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In vitro caries formation in primary tooth enamel

Role of argon laser irradiation and remineralizing solution treatment

Gary H. Westerman, DDS, MS; M. John Hicks, DDS, MS, PhD, MD; Catherine M. Flaitz, DDS, MS; G. Lynn Powell, DDS

Despite the decline in dental caries prevalence in the primary dentition, as documented in the First and Third National Health and Nutrition Examination Surveys,¹⁻³ dental caries remains the most common chronic childhood disease.⁴ This transmittable bacterial disease affects more children than any other disorder and is particularly prevalent in families with low socioeconomic status⁵⁻⁹ and in immunocompromised children.^{10,11} Early childhood caries appears to be on the rise and is considered by some to be an epidemic.^{12,13} The American Academy of Pediatric Dentistry Policy Statement on the Dental Home advocates early oral health examinations and early dental treatment for preventive measures and restorative needs.¹⁴ This policy encourages establishing a “dental home” early in life and recommends that the first dental visit should occur before 12 months of age.

Prevention of the complex multifactorial disease dental caries requires a risk assessment for future caries development and the institution of appropriate preventive modalities and oral hygiene

ABSTRACT

Background. The authors evaluated the effects of argon laser (AL) irradiation and remineralizing solution (RS) treatment alone and in combination on carieslike lesion formation in primary tooth enamel in an in vitro study.

Materials and Methods. The authors divided 10 caries free primary tooth enamel surfaces into four segments and assigned them to one of four treatment groups: no treatment control, AL irradiation alone at 13.5 joules per square centimeters (0.270 watts, 5-millimeter beam, 10 seconds), RS treatment alone for two minutes and AL irradiation before RS treatment. The authors created in vitro caries using a modified ten Cate solution. They evaluated longitudinal sections (three per tooth segment, 30 per treatment group) for mean lesion depth.

Results. After lesion formation, mean lesion depths (\pm standard deviation) were 179 ± 16 micrometers for the no treatment controls, $137 \pm 19 \mu\text{m}$ for AL irradiation alone, $87 \pm 9 \mu\text{m}$ for RS treatment alone and $68 \pm 12 \mu\text{m}$ for AL irradiation before RS treatment. All treatment groups had mean lesion depths that were significantly less than those for the matched no-treatment control group (analysis of variance [ANOVA], Duncan multiple range [DMR] test, $P < .05$). AL irradiation before RS treatment significantly reduced lesion depth compared with AL irradiation alone or RS treatment alone (ANOVA, DMR test, $P < .05$).

Conclusions. The maximum reduction in lesion depth in primary tooth enamel was achieved when the RS—which contained calcium, phosphate and fluoride in a carbopol base—was combined with AL irradiation.

Clinical Implications. It would appear that to improve clinical caries resistance to enamel dissolution, AL irradiation before RS treatment could be used.

Key Words. Primary teeth; enamel; caries; argon laser; calcifying solution; remineralization; demineralization; artificial caries.

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education.^{13,15-19} Preventive modalities include use of systemic and topical fluoride, application of fluoride varnish, reduction of dietary cariogenic refined carbohydrates, use of improved plaque removal and oral hygiene techniques, placement of pit and fissure sealants, use of fluoride-releasing preventive and restorative materials, and the prescribing of antimicrobials such as chlorhexidine gluconate. A relatively simple and noninvasive caries preventive regimen is treating primary and permanent tooth enamel with low-fluence argon laser (AL) irradiation, either alone or in combination with topical fluoride treatment, resulting in reduced enamel solubility and dissolution rates.²⁰⁻³¹

A recent *in vitro* study³² evaluated the influence of a commercially available remineralizing solution (RS) on caries formation and its progression in permanent tooth enamel. The broad-spectrum RS—which contained calcium, phosphate and fluoride ions in a carbopol base—enhanced enamel resistance to initial caries formation and caries progression. The ability of the enamel to resist *in vitro* caries formation was improved significantly over that of matched enamel surfaces treated with an acidulated phosphate fluoride (APF) gel.

The effect of the RS on primary tooth enamel is not known. Therefore, we conducted this *in vitro* study to evaluate the effects of AL irradiation alone and RS treatment alone and in combination on caries-like lesion formations in primary tooth enamel using polarized light microscopic techniques.

MATERIALS AND METHODS

We selected 10 extracted or exfoliated primary teeth with macroscopically caries-free buccal and lingual enamel surfaces, as determined with a stereo-zoom dissecting microscope (original magnification $\times 16$, for this *in vitro* study. After we performed soft-tissue débridement and fluoride-free prophylaxis, we applied an acid-resistant varnish to the teeth, leaving two buccal and two lingual windows of sound enamel exposed. We divided the teeth into four segments (distobuccal, mesiobuccal, distolingual and mesiolingual) to provide four segments with sound enamel windows from each tooth and assigned each segment to one of the four treatment groups. This allowed each tooth to serve as a matched internal control for each of the treatments. The four treatment groups were no-treatment control, AL irradiation

alone, RS treatment alone and AL irradiation before RS treatment.

We carried out AL irradiation using an AL unit (ARAGO, LaserMed, West Jordan, Utah) with low-fluence irradiation at 13.5 joules per square centimeter (0.270 watts, 5-millimeter beam, 10 seconds). We applied the RS (Remin+, Raintree Essix, Metairie, La.) to the enamel surface for two minutes per the manufacturer's recommendation, followed by copious air-water spray rinsing. The RS contained calcium, phosphate and sodium fluoride in a carbopol base (trisodium phosphate, calcium phosphate, calcium chloride, dibasic sodium phosphate, sodium fluoride, carbopol).

We rinsed the tooth specimens in distilled/deionized water and then exposed them to synthetic saliva (20 millimolar sodium carbonate, 3 mmol/L phosphate, 1 mmol/L calcium, pH 7.0) for 24 hours. We created *in vitro* caries-like enamel lesions using a modified ten Cate solution (2.2 mM calcium, 2.2 mM phosphate, 5.0 mM fluoride, pH 3.9). After exposing the specimens to the artificial caries solution for seven days, we prepared three longitudinal sections from each tooth segment, resulting in 30 caries-risk sites per treatment group. We imbibed the longitudinal sections with water and examined them with polarized light microscopy in a blinded fashion. We captured and evaluated images of the lesions using a computer-interfaced image software program (UTHSCSA ImageTool, Version 3.0 Final, University of Texas Health Science Center at San Antonio, "http://ddsdx.uthscsa.edu/dig/download.html") for mean lesion depth determination. We made comparisons among the four treatment groups (no treatment control, AL irradiation alone, RS treatment alone, AL irradiation before RS treatment) using analysis of variance (ANOVA) and Duncan multiple range test analysis for paired samples (alpha level of $\geq P < .05$).

RESULTS

The table shows mean lesion depths for the four treatment groups. When compared with the matched no-treatment control group (179 ± 16 micrometers), mean lesion depths were significantly less for AL-irradiation-alone group (137 ± 19 μm), RS-treatment-alone group (87 ± 9 μm) and the AL-irradiation-before-RS-treatment group (68 ± 12 μm). Mean lesion depths decreased by 23 percent for the AL-irradiation-alone group compared with the matched no-treatment control

TABLE

Lesion depth by treatment group.				
TREATMENT GROUP (NO. OF LESIONS)	MEAN LESION DEPTH (\pm STANDARD DEVIATION)	REDUCTION IN LESION DEPTH (%) VERSUS CONTROL	REDUCTION IN LESION DEPTH (%) VERSUS AL* ALONE	REDUCTION IN LESION DEPTH (%) VERSUS RS† TREATMENT ALONE
No-Treatment Control (30)	179 \pm 16 micrometers	—	—	—
AL Irradiation Alone (30)	137 \pm 19 μ m	23‡	—	—
RS Treatment Alone (30)	87 \pm 9 μ m	51‡	36§	—
AL Irradiation Before RS Treatment (30)	68 \pm 12 μ m	62‡	50§	22¶

* AL: Argon laser.
† RS: Remineralizing solution.
‡ Analysis of variance and Duncan multiple range test: $P < .05$ compared with the no treatment control group.
§ $P < .05$ compared with the AL irradiation alone group.
¶ $P < .05$ compared with the RS alone group.

group ($P < .05$). A comparison of mean lesion depths between the no-treatment control and the RS-treatment-alone groups revealed a 51 percent reduction in mean lesion depth ($P < .05$). The mean lesion depth for the AL-irradiation-before-RS-treatment group was 62 percent less than that for the no-treatment control group ($P < .05$). When compared with the AL-irradiation-alone group, the RS-treatment-alone group had an additional 36 percent reduction in mean lesion depth ($P < .05$). AL irradiation before RS treatment significantly reduced mean lesion depth compared with either AL irradiation alone (50 percent reduction, $P < .05$) or RS treatment alone (22 percent reduction, $P < .05$).

The histopathologic appearances of the representative carieslike enamel lesions from each group (Figures 1-4) may be correlated readily with the mean lesion depth findings among the four treatment groups. The representative lesions from each of the three treatment groups (Figures 2-4) demonstrated dramatic decreases in the body of the lesion depths compared with the representative lesion from the no-treatment control group (Figure 1). Not only were lesion depths affected, but also we noticed certain qualitative differences with the lesions. The body of the lesion in the AL-irradiation-before-RS-treatment group (Figure 4) showed a qualitatively decreased degree of positive birefringence (> 5 percent pore volume) and a negatively birefringent surface zone (5 percent pore volume). This indicates a lessened degree of demineralization. In contrast, the no-treatment control lesion (Figure 1) showed a relatively high degree of positive birefringence qualitatively,

with loss of the typical striae of Retzius and prism markings within the body of the lesion.

While the surface enamels overlying the carieslike lesions for all four groups were intact, we noted a certain degree of irregularity in the surface enamel in the no-treatment control (Figure 1) and AL-irradiation-alone (Figure 2) groups. The lesions in these groups had positively birefringent surface layers (> 5 percent pore volume) that merged with their underlying positively birefringent bodies. In contrast, with the lesion in the RS-treatment-alone group (Figure 3), the surface zone was composed of interspersed areas of pseudoisotropy (5 percent pore volume) and negative birefringence (< 5 percent pore volume), which are indicative of a lessened mineral loss, compared with the lesions in the no-treatment control (Figure 1) and AL-irradiation-alone (Figure 2) groups. With the lesion in the AL-irradiation-before-RS-treatment group (Figure 4), the surface zone was uniformly negatively birefringent (< 5 percent pore volume) and composed a considerable proportion of the entire lesion depth (at least 50 percent).

DISCUSSION

Although the majority of clinicians associate laser use in clinical practice with soft tissue disease management, the U.S. Food and Drug Administration has approved lasers for several hard-tissue applications. These include, but are not limited to, polymerization of visible light-cured preventive and restorative materials, tooth-whitening procedures and cavity preparations for restoration placement.^{15,16,33,34} In the past 13 years,

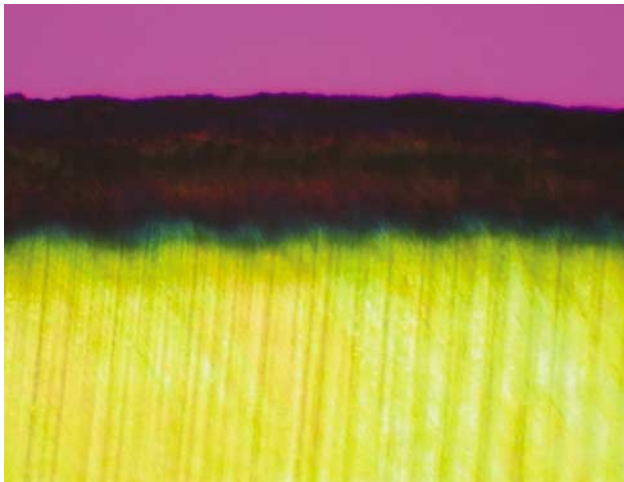


Figure 1. No-treatment control representative lesion (polarized light microscopy, water imbibition, original magnification $\times 200$).

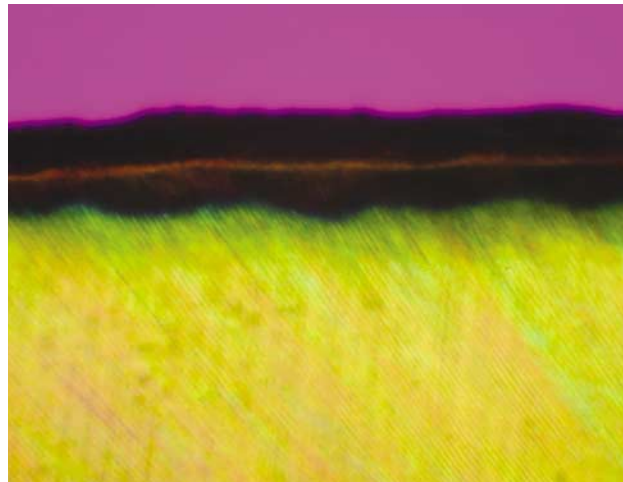


Figure 2. Argon-laser-irradiation-alone representative lesion (polarized light microscopy, water imbibitions, original magnification $\times 200$).

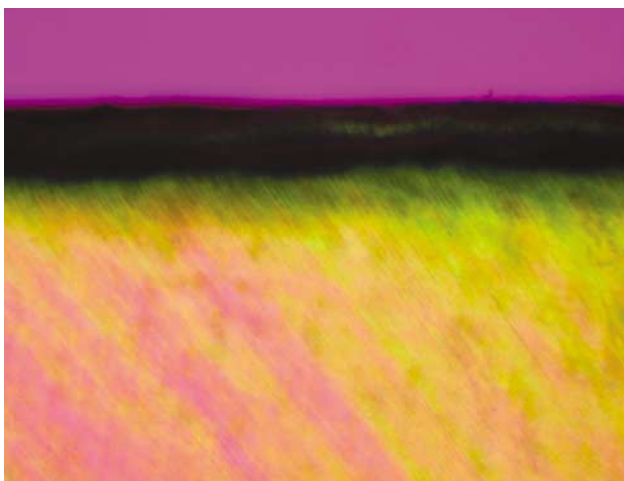


Figure 3. Remineralizing-solution-treatment alone representative lesion (polarized light microscopy, water imbibitions, original magnification $\times 200$).

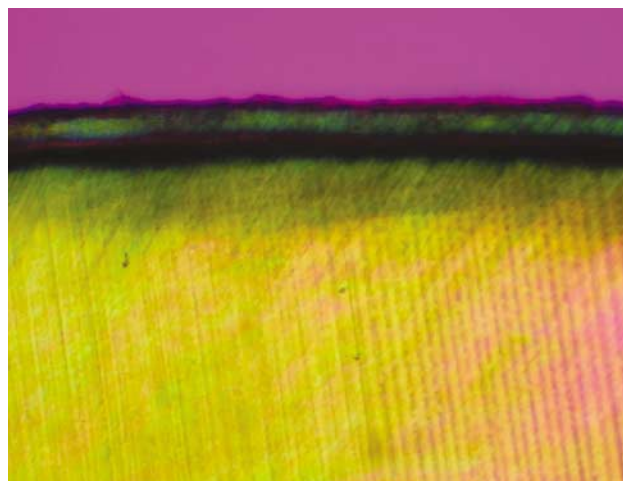


Figure 4. Argon-laser-irradiation-before-remineralizing-solution-treatment representative lesion (polarized light microscopy, water imbibitions, original magnification $\times 200$).

researchers have shown that lasers improve enamel's resistance to dissolution, enhance microhardness, and lessen in vitro and in vivo caries formation and progression.²⁰⁻³¹ In particular, the AL used at low-fluence irradiation levels significantly improves the ability of sound enamel, dentin and root surfaces to ward off a caries like challenge. In vitro caries models that create lesions closely resembling enamel caries have consistently shown a 30 to 40 percent reduction in mean lesion depth after caries initiation.^{20-26,31} Investigators have noted similar reductions when the lesions have undergone in vitro caries progression.^{23,24,31} This reduction in lesion progression was afforded by only a single 10-second exposure of the sound enamel to AL irradiation before

lesion formation. The effect of the AL on sound enamel would appear to endure for a considerable time, as demonstrated by the effect of AL irradiation on in vitro lesion progression.

The mechanisms for the caries resistance^{20,21,31,35} imparted by low-fluence AL irradiation may be related to the creation of a microsieve network within tooth mineral substance. This network within the tooth mineral substance may promote rapid redeposition of calcium and phosphate mineral phases mobilized during a cariogenic challenge in an acidic environment. Also, researchers have suggested that the crystalline structure of tooth mineral substance may be altered and become more resistant to demineralization by removal of organic material and carbonate and

reduction in internal crystalline strain. Caries resistance may be enhanced further by the increased fluoride uptake that occurs with laser treatment. The presence of increased fluoride within tooth mineral substance may result in the formation of less acid-soluble fluoridated hydroxyapatite (FHAP) from more soluble tooth mineral substance phases, such as octacalcium phosphate (OCP), dicalcium phosphate dihydrate (DCPD) and tricalcium phosphate (TCP).^{17-19,32-40} Researchers also have shown that swelling of the organic matrix of tooth mineral substance occurs, and this may make the pore structure of tooth mineral substance less accessible to organic acids produced by dental plaque.^{13,15,16,20,21,31,34,35}

The ability of laser irradiation to affect enamel solubility is reflected by the change in the critical pH at which enamel dissolution occurs⁴¹⁻⁴³; the critical pH for sound enamel is pH 5.5. After laser exposure, the critical pH is reduced to pH 4.8. This means that a fivefold increase in organic acid would be necessary to start demineralization of lased enamel. Using the results from our study, it is evident that AL irradiation of primary tooth enamel alone provided a significant level of caries resistance compared with untreated primary tooth enamel from the matched no-treatment controls ($P < .05$).

Clinical pilot studies^{25,27-29} have confirmed the in vitro caries-resistant effect of AL irradiation.^{20-24,26,30,31} These investigations have taken advantage of the well-established method for creating accelerated natural caries of placing orthodontic bands with open plaque-retentive slots on teeth scheduled for extraction before orthodontic therapy. Well-defined natural caries develop within the enamel adjacent to these plaque-retentive slots during a four- to six-week period. After tooth extraction, this methodology allows for laboratory assessment of the effects of experimental treatment within the confines of the oral environment. These in vivo studies, using the accelerated natural caries model, have found that sound enamel exposed to low-fluence irradiation AL for 10 seconds resulted in a 29 to 44 percent reduction in lesion depth, compared with matched untreated sound enamel.^{25,27-29} When low-fluence AL irradiation was combined with topical fluoride treatment, there was a 62 percent reduction in lesion depth in this accelerated natural caries model. The caries-protective effect of AL irradiation found in these clinical studies is similar to that reported in laboratory investiga-

tions using artificial caries media to produce in vitro enamel lesions.^{20-24,26,30,31} It appears that in vitro techniques successfully mimic the accelerated in vivo natural caries model and provide a certain degree of validity to laboratory in vitro caries methods for evaluating caries-preventive agents.

Recently, an RS (Remin+) became commercially available for use in clinical practice. It contains a proprietary formulation of calcium, phosphate and sodium fluoride in a carbopol base (trisodium phosphate, calcium phosphate, calcium chloride, dibasic sodium phosphate, sodium fluoride and carbopol) and is marketed primarily to orthodontists for the prevention of white-spot lesions adjacent to orthodontic brackets and appliances. Promising results, with respect to enhancing the caries resistance of sound permanent tooth enamel, have emerged from an in vitro caries study comparing the RS with traditional topical APF treatment.³² After caries formation, the RS-treatment-alone group had a 69 percent reduction in lesion depth compared with the matched no-treatment control group and a 46 percent reduction in lesion depth compared with the APF treatment group. The study yielded similar findings when the in vitro carieslike enamel lesions underwent lesion progression. Lesion depth was reduced by more than 50 percent with RS treatment compared with matched no-treatment controls and by slightly less than 40 percent when compared with APF treatment.

In our study of primary tooth enamel, the RS-treatment-alone group had a slightly greater than 50 percent reduction in lesion depth compared with the matched no-treatment control group, and a 36 percent reduction when compared with the AL-irradiation-alone group. The RS provides a source of calcium, phosphate and fluoride in remineralizing hypomineralized or clinically undetectable demineralized sound enamel. The mineral contained in the RS may result in precipitation of fluoride-rich calcium and phosphate mineral phases that could form stable, less soluble mineral phases within the superficial enamel or result in fluoride-rich mineral deposits on the enamel surface that become mobilized during cariogenic challenges.^{13,15,16,19,36-40} These fluoride-rich mineral phases may affect the dissolution rate of enamel.

It is well-known that only a small concentration of fluoride is required for remineralization to be favored over demineralization. Dental plaque

and salivary fluoride concentrations in the range of 0.03 to 0.08 parts per million are required for remineralization to be favored over demineralization, even in acidic conditions.^{13,15-19} Fluoride also may act as a catalyst in the conversion of more soluble, less stable mineral phases (DCPD, TCP, OCP) to less soluble, more stable mineral phases (hydroxyapatite [HAP], FHAP, fluorapatite).^{19,36-40} It also is possible that calcium fluoride may be formed from the mineral and fluoride components in the RS. Calcium fluoride on the tooth surface and within the superficial layers of the enamel acts as a reservoir for fluoride release to the adjacent enamel, dental plaque or saliva.^{19,36-40} During an acidic cariogenic attack, fluoride is released from the calcium fluoride and has been shown to inhibit HAP dissolution and enhance FHAP formation.^{19,36-40} Interestingly, investigators have found that calcium fluoride may be hydrolyzed to partially FHAP in the presence of acid phosphate or phosphate ions.^{19,36-40}

We noted a synergistic effect on in vitro caries formation in primary enamel when AL irradiation took place before RS treatment in our study. This combined treatment of sound primary tooth enamel surfaces resulted in the greatest reduction in lesion depth. With the AL irradiation before RS treatment, carieslike enamel lesion depths were reduced by 62 percent when compared with matched no-treatment controls, by 50 percent when compared with AL irradiation alone, and by 22 percent when compared with RS treatment alone ($P < .05$). The mechanisms^{13,15,16,19-21,31,34-43} for increasing the resistance of tooth mineral substance to cariogenic challenges previously described for lasers and RS no doubt play a role in the lesion depth reductions with primary tooth enamel found in our study. A similar synergistic effect has been reported between AL irradiation and topical APF application both in vitro laboratory studies^{20-24,26,30,31} and in vivo clinical pilot studies.^{25,27-29} As we noted earlier, AL irradiation of permanent and primary tooth enamel leads to a 30 to 40 percent reduction in lesion depth. When AL irradiation occurs either before or after topical APF application, a 50 percent to slightly greater than 60 percent reduction in lesion depth occurs. It would appear that dentists should combine AL irradiation with a remineralizing agent for optimal caries resistance.

CONCLUSIONS

AL irradiation alone or RS treatment alone provided significant caries reduction in primary tooth enamel when compared with matched no treatment controls ($P < .05$). The susceptibility of primary tooth enamel to a continuous cariogenic challenge decreased significantly with RS treatment alone compared with AL irradiation alone ($P < .05$). We achieved the maximum reduction in lesion depth in primary tooth enamel when we combined the RS, which contained calcium, phosphate and fluoride in a carbopol base, with AL irradiation ($P < .05$). ■

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1. National Technical Information Service, Division of Health Examination Statistics. National health and nutrition examination survey (NHANES I), 1971-1974. Hyattsville, Md.: U.S. Dept. of Commerce; 1979. Dental data tape catalog 4235.
2. Thearmentree A, Eklund SA. Comparison between NHANES I and NHANES III: comparable NHANES I tooth and surface data (abstract 2076). *J Dent Res* 1999;78(special issue):365.
3. National Center for Health Statistics. Third National Health and Nutrition Examination Survey, 1988-1994, NHANES III Examination Data File (CD-ROM). Hyattsville, Md.: U.S. Dept. of Health and Human Services; Centers for Disease Control and Prevention; 1996. Public use data file documentation 76,220.
4. National Institute of Dental and Craniofacial Research. Oral health in America: A report of the surgeon general. Rockville, Md.: U.S. Department of Health and Human Services, U.S. Public Health Service; 2000.
5. Gillcrist JA, Brumley DE, Blackford JU. Community socioeconomic status and children's dental health. *JADA* 2001;132:216-22.
6. Quartey JB, Williamson DD. Prevalence of early childhood caries at Harris County clinics. *ASDC J Dent Child* 1999;66:127-31, 85.
7. Blen M, Narendran S, Jones K. Dental caries in children under age three attending a university clinic. *Pediatr Dent* 1999;21:261-4.
8. Tinanoff N. Introduction to the Early Childhood Caries Conference: initial description and current understanding. *Community Dent Oral Epidemiol* 1998;26(supplement 1):5-7.
9. Vargas CM, Crall JJ, Schneider DA. Sociodemographic distribution of pediatric dental caries: NHANES III, 1988-1994. *JADA* 1998;129:1229-38.
10. Tofsky N, Nelson EM, Lopez RN, Catalanotto FA, Fine DH, Katz RV. Dental caries in HIV-infected children versus household peers: two-year findings. *Pediatr Dent* 2000;22:207-14.
11. Hicks MJ, Flaitz CM, Carter AB, et al. Dental caries in HIV-infected children: a longitudinal study. *Pediatr Dent* 2000;22:359-64.
12. Featherstone JD, Adair SM, Anderson MH, et al. Caries management by risk assessment: consensus statement, April 2002. *J Calif Dent Assoc* 2003;31:257-69.
13. Featherstone JD. The caries balance: contributing factors and early detection. *J Calif Dent Assoc* 2003;31:129-33.
14. American Academy of Pediatric Dentistry's policy statement on the dental home. *Pediatr Dent* 2001;23(supplement):10.
15. Featherstone JD. The science and practice of caries prevention. *JADA* 2000;131:887-99.
16. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride. *Community Dent Oral Epidemiol* 1999;27(1):31-40.
17. Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental caries: role of saliva and dental plaque in the dynamic process of demineralization and remineralization (part 1). *J Clin Pediatr Dent* 2003;28:47-51.
18. Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental caries: role of saliva and dental plaque in the dynamic process of demineralization and remineralization (part 2). *J Clin Pediatr Dent* 2003;28:119-24.
19. Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental

- caries: role of saliva and dental plaque in the dynamic process of demineralization and remineralization (part 3). *J Clin Pediatr Dent* 2004;28:203-14.
20. Hicks J, Flaitz C, Ellis R, Westerman G, Powell L. Primary tooth enamel surface topography with in vitro argon laser irradiation alone and combined fluoride and argon laser treatment: scanning electron microscopic study. *Pediatr Dent* 2003;25:491-6.
21. Westerman GH, Hicks MJ, Flaitz CM, Ellis RW, Powell GL. Argon laser irradiation and fluoride treatment effects on caries-like enamel lesion formation in primary teeth: an in vitro study. *Am J Dent* 2004;17:241-4.
22. Westerman GH, Hicks MJ, Flaitz C, Powell GL. In vitro enamel caries formation: argon laser, light-emitting diode and APF treatment effect. *Am J Dent* 2004;17:383-7.
23. Hicks MJ, Flaitz CM, Westerman GH, Blankenau RJ, Powell GL, Berg JH. Enamel caries initiation and progression following low fluence (energy) argon laser and fluoride treatment. *J Clin Pediatr Dent* 1995;20:9-13.
24. Westerman GH, Flaitz CM, Powell GL, Hicks MJ. Enamel caries initiation and progression after argon laser irradiation: in vitro argon laser system comparison. *J Clin Laser Med Surg* 2002;20:257-62.
25. Hicks J, Winn D 2nd, Flaitz C, Powell L. In vitro caries formation in enamel following argon laser irradiation and combined fluoride and argon laser treatment: a clinical pilot study. *Quintessence Int* 2004;35:15-20.
26. Haider SM, White GE, Rich A. Combined effects of argon laser irradiation and fluoride treatments in prevention of caries-like lesion formation in enamel: an in vitro study. *J Clin Pediatr Dent* 1999;23:247-57.
27. Blankenau RJ, Powell G, Ellis RW, Westerman GH. In vivo caries-like lesion prevention with argon laser: pilot study. *J Clin Laser Med Surg* 1999;17:241-3.
28. Anderson AM, Kao E, Gladwin M, Benli O, Ngan P. The effects of argon laser irradiation on enamel decalcifications: an in vivo study. *Am J Orthod Dentofacial Orthop* 2002;122:251-9.
29. Anderson JR, Ellis RW, Blankenau RJ, Beiraghi SM, Westerman GH. Caries resistance in enamel by laser irradiation and topical fluoride treatment. *J Clin Laser Med Surg* 2000;18(1):33-6.
30. Westerman GH, Ellis RW, Latta MA, Powell GL. An in vitro study of enamel surface microhardness following argon laser irradiation and acidulated phosphate fluoride treatment. *Pediatr Dent* 2003;25:497-500.
31. Hicks MJ, Flaitz CM, Westerman GH, Blankenau RJ, Powell GL, Berg JH. Caries-like lesion initiation and progression around laser-cured sealants. *Am J Dent* 1993;6:176-80.
32. Hicks J, Flaitz C. Role of remineralizing fluid in in vitro enamel caries formation and progression (abstract). *Pediatr Dent* 2004;26(2):189.
33. Powell GL, Blankenau RJ. Laser curing of dental materials. *Dent Clin North Am* 2000;44:923-30.
34. Featherstone JD. Caries detection and prevention with laser energy. *Dent Clin North Am* 2000;44:955-69.
35. Oho T, Morioka T. A possible mechanism of acquired acid resistance of human dental enamel by laser irradiation. *Caries Res* 1990;24:86-92.
36. Legeros RZ. Calcium phosphates in demineralization and remineralization processes. *J Clin Dent* 1999;10(2):65-73.
37. ten Cate JM, van Loveren C. Fluoride mechanisms. *Dent Clin North Am* 1999;43:713-42.
38. ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. *Acta Odontol Scand* 1999;57:325-9.
39. ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. *Eur J Oral Sci* 1997;105:461-5.
40. Ogaard B. The cariostatic mechanism of fluoride. *Compend Contin Educ Dent* 1999;20(supplement 1):10-17, 34.
41. Fox JL, Yu D, Otsuka M, Higuchi W, Wong J, Powell GL. Initial dissolution rate studies on dental enamel after CO₂ laser irradiation. *J Dent Res* 1992;71:1389-98.
42. Fox JL, Yu D, Otsuka M, Higuchi WI, Wong J, Powell G. Combined effects of laser irradiation and chemical inhibitors of the dissolution of dental enamel. *Caries Res* 1992;26:333-9.
43. Hsu J, Fox JL, Wang Z, Powell GL, Otsuka M, Higuchi W. Combined effects of laser irradiation/sodium fluoride ion on enamel demineralization (published correction appears in *J Clin Laser Med Surg* 1998;16:294-5). *J Clin Laser Med Surg* 1998;16:93-105.