn and CAFYN cDNAs were stably expressed into the SCC9β6 and SCC9 cells, respectively, using the Retro-X-system (Clontech).

Growth Conditions

Cells were routinely cultivated in Dulbecco's modified Eagle's medium (DMEM) with 10 percent fetal bovine serum.

Antibodies

Murine monoclonal antibodies to EGFR were purchased from Millipore (Chemicon division, Temecula, Calif.). Rabbit monoclonal antibody 4B5 is directed to the human b6 subunit and was a gift of Dr. Robert Pytela.

Immunohistochemistry

Multiple 5 µm serial sections from selected formalin-fixed, paraffin-embedded blocks were cut onto aminopropyltriethoxysilane-coated slides. The tissues were dewaxed in xylene and rehydrated. Heat-induced antigen retrieval in citrate buffer (three minutes in a pressure cooker) was followed by blockade of endogenous peroxidase activity with hydrogen peroxide. The sections were incubated with 10 percent goat serum in phosphate-buffered saline to reduce nonspecific binding and background staining. Anti-EGFR or anti-β6 were used at 1:50 dilution and then applied onto the slides and incubated one hour at room temperature. The slides were washed with Tris-buffered saline containing 0.05 percent Tween. This was followed

by incubation with secondary goat anti-mouse IgM conjugated with horseradish peroxidase, HRP, antibody at room temperature. The color change reaction was performed using diaminobenzidine, DAB, as substrate. The slides were then counterstained with haematoxylin, dehydrated, and cleared in xylene. Coverslips were applied with cytoseal 60 mounting medium.

Immunofluorescence Microscopy

A total of 2 x 10^5/ml cells were plated onto fibronectin (FN) — coated glass coverslips (10 µg/ml) for 24 hours, serum — free and fixed with 3 percent paraformaldehyde, permeabilized with 0.1 percent Triton X-100. The cells were incubated first with monoclonal antibodies to EGFR or β6 integrin for one hour then rinsed with phosphate-buffered saline, and incubated with biotin-conjugated goat anti-mouse IgG (1:50) for 30 minutes at room temperature, followed by an additional rinse with phosphate-buffered saline. The cultures were then incubated with fluorescein isothiocyanate (FITC)-conjugated streptavidin (1:100) (Amersham, Piscataway, N.J.) for 30 minutes at room temperature, washed with PBS, and mounted with Vectashield (Vector Laboratories, Burlingame, Calif.). The cultures were then examined for expression of green-fluorescence using immunofluorescence microscopy.

Immunofluorescence In Vivo

5-µm thick frozen sections were brought to room temperature and air dried for 30 minutes, then fixed in cold acetone (-20 degrees Celsius) for 10 minutes. After rinsing with phosphate-buffered saline, the slides were then blocked for 20 minutes with phosphate-buffered saline containing 2 percent fetal bovine serum to prevent nonspecific staining. Primary antibodies were added for one hour at room temperature and rinsed with phosphate-buffered saline, and then incubated with an fluorescein-conjugated secondary goat anti-mouse IgG (1:50) (Jackson ImmunoResearch Laboratories, West Grove, Penn.) for 30 minutes at room temperature followed by an additional rinse with phosphate-buffered saline. Glass coverslips were mounted with Vectashield (Vector Laboratories). The slides were then examined for expression of green-fluorescence using immunofluorescence microscopy.

Western Blotting

Cells were serum-starved for 24 hours and then plated onto FN (10 µg/ml) for 24 hours. The cells were then lysed in Nonidet P-40 lysis buffer (1.5 percent Nonidet P-40, 150 mM, NaCl, 0.2 percent SDS, 1 mM EDTA, 20 mM Tris-HCl, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml leupeptin,10 µg/ml aprotinin,1 mM Na_3VO_4, 50 mM NaF).

Protein concentration was determined by BCA Protein Assay Kit (Pierce, Rockford, Ill.). The proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Micron Separation Inc, Westborough, Mass.), using a semi-dry blotting apparatus (Bio-Rad, Hercules, Calif.) as described elsewhere. The membranes were then developed using the ECL Chemiluminescence Kit (Amersham) and bands were detected by exposure to X-ray film. The blots were quantified and assigned relative value units (rvu) using an image analysis program (NIH Image).

Results

EGFR and αvβ6 Are Localized to the Leading Edge of the Invasive Unit

Tissue sections from an invasive oral squamous cell carcinoma were examined for the expression of EGFR and the integrin αvβ6 (**FIGURES 1A-B**). In this study, the authors wanted to compare relative localization of EGFR with that of β6. Briefly, frozen tissue sections were incubated with monoclonal antibodies to EGFR or αvβ6 overnight at 4 degrees Celsius. The specimens were washed and incubated with goat-anti-mouse secondary antibody conjugated to horseradish peroxidase. When analyzed the relative distribution of EGFR andαvβ6 was similar (**FIGURE 1**). The tissue sections epithelial clusters invading into the surrounding extracellular matrix. The invading finger-like tumor projections was positive for EGFR with an apparent localization to the leading edge of the lesion (**FIGURE 1A, SEE ARROWS**).

Similarly, the expression of the αvβ6 integrin is also distributed throughout the lesion with localized concentration at the matrix/tumor interface (**FIGURE 1B, SEE ARROWS**). Results demonstrated that both EGFR and αvβ6 are situated where the tumor nest meets the extracellular matrix and this strongly suggests the possibility of crosstalk between the two molecules. This temporal-spatial co-distribution of αvβ6 and EGFR may be important for tumor cell invasion.

Localization of EGFR by Immunofluorescence Microscopy In Vivo

The authors wanted to define the parameters of EGFR expression using immunofluorescence microscopy. Frozen specimens (5 µm thickness) were incubated with antibodies to EGFR and β6 overnight at 4 degrees Celsius. The tissue was rinsed and processed for immunofluorescence. The slides were then incubated with goat-anti-mouse secondary antibodies which were tagged with FITC. Note that these results clearly showed the expression of the EGFR (**FIGURE 2A**) and integrin β6 (**FIGURE 2B**) were localized to the cell membrane in a continuous fash-

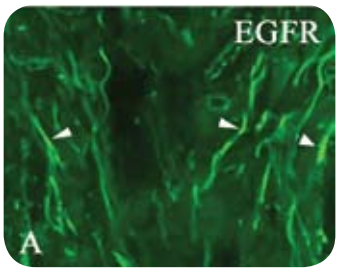

FIGURE 2A.

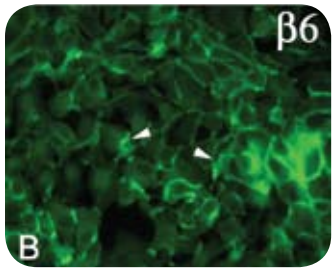

FIGURE 2B.

FIGURE 2. Immunofluorescent detection of EGFR and αvβ6 in SCC tissue sections. 5-μM thick tissue sections were prepared again using antigen retrieval for verification staining. The tissue sections were then processed for staining with antibodies to EGFR **(A)** and β6 **(B)** and visualized by secondary antibody conjugated to FITC. Coverslips were mounted with Cytoseal. The staining for EGFR was completely tumor contained and totally separated from the surrounding tissue **(A)**. The expression of β6 was also restricted to the epithelial component **(B)**.

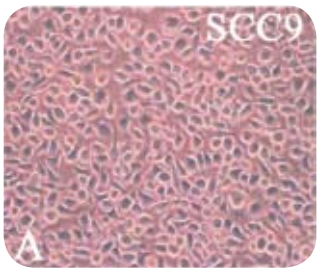

FIGURE 3A.

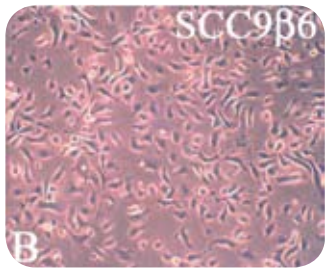

FIGURE 3B.

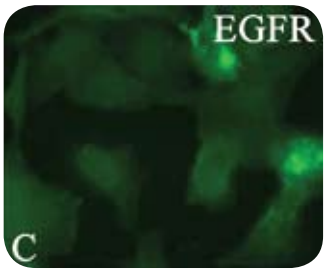

FIGURE 3C.

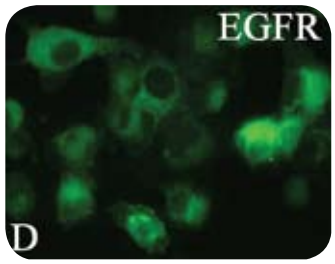

FIGURE 3D.

FIGURE 3. Differential expression of EGFR in vitro. Poorly invasive SCC9SN **(A)** and highly invasive SCC9β6 **(B)** cells were placed on fibronectin substrates for six hours and photographed for morphology. **(A)**. SCC9SN: epithelial; **(B)**. SCC9β6; mesenchymal. The cells were then processed and stained for immunofluorescence using monoclonal antibodies to EGFR. Note the lack of staining in the β6-negative SCC9SN cells **(C)**. In contrast, note the high-level staining for EGFR in the SCC9β6 cells **(D)**.

ion. This multilayered clustering of EGFR and αvβ6 demonstrate the fluidity of the receptors within the cell membrane.

Differential Localization of EGFR in Oral SCC Cells in Culture

αvβ6 negative SCC9SN cells are epithelial in appearance (**FIGURE 3A**). Expression of the β6 integrin into SCC9SN cells converts the cells into invasive mesenchymal-type cells (**FIGURE 3B**).[8,5] The authors evaluated both cell lines for the expression of EGFR. Expression of EGFR paralleled that of αvβ6 integrin. The SCC9β6 cells were positive highly reactive with antibodies to EGFR (**FIGURE 3D**), whereas the poorly invasive oral SCC9 cells reacted minimally with anti-EGFR antibodies (**FIGURE 3C**). The expression of EGFR was significantly higher in the αvβ6 positive SCC9β6 cells when compared to the β6 negative SCC9SN cells. This demonstrates that the neoexpression of β6 is the coincident with expression of EGFR and that perhaps there is communication between the two molecules.

Expression of EGFR Is Regulated by the Fyn Kinase

The authors previously showed that β6 ligand binding initiates Fyn-kinase signaling cascade, which is propagated through Ras/RAF/MAP Kinase.[9] This pathway results in increased MMP3 activation, invasion and metastasis. The SCC9β6KDFyn cell line was previously established by transfecting the SCC9β6 cells with a "kinase dead" Fyn.[9,8] Similarly, the SCC9CAFyn cells were established by overexpressing a constitutively active Fyn into the poorly invasive SCC9 cells.[9,8] Western blotting was performed to evaluate the expression of EGFR. Briefly, the SCC9SN, SCC9β6, SCC9CAFyn, and SCC9β6KDFyn cells were plated onto fibronectin substrates (10 μg/ml) for six hours, lysed, separated by SDS PAGE and analyzed by Western blot for EGFR. The blot was scanned and assigned relative value units (rvu) (see Materials and Methods section).

Actin was used as a loading control to assure that equivalent protein was loaded. Expression of EGFR was threefold higher in the SCC9β6 cells when compared to the parental SCC9SN cells (**FIGURE 4**). In addition, when SCC9SN cells are transfected with a constitutively active Fyn the expression of EGFR is increased by threefold (**FIGURE 4**). As the SCC9SN cells are β6 negative, this implicated Fyn kinase in modulating EGFR expression. In direct contrast, the expression of EGFR by the SCC9β6KDFyn cells was reduced sevenfold (**FIGURE 4**). These results demonstrate

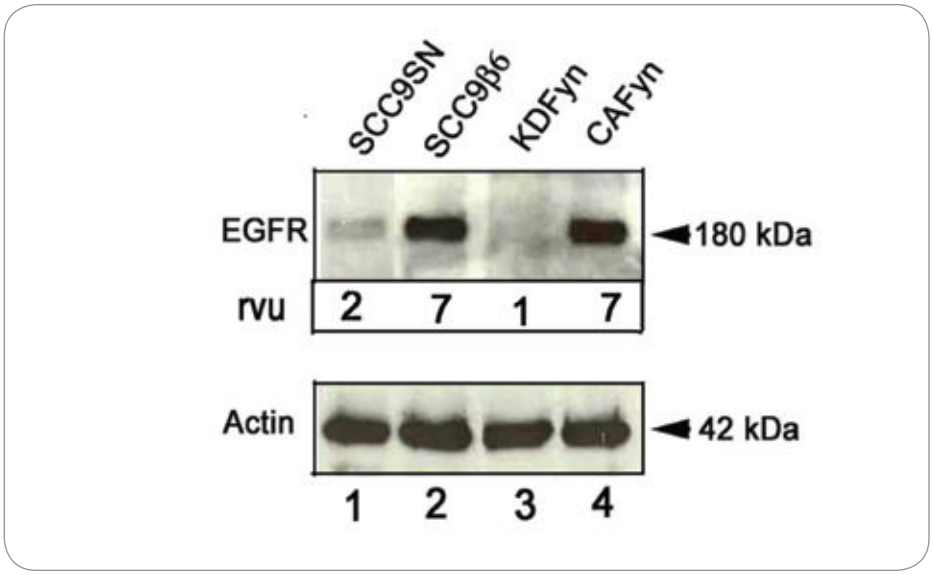

FIGURE 4. Expression of EGFR is modulated by Fyn Kinase. SCC9SN (lane 1); SCC9β6 (lane 2); SCC9β6KDFyn (lane 3) and SCC9CAFyn (lane 4) were plated onto 10μg/ml of fibronectin for 6h. The cells were lysed and separated by SDS-PAGE, and analyzed by Western blot. Relative value units (rvu) were assigned. Note the threefold increase in EGFR when SCC9β6 are compared to SCC9SN (lanes 2 and 1, respectively). Note the almost complete loss of EGFR when cells express kinase dead Fyn (lane 3) compared to the SCC9β6 cells. These results clearly demonstrate the expression of EGFR is sensitive to Fyn kinase activation.

that the expression of EGFR is regulated by activation of Fyn independent of β6 integrin ligation. The significant increase in EGFR expression by the SCC9CAFyn cells shows the importance of the downstream signaling event as the SCC9CAFyn cell line is αvβ6 negative.

Discussion

The progression of oral cancer to an invasive and metastatic stage is the cause of high morbidity and mortality of this disease. The process of cells moving between mesenchymal and epithelial phenotypes is required to promote and direct the invasive process. Metastability is defined as cells with features of both epithelial and mesenchymal cells and this feature is inherent in many oral SCC cells.[5]

One of the defining moments in tumor cell invasion is the breaking off of cells from the primary tumor. From that point forward, interactions with the extracellular matrix are continually modified and result in a variety of novel signals to the tumor cells, via specific cell surface receptors. A variety of growth factors and extracellular matrix molecules also contribute to this process.[10] The authors supported this idea and previously documented the relevance of growth factors such as TGFβ1 in oral cancer progression.[11]

The majority of human epithelial cancers are marked by the activation of a variety of growth factors and receptors of the epidermal growth factor receptor family.[12] EGFR was the first growth factor receptor to be proposed as a target for cancer therapy more than 20 years ago.[12] EGFR exists on the cell surface and is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α (TGFα). As more than 90 percent of EGFR mutations affect the kinase domain, a host of drugs targeting the EGFR ATP binding site have been in use for some time. For example, Cetuximab has been used as adjunct therapy with radiation.[12]

In addition, several small molecule inhibitors directed at the kinase activity of EGFR have also been employed. Although initially impressive, the long-term effectiveness of these drugs has not been as promising. The authors were curious to understand the problem using conventional anti-EGFR therapy. In order to do this, the authors wanted to characterize the expression of EGFR using αvβ6, an established marker of invasiveness as a reference point. The authors identified both EGFR and αvβ6 to the leading edge of the tumor nests, thus being in the perfect position to take advantage of released growth factors as the ECM is modified by MMP activity or simple mechanical distortion. Tissue section immunofluorescence microscopy clearly demonstrated the solid front these two molecules occupy, embedded within the cell membrane as it warps itself manipulating and modifying the surrounding matrix in vivo.

The preliminary portion of the study identified a situation in vivo that the authors wanted to evaluate in a controlled cell culture environment. When plated on fibronectin the SCC9β6, cells assumed a fibroblast-like morphology, and expressed a variety of molecules associated with the mesenchymal phenotype, such as vimentin and MMP3. This contrasted with the epithelial appearing SCC9SN cells. Antibodies to EGFR were highly reactive with the SCC9β6 cells and not with the SCC9SN cells. The authors reviewed this biochemically by Western blot and found that overexpression of β6 increased EGFR expression through activation of Fyn kinase. Interestingly, the expression of a constitutively active Fyn, even in the absence of αvβ6, stimulates EGFR expression.

In conclusion, the authors' findings outline a mechanism by which invasive oral SCC cells modulate EGFR expression. Most invasive oral cancer cells are positive for the αvβ6 integrin. The authors now implicate integrin β6 as the founding step in promoting EGFR expression, which is perpetuated via Fyn kinase. Work by others has previously associated EGFR signaling through Fyn kinase and α6β4 integrin.[13] In their study, they observed that EGFR causes

disassembly of hemidesmosomes by activation of Fyn, which, in turn phosphorylated β_4 integrin cytoplasmic domain. It is likely that EGFR function is intimately associated with Fyn kinase activation via integrin $\alpha 6\beta_4$ and $\alpha v\beta 6$. These results indicate that Fyn kinase needs to be considered when targeting EGFR with future chemotherapeutic agents. ■■■■

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UNIVERSITY OF SOUTHERN CALIFORNIA

The University of Southern California's School of Dentistry strives for research to be an integral component of the dental school education and encourages dental students to participate in basic, clinical, or biobehavioral research.

USCSD sponsors a Summer Research Fellowship Program for a select group of incoming, highly motivated DDS students who apply for positions as research assistants. If selected, the students are placed with a faculty mentor who guides them on an independent research project for 10 weeks during the summer. The fellows periodically meet with faculty and other student researchers to discuss their projects and to highlight their findings. A goal of each fellow is to produce data sufficient for a presentation at a professional meeting and hopefully for a peer-reviewed paper in a research journal.

Role of the Transcription Factor NFIC in Odontoblast Gene Expression

MI YOUNG KIM; JULIA REYNA, PHD; LI-SHA CHEN, BS; AND MAGGIE ZEICHNER-DAVID, PHD

ABSTRACT The transcription factor NFI-C is essential for root development. Mice lacking NFI-C develop abnormal roots and lose their teeth, resembling radicular dentin dysplasia I in humans. The purpose of this study was to understand the role of NFI-C in dentinogenesis. The authors found statistically significant increases in the expression of several mRNAs in cells lacking NFI-C, suggesting that these molecules might interfere with odontoblast cell migration and differentiation, and consequently with root development.

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Transcription factors are proteins that bind to specific areas of the DNA sequence and control the transcription of DNA to RNA, therefore determining the phenotype of that particular cell. The nuclear factor I (NFI) family of transcription factors were first described as being required for the replication of viruses and for the transcription of many developmentally regulated and tissue-specific genes. NFIs are site-specific DNA binding proteins that bind to the consensus DNA sequence TTGGC(N)5GCCAA, for which are also known as CTF or CAAT box transcription factors. NFI binding sites have been identified in the promoter, enhancer, and silencer regions of more than 100 genes.[1] In vertebrates there are four members of the NFI family: NFI-A, NFI-B, NFI-C and NFI-X, that appear to be expressed in overlapping, as well as distinct patterns during mouse embryogenesis and adult tissues.[2]

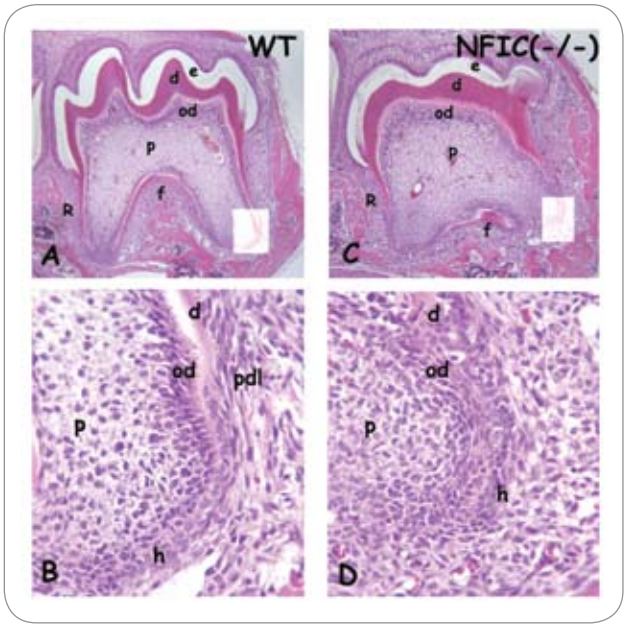

FIGURE 1. Histological comparison of normal and NFI-C lacking developing molars. Mouse maxillary first molars at 14 days postnatal were obtained from wild-type (WT) mice (**A AND B**) and mice lacking NFI-C [NFIC (-/-)] in **C AND D**. Samples were fixed in formalin, embedded in paraffin and processed for histological analysis after staining with H&E. An equivalent apical end of the growing root (clear squares in **A AND C**) from each molar was observed at higher magnification (40x) and shown in pictures **B AND D**. Different structures labeled in the pictures are: e=enamel; d=dentin; od=odontoblasts, p=dental pulp; f=furcae; R=roots; pdl=periodontal ligament and h=Hertwig's epithelial root sheath.

The ability to remove (knock down) or add (overexpression) genes to animal models, particularly mice, has been crucial to understand the function of many proteins and mice lacking each one of the NFI family members have been created showing strikingly different phenotypes. More than 95 percent of mice lacking NFI-A died shortly after birth, the few survivors developed hydrocephalus and tremors because they lacked a corpus callosum (major fiber tract connecting the two brain hemispheres); no other major defects were noticed.[3] Mice lacking NFI-B died after a few hours of being born and displayed severe lung hypoplasia.[4] The NFI-X null animals died after three weeks of being born and presented with hydrocephalus and partial agenesis of the corpus callosum.

Additionally, these animals had skeletal problems due to a delay in ossification and decreased mineralization with progressive degeneration of intervertebral disks that resulted in deformation of the spine. It appeared that down-regulation of tetranectin (a plasminogen binding protein involved in mineralization) was responsible for the skeletal defects in these mice.[5] Mice null for tetranectin showed the same skeletal abnormalities.[6] Mice lacking NFI-C were accidentally found to be essential for tooth development, particularly roots formation.[7] The NFI-C null mice appeared normal for a few weeks and then they started losing weight, failed to thrive, and died of starvation. A closer analysis of these mice indicated that mandibular incisors and mandibular and maxillary molars lacked roots, therefore, there was no periodontium, no attachment, the teeth exfoliated easily, and the mice could not eat. Once the animals were placed on a soft diet, they thrived like their normal littermates. No defects in any other organ were detected.[7]

Histological analysis of the molars of mice lacking NFI-C [NFIC(-/-)] demonstrated no changes in crown development, a normal layer of enamel and dentin were clearly seen. However, cellular and morphological changes associated with root development were evident; roots were very short compared to the normal wild-type (WT) roots, there was no furcation (f) demarcating the roots, no clear layer of dentin, and there was disorganization of the structures in the developing roots (**FIGURE 1**). Since the crown of the molars were not affected, these results indicated that the transcription factor NFI-C represented the first transcription factor associated exclusively with root development, and it appeared to be a key regulator of this process.

There are human genetic diseases associated with short or absent roots such as radicular dentin dysplasia type I (DDI), also known as "rootless teeth," a rare dentin defect that appears to be inherited as an autosomal dominant condition with a reported frequency of 1:100,000 persons. Both primary and permanent teeth were affected. Clinically, the dental crowns appeared normal while radiographically the teeth were characterized by pulpal obliteration and short, blunted roots.[8]

The teeth were mobile, frequently abscessed, and were lost prematurely. On light microscopic examination of the permanent teeth, the coronal dentin was normal, but further apically became irregular, filled the pulp chamber, and had a "sand dune" morphology.[9,10] There was no known specific treatment approach for DD type I except for preventive measures to increase longevity of the dentition such as trying to keep occlusal forces to a minimum and avoiding orthodontic treatment for the misaligned teeth. Recent studies in a family with RDD I suggested that there is a mutation in the NFI-C gene.[11]

Given the important functional role of NFI-C and its clinical relevance in human disease, it becomes very important to understand the cellular and molecular effects of NFI-C in root development. Since there are numerous genes that can be induced or repressed by NFI-C, and NFI-C is expressed in several odontogenic cells, and radicular dentin is particularly affected, in this study, the authors determined the genes affected by NFI-C in odontoblast cells using DNA microarray technology.[7,12-15]

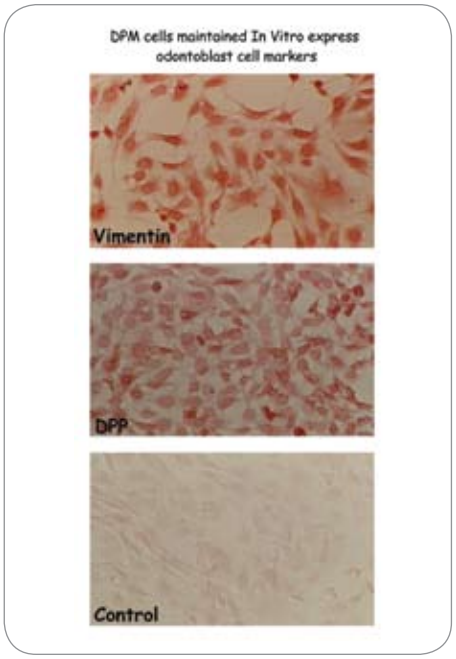

FIGURE 2. Expression of odontoblast-markers by DPMA-5 cells. The DPMA-5 cell line was grown in culture under differentiation conditions. Antibodies against specific markers for mesenchymal cells (Vimentin) and odontoblast cells (dentin phosphoprotein (DPP) were used for immunocytochemical characterization of these cells. The control was subjected to the same treatment except that no primary antibody was used to rule out nonspecific staining, 100x magnification.

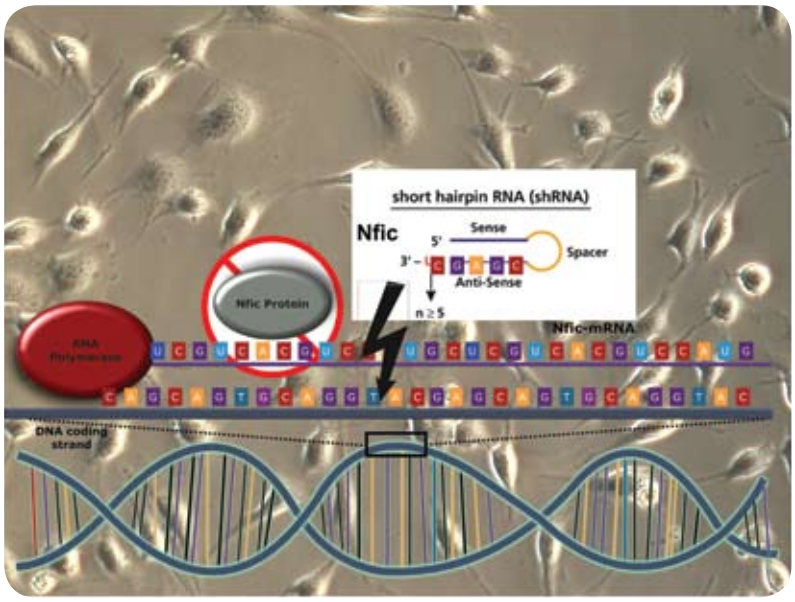

FIGURE 3. Diagram representing the RNA silencing (shRNA) technology. This diagram represents how the expression of the transcription factor NFI-C can be silenced using short hairpin RNA (shRNA). Once the NFIC-shRNA binds to the complementary sequence of the NFIC-mRNA, it destroys the mRNA and therefore there is no protein made.

Histological Analysis of Growing Roots in NFIC-Containing and NFIC-Lacking Molars

The role of NFI-C in odontoblast cell differentiation during root formation was first analyzed at the histological level using light microscopy by comparing normal (WT) vs. NFIC (-/-) developing mice maxillary first molars at 14 days postnatal. As can be seen in **FIGURE 1**, the growing apical portion of the roots in the WT molars (square in **FIGURE 1A** shown in high magnification in **FIGURE 1B**) shows a well-structured layer of polarized odontoblast cells, a well-defined layer of predentin and dentin, and a clear HERS structure. In contrast, the forming root of the NFIC (-/-) shows great structural disorganization starting in the cervical region, there was no apparent structure, there was no well-defined layer of HERS cells, the odontoblasts seem short, not polarized, and appeared in multiple layers bound by some kind of nonmineralized extracellular matrix (**FIGURES 1B AND 1C**). There appeared to be formation of some dentin but looked irregular and sparse, and there was no predentin. HERS were not easily identified and there was no organized structure leading to root formation at the apical end. This data suggested there might be a problem with odontoblast cell polarization and secretion of the extracellular matrix.

Down-Regulation of NFI-C in an Odontoblast-Like Cell Line

Next, the authors wanted to determine changes in the genes expressed by odontoblast cells that might be responsible for the morphological and functional changes seen in the absence of NFI-C. Given the lack of cellular organization in the NFIC (-/-) mice roots, it will be very difficult to isolate pure odontoblast cells, without contamination from other odontogenic cells, to determine changes in gene expression only in these cells. Therefore, the authors decided to use a pure and fully characterized odontoblast-like cell line (DPMA-5) established in their laboratory.[16] This cell line was derived from dental pulp mesenchymal cells that can be maintained in culture indefinitely under proliferation conditions and allowed to differentiate into odontoblast-like cells under differentiation conditions.[16] These cells were neural crest-derived mesenchymal cells that express vimentin (**FIGURE 2**) and they express dentin phosphoprotein (DPP), a well-known marker for odontoblast differentiation.

To determine the effect of NFI-C in odontoblast gene expression, the authors compared gene expression in cells containing NFI-C with those cells lacking NFI-C. Since the DPMA-5 cell line express NFI-C, the authors needed to down-regulate the expression of NFI-C to make these cells similar to the odontoblasts present in the NFIC (-/-) molars. This was achieved using the silencing RNA technology using a short hairpin RNA (shRNA).[17] This method (depicted in **FIGURE 3**) allowed deleting or down-regulating a specific mRNA using a small RNA, which is complementary to a region of the mRNA that needs to be

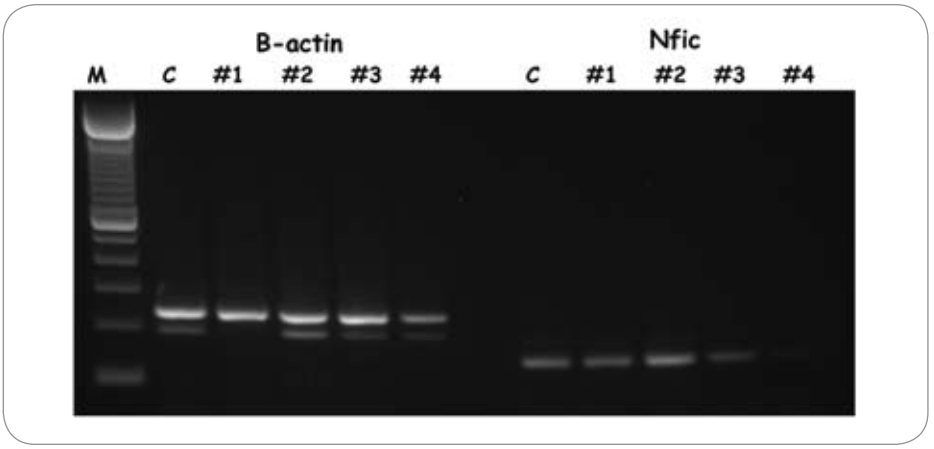

FIGURE 4. Absence of NFIC-mRNA in DPM A-5 cells expressing NFIC-shRNA#4. Four different NFIC-shRNAs were tested for their ability to destroy the NFI-C mRNA in DPMA-5 cells maintained in culture. The expression of Beta-actin (control) and NFI-C were determined using reverse transcription-polymerase chain reaction (RT-PCR) followed by agarose gel electrophoresis. C (control) represents the original DPMA-5 cells and #1-#4 the different NFIC-shRNAs tested. All cells express actin and all cells express NFI-C except the cells treated with NFIC-shRNA#4.

down-regulated (in this case the NFIC mRNA). The shRNA was introduced into the cells using a vector that was then passed to daughter cells, allowing the silencing gene to be inherited.

The shRNA hairpin structure was cleaved by the cellular machinery into siRNA (silencing RNA) that binds and cleaves the target mRNA. Since not all regions of the mRNA respond similarly, different shRNAs to different regions of the mRNA needed to be tested. Four different NFIC-shRNAs were produced and tested using RT-PCR for the presence of NFIC mRNA. **FIGURE 4** shows that the most effective shRNA was NFIC-shRNA 4, eliminating almost all of the NFIC mRNA. The mRNA for β-actin was used as a control to ensure that the NFIC-shRNAs only had an effect on NFIC since β-actin mRNA was still present. A random noncoding shRNA was also used as a control to ensure that the effects seen are due to the elimination of NFI-C in these cells and not by the introduction of a random shRNA (data not shown). These experiments indicated the authors successfully down-regulated the NFIC mRNA with NFIC-shRNA 4 and this shRNA was used to create a stable odontoblast-like cell line lacking NFIC mRNA, which was used in the next experiments.

DNA Microarray Comparison of Gene Expression

Experiments to determine which genes are regulated by NFIC were done using DNA microarray technology. At the present time, DNA microarray technology can determine changes in more than 28,000 mouse genes in one single experiment. However, interpretation of these results can be complicated given the large number of genes that can be differentially expressed. Since previous data suggested there might be a problem with the formation of the extracellular matrix, the authors decided to first use more focused pathway microarrays like the Extracellular and Cell Adhesion mRNAs (SABiosciences, Frederick, Md). This particular type of array uses real-time PCR to measure the amount of mRNA for a specific protein present in the sample to be tested. The Mouse Extracellular Matrix and Adhesion Molecules RT²Profiler PCR Array profiles the expression of 84 genes associated with cell-cell and cell-matrix interactions.

This array contained extracellular matrix (ECM) proteins including basement membrane constituents, collagens, and genes playing a role in ECM structure. Proteases involved in remodeling of the ECM were included, as well as their inhibitors. This array also represented molecules important to cell adhesion including molecules involved in cell-cell and cell-matrix adhesion, trans-membrane molecules, etc. For these experiments, mRNA from the DPM-NFIC-shRNA 4 cell line was extracted and compared with mRNA obtained from the original DPM cell line used as control. Experiments were done in triplicate and the results analyzed using the software provided by the manufacturers that use the Student T-test to calculate the significance of the results. Changes that were statistically significant (P=<0.05) and at least a twofold over or under were summarized in **FIGURE 5,** where mRNAs presented in higher concentrations in the cells lacking NFI-C compared to the control are in red, and mRNAs, whose concentration is lower in the cell lacking NFI-C, are in blue. The major change seen is in the transforming growth factor beta-induced (Tgfbi), which is considerably increased (22.28 fold) in the DPM-NFIC-shRNA 4 as compared with the DPM cells that express NFIC. In addition of Tgfbi, mRNAs for Tnc (tenascin C), Vcan (versican), Col6a1 (collagen, type VI, alpha 1), Lamb2 (laminin, beta 2), Col3a1 (collagen, type III, alpha 1), Timp3 (tissue inhibitor of metalloproteinase 3), Mmp2 (matrix metallopeptidase 2), Ecm1 (extracellular matrix protein 1), thbs2 (thrombospondin 2), and col5a1 (collagen, type V, alpha 1) were also increased. In contrast, mRNAs for Itga3 (integrin alpha 3), Lama1 (laminin, alpha 1) and Ctgf (connective tissue growth factor) were decreased in the DPM cells lacking NFIC.

Results shown as a volcano plot facilitate seeing the major statistically significant changes as can be seen in **FIGURE 6**. The volcano plot graphs the log2 of the fold change in each gene's expression between the samples versus its p value from the t-test. The horizontal middle line indicates fold changes of one.

FIGURE 5. DNA microarray results. The results of the DNA microarrays are presented in this graphic as fold changes of mRNA present in the cells lacking NFI-C or containing NFI-C. Overexpressed mRNAs in NFIC-shRNA cells, compared to normal cells, are in red and underexpressed mRNAs are in blue. The numbers indicate the fold change. Only changes above twofold and statistically significant (P=<0.05) are presented.

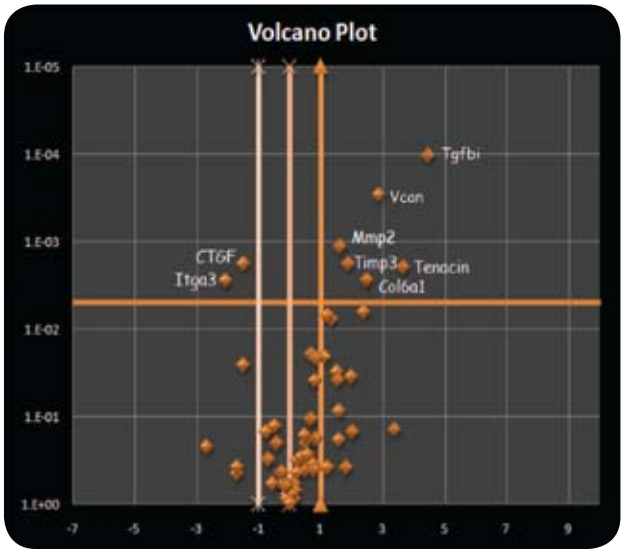

FIGURE 6. Volcano plot graphic of the DNA microarray results. The volcano plot facilitates seeing the major statistically significant changes. The volcano plot graphs the log2 of the fold change in each gene's expression between the samples versus its p value from the t-test. The horizontal middle line indicates fold changes of 1. The lines on each side indicate the desired fold change in gene expression threshold, which were set at twofold. The horizontal line indicates the p value of the t-test threshold, which was set at p=<0.05.

The lines on each side indicate the desired fold change in gene expression threshold, which were set at twofold. The horizontal line indicates the p value of the t-test threshold, which was set at p=<0.05. As can be seen, again, the most significant change is in the increase of Tgfbi followed by Tenascin, Vcan, Mmp2, and Timp3, and the decrease of Ctgf and Itga3.

Conclusions

From the data presented in this study, it is clear that the transcription factor NFIC has no major role in tooth crown formation but it is essential for proper root formation. Without it, there are major problems in the roots, including very short malformed roots, defective dentin, no cementum, and no periodontal ligament, and therefore, no attachment and tooth loss. What is not clear yet is the mechanism by which NFI-C controls cell organization, differentiation, and morphogenesis. In this study, the authors looked at the expression of ECM and cell adhesion proteins regulated by NFI-C in odontoblast cells and found that in the absence of NFI-C expression there was an increase in the expression of several of these mRNAs in the odontoblast-like cell line.

It is well-known that odontoblast cells are in charge of producing and secreting the matrix proteins that form the dentin extracellular matrix, and it is known that dentin is a collagen-based ECM, with collagen type I constituting more than 90 percent of the organic matrix. Furthermore, mutations in the gene for collagen type I were responsible for the hereditary form of dentinogenesis imperfecta type I, which is associated with osteogenesis imperfecta (reviewed in Barron et al., 2008). It is believed that collagen type I defines the framework for mineral deposition and that dentin phosphoprotein binds to this collagen to facilitate nucleation of hydroxyapatite and mineralized dentin formation.[18]

In addition of collagen type I, the expression of collagens type 3, 4, 5, and 6 in odontoblast cells has long been demonstrated.[19] The exact role of these collagens in dentin formation is not yet known. The authors' study found no changes in collagen type I; however, there was a statistically significant increase in the expression of collagens 3a1, 5a1, and 6a1 in odontoblast-like cells lacking expression of the NFI-C factor. This increase might explain the presence of a nonmineralized ECM in the area where preodontoblast cells were supposed to differentiate in the growing apical root of NFIC (-/-) molars.

The transforming growth factor beta-induced (TGFBI) gene encodes the transforming growth factor beta-induced protein (also known as βig-h3, keratoepithelin, RGD-CAP, and MP78) was first identified in an adenocarcinoma cell line where it was found to be upregulated upon addition of transforming growth factor beta (TGF-β). It has now been shown that it is expressed in a wide range of tissues in several species. Elevated expression levels are particularly observed in zones of active growth and high levels of Tgfbi are found in the skin, bone, kidney, and cornea. Tgfbi is thought to function as a cell adhesion protein because of the presence of

structural elements like the FAS domains, which are known to possess cell binding properties and because of the presence of the integrin binding RGD motif.

Furthermore, in vitro studies have shown that Tgfbi mediates cell adhesion and/or spreading through integrins $\alpha_1\beta_1$, $\alpha_3\beta_1$, $\alpha v\beta_3$, $\alpha v\beta_5$, $\alpha 6\beta_4$ and $\alpha_7\beta_1$. In addition to integrins, Tgfbi interacts with several components of the ECM like collagens, fibronectin, decorin, and biglycan, thus suggesting that Tgfbi may be involved in cell-matrix interactions, cell adhesion, migration, and differentiation. Mutations in Tgfbi have been found in several distinct autosomal dominant genetically determined corneal disorders.[21] These diseases are characterized by the progressive accumulation of cloudy material in the cornea leading to impaired vision. Tgfbi is overexpressed in colon, pancreatic, and liver cancers.[22]

Perhaps the excess amount of Tgfbi in odontoblast-like cells lacking NFI-C might make the cells more "sticky" and delay migration and differentiation of these cells during root formation. The expression of Tgfbi in preodontoblast or odontoblast cells or any other odontogenic cells has not been reported suggesting it is not expressed during normal tooth or root development. The expression of this protein in the absence of NFI-C implied that NFI-C might be a repressor of Tgfbi expression. Further experiments are required to test this hypothesis.

Tenascins are a family of large ECM proteins. There are four tenascins termed tenascin-C, -R, -X and -W present in connective tissues and each has a specific expression pattern. Tenascins promote only weak cell adhesion, do not activate cell spreading, and they have been classified as anti-adhesive, adhesion-modulating, or even repellent ECM proteins. Tenascin-C deficient mice show abnormalities in the nervous system and defects in several regenerative processes while overexpression is found in tumor stroma.

Tenascins are also known to influence cell shape, migration, and growth.[23] Tenascin-C is transiently expressed in the condensed dental mesenchyme during initial stages of tooth development and reappears later in the dental papilla mesenchyme where it persists in the dental pulp but is down-regulated in odontoblasts.[24] Perhaps the increase in tenascin-c expression in odontoblast-like cells lacking NFI-C suggest a repressor activity for this transcription factor and might be associated with the lack of polarization and differentiation of typical odontoblast cells during root dentin formation.

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Versican is a large chondroitin sulfate proteoglycan and it is considered an anti-adhesive molecule. It is believed to be able to bind hyaluronan to form large aggregate structures and it is present in dental pulp, being more abundant in the coronal pulp and subodontoblastic layer of the coronal pulp by the completion of crown formation and less abundant in the radicular pulp.[25] Nevertheless, its role in tooth development and dentin formation is still unknown and the significance of its increased expression in cells lacking NFI-C remains to be determined.

ECM1 has been found to inhibit the activity of MMP9 by binding directly with this protease.[26] It has also been suggested that it has angiogenic properties and is present in breast tumor cells. ECM1 has been shown to regulate endochondral bone formation, stimulate the proliferation of endothelial cells, and induce angiogenesis. In low doses it can stimulate alkaline phosphatase activity while at higher concentrations, it can inhibit alkaline phosphatase activity and mineralization.[27] The role of ECM-1, if any, in tooth development is unknown.

The MMP2 gene codes for a matrix metalloproteinase, MMP, which is involved in the breakdown of extracellular matrix in normal physiological processes. MMPs are responsible for regulating the breakdown of ECM during development, and an increase in MMP2 has been found in processes such as tooth eruption and root resorption.[28]

It has been also found in the dental papilla, and it is believed to be involved in the remodeling of these tissues.[29] Furthermore, it has been reported that MMP2 and MMP20 are responsible for the processing of DSPP into DSP and DPP, which is required for dentin formation.[30] The fact that MMP2 is up-regulated significantly in NFI-C silenced cells might suggest an increase in ECM remodeling. However, TIMP3, which is an inhibitor of MMPs, is also up-regulated and might neutralize the effects of MMP2.

The laminin family is composed of basement membrane proteins that have been implicated in diverse functions of epithelial and mesenchymal cells. During tooth development, the mRNA of three laminin chains, 1, 2, and 4, are expressed in tooth mesenchymal cells, whereas two

other types, laminin 3 and 5 chain mRNA, are found in epithelial cells.[31] The role of Lama1 in odontoblast cell differentiation is not known; however, Lama2 has been shown to be essential for odontoblast cell differentiation.[32] Perhaps the down-regulation of Laminin a1 might play a role in the delayed differentiation of odontoblasts in the NFI-C null roots. However, since the increase in Lamb2, and the decrease in Lama1, Itga3 and ctgf are also seen in cells treated with the noncoding shRNA, the authors assume this effect is associated with the process of shRNA expression but not necessarily with the effect of NFI-C on these cells.

In conclusion, taken all together the authors' data indicates that the transcription factor NFI-C has a direct effect of the expression of genes associated with the ECM and cell adhesion in odontoblast-like cells and this effect appears to be that of repressor of their expression. Perhaps an increase in these molecules interferes with the proper migration and differentiation of odontoblast cells during radicular denting formation. This might explain the defects seen in the patients affected with RDD-I. Other aberrant changes in odontoblasts cells during root development and loss of intercellular junctions and the decreased expression of ZO-1 and occluding have been reported.[12,14,15]

Nevertheless, since odontoblast differentiation is the result of epithelial-mesenchymal interactions, the epithelial influence odontoblast differentiation can't be ignored, and in the case of root development, that will be the role of the Hertwig's epithelial root sheath (HERS). This might explain the difference between the normal crown dentin and the abnormal radicular dentin. Another possibility is that crown and root odontoblasts are different. These possibilities are currently being explored in the authors' laboratory. ■■■■

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Mouthin' Off

The Navajo language has no alphabet or symbols, is unwritten, and is of extreme complexity.

Robert E. Horseman, DDS

ILLUSTRATION BY CHARLIE O. HAYWARD

During World War II when secret military codes were being invented and successfully decoded by both sides on a daily basis, there was a continual search for a language that could not be deciphered by the enemy. It wasn't until Pig Latin was finally solved by shrewd German cryptographers, that the Allies came upon a communication tool that could not be understood by anybody but a handful of Navajo Indians, called "code talkers." The Navajo language has no alphabet or symbols, is unwritten, and is of extreme complexity. A Navajo's horse might understand it — at least to the extent of obeying "stop" and "go," or working out that its rider wanted to go to Flagstaff, not Tucson — but to the rest of the non-Navajo world, it was no more intelligible than a Robin Williams unscripted riff.

Secrecy was de rigueur during wartime, but was the exact opposite of the goal of early 1800s America when the term "melting pot" was applied to a burgeoning nation. Disambiguation, a six-syllable word roughly translated as "everybody speak American/English so we can all understand each other," was not 100 percent successfully promoted. Ethnic groups tended to respond with their version of "This has the odor of a three-day-old fish!"

Unfortunate misinterpretations between citizens of states such as New Jersey and Mississippi occurred when the spelling of words had no relation to the pronunciation. "Noo Joisey" and the word "yawl" that perversely did not refer to a boat, but a group of people, were typical points of contention.

Offering a simple solution to the dilemma of so many diverse languages and dialects was Dr. Ludovic Lazarus Zamenhof, a Jewish ophthalmologist, writing a book titled *Unua Libro* under the pseudonym Doktoro Esperanto. In it, he proposed an entirely new language,

CONTINUES ON 917

DR. BOB, CONTINUED FROM 918

Esperanto, that in Esperanto means "one who hopes." It would serve as a universal, flexible second language to foster peace and international understanding. Published in 1887, Esperanto has not been as successful in fostering peace and international understanding as hoped, according to reports from the Middle East.

For example, this sentence in Esperanto reads: En multaj lokoj de Ĉinio estis temploj de drako-regô. Esperantists claim this means "In many places in China there were temples of the dragon king."

To others it sounds like "the pen lies on the table of my aunt" essayed by a first-year French student with peanut butter appliquéd to his palate. Obviously, Docktor Esperanto's concept needs a little more time.

As a student at Frances E. Willard Junior High School in the early '30s, I had successfully transited the rite of passage for boys of that age wherein we traded in our baggy, buckled-at-the-knee "knickerbockers" for long pants. Possibly the dorkiest outfit since the demise of the Lord Fauntleroy suit, wearing knickers beyond the sixth grade would have gotten you beaten up after school faster than having a name like Percy. Without access to tattoos or lip piercings, a youth needed more than long pants to cope with puberty.

Nowhere in the narrow adolescent view of the Frances E. Willard student body was a desire to embrace a new language designed to be spoken and understood by everyone. Quite the opposite. Esperanto, had we even been aware of it, would have occupied a position well south of Latin and algebra, especially if there was homework involved. Instead, having exhausted the exclusiveness of Pig Latin, which had now been mastered by 4-year-olds, we perfected a language of our own and became the code talkers for our generation, or at least those who attended our school. The language had no name, nobody took credit for devising it or knew where it came from, but once it was common knowledge that adults could not understand it or learn it, it was ours to protect and cherish. Mrs. Willard, suffragist and president of the United States Christian Temperance Union, would have been proud of our pursuit of knowledge had she lived an additional 36 years to bear witness.

Unfortunately, NATO and the Department of Homeland Security have kindly, but firmly rejected my offer to teach the vanishing patois of my youth to our cryptologists in the present international unrest. In offering the secret code to you, please be aware of an obligation — should I be unavailable — to accompany a couple of large men in black suits tethered to a brace of AKC-registered Dobermans who may appear suddenly on your doorstep one day.

Please pay attention, I shall say this but once: The basic elements are "la" and "f." The word "basic" then becomes bala-fasic, pronounced bayla-faysic. "Elements" becomes ela-felaments. Multiple syllable words have to be broken down a little more. "United States government" is U-nila-fited Stayla-fates gula-fuvern-ment. Practice this at home. Soon you will notice that few, if any, people will understand you unless they are alumni of Frances E. Willard Junior High School.

When you get to the point where a téte-a-téte held with a Navajo results in neither of you understanding the other, you will know your efforts have not been in vain. Your children will regard you with new respect and you can write your own tilafickit. ■■■■