

An in vitro evaluation of fluorescein for testing the permeability of white spots on tooth enamel

Roald J. Shern, DDS, MPH, MS John B. Kennedy, BS
Michael W. Roberts, DDS, MScD

Abstract

This investigation demonstrates the reliability of fluorescein for detecting the permeability of incipient dental caries (white spots). Artificial white spots were created on the buccal surface of 12 human bicuspid teeth by viscous lactic acid (pH 4). Permeability of these lesions was assessed and reassessed before and after 24 and 48 hr of acid challenge using two disclosants: sodium iodide and sodium fluorescein. Estimates obtained from both disclosants showed that the microvoid volume approximately doubled as the decalcification time doubled. The two disclosants exhibited good intraclass reliability and their scores were correlated ($r = 0.69$ to $r = 0.91$). However, only fluorescein disclosed the extent of porous white spot lesions. Thus, fluorescein should be considered when the objective is to detect the location and permeability of incipient lesions.

Introduction

Several methods for detecting clinically the location and the amount of hard tissue loss due to incipient dental caries have been proposed (Marthaler 1965; Backer Dirks 1966; von der Fehr et al. 1970; Hefferren et al 1971; Edgar et al. 1978; Kleinberg et al. 1978). Some of these methods have been modified for in vivo as well as in vitro application. The ability to detect these reversible early lesions offers several advantages, including opportunities to study their pathogenesis, to shorten the time required for clinical trials, and to predict cavitation unless corrective care is initiated.

Investigators at Forsyth Dental Center have demonstrated a relatively simple, yet reliable, method for measuring porosity of the incipient enamel caries and enamel white spots (Bakhos et al. 1977; Bakhos and Brudevold 1982; Brudevold et al. 1984; Tavares et al. 1984). Potassium iodide is applied to the enamel surface and the excess removed in this noninvasive system. Permeability is estimated by measuring the quantity of

iodide that can be eluted from the test area. This method has been adapted for use both in vitro and in vivo (Bakhos et al. 1977; Brudevold et al. 1984; Kashket et al. 1989).

Although incipient dental caries often appears as white spots, not all white spots represent active caries with resultant porosity. Porous white spots can imbibe solutions. Accordingly, Rawles and coworkers (1978) used fluorescent dye uptake as an aid to early diagnosis of incipient lesions. This fluorescent method facilitates visualization of the surface extent of the active lesion, but does not estimate the amount of mineral loss.

Fluorescent dyes offer a possible alternative to potassium iodide for quantifying enamel loss in incipient lesions and, in addition, prove useful for visualizing the porous area of a white spot. There are many fluorescence-inducing light sources available. For example, fluorescence can be stimulated using the Plak-Lite™ (Brilliant International Inc., Bala Cynwyd, PA), certain composite-resin curing lamps, and incandescent lamps whose output has been converted to the appropriate wave lengths with commercially available photographic filters (Shern et al. 1981).

Fluorescein has several characteristics that make it suitable for clinical use. Fluorescein has been used widely in dentistry and ophthalmology because it is relatively nontoxic and pharmacologically inactive. Furthermore, dilute solutions may be used because of the sensitivity of the fluorimetry methods and the ability of dilute fluorescein solutions to fluoresce. Fluorescein also has favorable cosmetic properties; the staining is transient and almost imperceptible under conventional illumination.

The purpose of the present study is to compare the intraclass reliability of sodium fluorescein with potassium iodide to disclose the porosity of an artificial incipient lesion.

Methods

A circular area (diameter 3 mm) on the midbuccal surface of each of 12 extracted human bicuspid teeth was sampled before and after decalcification for 24 and 48 hr. A random subsample of six of the 24-hr samples, and all 12 of the 48-hr samples, were assessed for disclosant uptake within the circular area. Viscous lactic acid (pH 4) was used to create white lesions as described by Clark and coworkers (1986). Test areas were prepared and evaluated for iodide permeability as described by Tavares et al. (1985). In brief, 5 μ l of 2 M potassium iodide was placed on the cleaned and prepared test area. Excess potassium iodide was wiped from the test area with a cotton roll after 40 sec. The area was wiped with a second dry cotton roll, followed by a cotton roll premoistened with an aqueous solution of 0.1 M Na_2HPO_4 and 0.0025 M hexametaphosphate, and finally with another dry cotton roll. The iodide was extracted from the test area using 10 μ l of deionized water pipetted onto the test area for 40 sec, recovered with the pipette and placed in a collection tube. This extraction procedure was repeated three additional times and the resultant 40 μ l collection was diluted to 2 ml with 0.02 M potassium acetate. The iodide concentration was detected with an ion specific electrode (#945300, Orion Research Inc., Boston, MA) and displayed (Orion Ionanalyzer, #901) according to the manufacturer's instructions. Iodide permeability was reassessed before decalcification and after 48 hr.

Similar procedures were used for testing the permeability of enamel by fluorescein. Reassessments were made before decalcification, after 24 and 48 hr. An aqueous solution of 0.036 M sodium fluorescein (A833 Fisher Scientific Co., Fair Lawn, NJ) was used as the disclosant and it was extracted in the same manner as iodide. Specifically, the fluorescein was extracted from the test area using 10 μ l of distilled water pipetted onto the test area for 40 sec, recovered with the pipette, and placed in a collection tube. This procedure was repeated three times. The resultant 40 μ l of back diffusant was diluted to 3 ml with an aqueous solution containing 0.01 M Na_2HPO_4 (adjusted to pH 8 with NaOH) and 20% glycerol v/v. Fluorescein levels in the recovered solution were read with an Aminco Fluoro-colorimeter (J4-7439, American Instrument Co., Silver Spring, MD). Wave lengths were 490 nm for excitation and 535 nm for emission.

Subsequently, selected assessment areas were recorded with fluorescent microphotography, as were examples of naturally occurring white spots on extracted teeth not included in the present study. One per cent fluorescein was again applied to the study teeth and excess removed with a dry cotton

roll. Fluorescence was excited by a blue light produced by an illuminator fitted with a blue filter (Wratten 47A — Eastman Kodak Co., Rochester, NY). Two barrier filters (Wratten #2E and #6 — Eastman Kodak Co., Rochester, NY) were attached to objective lenses of the microscope to remove unwanted reflected light.

The data were summarized, and intraclass correlation coefficients were calculated (Shrout and Fleiss 1979).

Results

Uptake of the disclosants by the test area was negligible before the acid challenge. Data obtained from both tests showed that enamel porosity volumes approximately doubled as the decalcification time increased from 24 to 48 hr (Table). Intraclass correlation coefficients exceeded 0.8 for estimates obtained after 48 hr decalcification with both disclosants.

The correlations between iodide and fluorescein scores ranged from 0.69 to 0.91. The fluorescein which penetrated the enamel microvoids fluoresced under a blue light, documenting the permeability of the white lesions (Figs 1 and 2, next page).

Discussion

Permeability tests using iodide proved to be reliable, as previously reported (Bakhos, Brudevold Aasenden 1977; Tavares et al. 1985). However, our absolute values were markedly higher than those observed by Bakhos and coworkers (1977). Differences in the source of dental enamel, longer decalcification time, and lower pH of the lesion-promoting solution probably contributed to the higher values observed in the present study.

However, the rate and degree of demineralization noted in the present study were in closer accord with the

TABLE. Intraclass Correlation of Fluorescein and Iodide Inbided by Enamel Following 24 and 48 Hours of Decalcification

24 Hours Decalcification (n = 6)			
Disclosant ($\mu\text{g cm}^{-2}$)	Test	Retest	Intraclass Correlation
Fluorescein	0.49(0.16)*	0.54(0.33)	0.58
Iodide	10.25(0.21)	—	—
Correlation between dye scores	0.91	—	
48 Hours Decalcification (n = 12)			
Disclosant ($\mu\text{g cm}^{-2}$)	Test	Retest	Intraclass Correlation
Fluorescein	0.93(0.22)*	0.97(0.29)	0.81
Iodide	21.53(0.30)	23.00(0.33)	0.85
Correlation between dye scores	0.69	0.77	

* Mean (coefficient of variation).

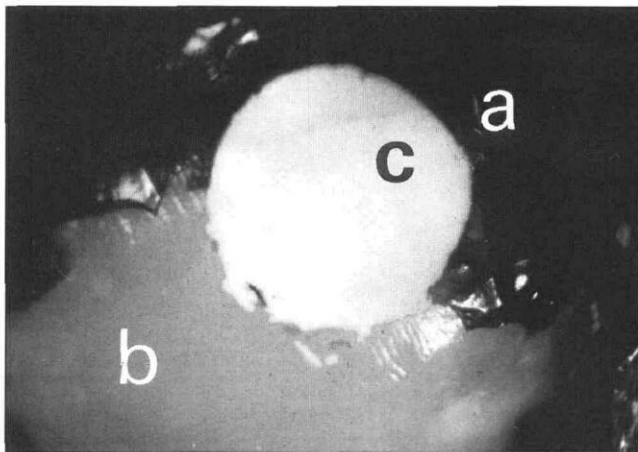


Fig 1. Buccal surface of extracted test tooth. Wax used to isolate enamel to be etched is shown (A) along with untreated enamel (B). The acid etched 3 mm fluorescing porous white spot is evident (C).

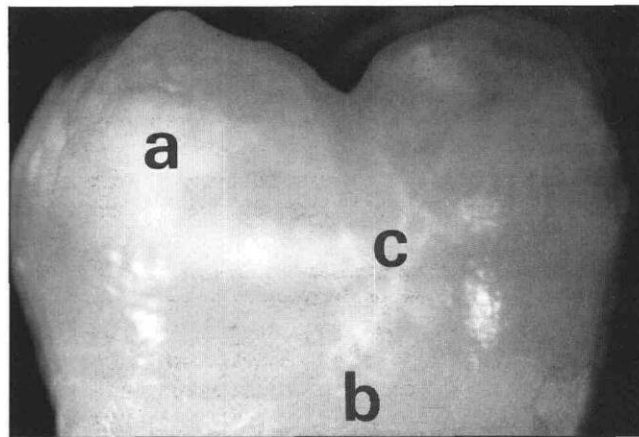


Fig 2. Lingual surface of extracted molar. The uptake of fluorescein demonstrates a porous enamel lesion (A). Organic debris on the root surface is easily detected (B) along with a surface crack in the enamel (C).

findings of Featherstone and Mellberg (1981), who measured imbibition of a fluorochrome (rhodamine B) using the same tooth type, surface preparation, and pH for the viscous acid challenge. The approximately five times higher uptake they observed may be due to the longer time allowed for diffusion (5 min).

Differences in the molecular structure and molarity may account for the difference in expected imbibition between the fluorescein and iodide. The smaller iodide molecules could be expected to pack more densely into the microvoids. Furthermore, the fluorescein solution was very dilute. In the present formulation, fluorescein may be less sensitive than iodide for measuring the effects of subtle acid challenge. If so, higher fluorescein concentration, greater contact time, and an improved penetrant system might enhance the dye penetration (Van de Rijke et al. 1989), permitting increased sensitivity.

Comparable reliability was exhibited by both disclosants, suggesting that fluorescein could be substituted for iodide when monitoring enamel permeability. This supposition was strengthened by the observation that the scores for the two disclosants were highly correlated ($r = 0.91$) after 24 hr.

Fluorescein has several characteristics that would be particularly useful in clinical application and studies. In addition to favorable pharmacologic and cosmetic properties, fluorescein can be used to visualize white spot lesions. This property allows an investigator or clinician to differentiate porous incipient lesions from healed lesions and other impervious white spots, e.g. mild fluorosis.

During our studies we noted two additional conditions, other than incipient dental caries, which allowed fluorescein to be retained: stress cracks and noncarious

enamel defects, and organic deposits such as dental plaque and debris. These potential causes of false positive estimates were easily identified and were avoided. The effects of these artifacts are much harder to avoid using nonfluorochromes.

Fluorescein possesses an additional benefit as a disclosant, as it increases the versatility of the enamel permeability tests. Disclosant selection can be guided by the availability of equipment and the nature of the assessments. Fluorescein would be particularly well suited for use in epidemiological trials and clinical practice, where it is necessary to discriminate porous from nonporous white spots without perceptibly discoloring the teeth.

Dr. Shern is senior staff dentist at the Clinical Investigations and Patient Care Branch of the National Institute of Research, Bethesda, MD. Mr. Kennedy is a biologist, Epidemiology and Oral Disease Prevention Program at the National Institute of Dental Research, Bethesda, MD. Dr. Roberts is associate professor in the Department of Pediatrics, School of Medicine and in the Department of Pediatric Dentistry, School of Dentistry at the University of North Carolina—Chapel Hill. Reprint requests should be sent to: Dr. Roald J. Shern, National Institutes of Health, Building 10, 1N-114, Bethesda, MD 20892.

Backer Dirks O: Posteruptive changes in dental enamel. *J Dent Res* 45:503-11, 1966.
 Bakhos Y, Brudevold F, Aasenden R: In-vivo estimation of the permeability of surface human enamel. *Arch Oral Biol* 22:599-603, 1977.
 Bakhos Y, Brudevold F: Effect of initial demineralization on the permeability of human tooth enamel to iodide. *Arch Oral Biol* 27:193-96, 1982.
 Van de Rijke JW, Busscher HJ, ten Bosch JJ, Perdok JF: Surface tension and contact angle on enamel of surfactant solutions for caries diagnosis with dyes. *J Dent Res* 68:1205-9, 1989.
 Brudevold F, Attarzadeh F, Tehrani A, van Houte J, Russo J: Development of a new intraoral demineralization test. *Caries Res* 18:421-29, 1984.
 Clark JW, More FG, Corpron RE, Korytnicki D, Kowalski CJ: Effects on artificial enamel lesions in vivo by exposure to NaF lozenges.

- Caries Res 20:465-72, 1986.
- Edgar WM, Rugg-Gunn AJ, Jenkins GN, Geddes DAM: Photographic and direct visual recording of experimental caries-like changes in human dental enamel. Arch Oral Biol 23:667-73, 1978.
- Featherstone JDB, Mellberg JR: Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. Caries Res 15:109-14, 1981.
- von der Fehr FR, Løe H, Theilade E: Experimental caries in man. Caries Res 4:131-48, 1970.
- Hefferren JJ, Cooley RO, Hall JB, Olsen NH, Lyon HW: Use of ultraviolet illumination in oral diagnosis. J Am Dent Assoc 82:1353-60, 1971.
- Kleinberg I, Chatterjee R, Domokos A, Castaldi CR, Fain M, Chebib F: An ultraviolet photographic technique for the early detection of carious lesions, in Methods of Caries Prediction, Bibby BG, Shern RJ, eds. Washington: IRL Press, 1978, p 271-79.
- Marthaler TM: The caries inhibiting effect of amine fluoride dentifrices in children during three years of unsupervised use. Br Dent J 119:153-63, 1965.
- Rawls HR, Robert KQ, Zimmerman EL, Owen WD: Fluorescent dye-uptake as an aid to early diagnosis of incipient carious lesions, in Methods of Caries Prediction, Bibby BG, Shern RJ, eds. Washington: IRL Press, 1978 p 261-65.
- Shern RJ, Monell-Torrens E, Couet KM, Kingman A, Bowen WH: Post-mortem evaluation of dental plaque in rats, in Animal Models in Cariology, Tanzer JM, ed. Washington: IRL Press, 1981 p 215-21.
- Shrout PE, Fleiss JL: Intraclass correlations: uses in assessing rater reliability. Psychol Bull 86:420-28, 1979.
- Tavares M, DePaola PF, Soparkar PM, van Houte J, Alman JE: The reliability of the iodide permeability test applied to the natural dentition. J Dent Res 64:1257-60, 1985.

Medical price tag for Northern California AIDS cases: \$33,000 per patient

As more AIDS cases are handled on an outpatient basis, health care costs have dropped in Northern California, according to a new study in the April, 1990 issue of *Archives of Internal Medicine*.

Robert A. Hiatt, MD, PhD, of the Division of Research at Kaiser Permanent Medical Care Program (KPMCP), Oakland, CA, reported that the Kaiser system in Northern California has handled 866 AIDS cases since the epidemic began in 1981. A random sample review of 71 patients whose conditions were diagnosed from 1984 through June 1987 determined the mean lifetime costs per patient were \$32,816. Overall costs have dropped, despite the significant increases in medications such as AZT. The authors believe the health care price tag for AIDS patients in Northern California is lower than in other parts of the country because of the extensive social support network that exists in the San Francisco Bay Area, and because of a low proportion of AIDS cases associated with intravenous drug use.