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New Directions in Cariology Research

Guest Editors: Alexandre R. Vieira, Marilia Buzalaf, and Figen Seymen

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Editorial Board

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Editorial

New Directions in Cariology Research

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Although there has been more than 100 years since Miller first postulated about the caries etiopathogenesis, this disease remains the most prevalent noncontagious infectious disease in humans. It is clear that the current approaches to decrease the prevalence of caries in human populations, including water fluoridation and school-based programs, are not enough to protect everyone. The US National Institutes of Health Consensus Development Program released a statement in 2001 entitled "Diagnosis and Management of Dental Caries Throughout Life: (NIH Consensus statement Online 2001 March 26–28; 18(1): 1–24) and listed six major clinical caries research directions.

(i) The "epidemiology of primary and secondary caries" needs to be systematically studied with population cohort studies that collect information on natural history, treatment, and outcomes across the age spectrum.

(ii) Research into "diagnostic methods", including established and new devices and techniques, is needed. Development of standardized methods of calibrating examiners is also needed.

(iii) "Clinical trials" of established and new treatment methods are needed. These should conform to contemporary standards of design, implementation, analysis, and reporting. They should include trials of efficacy.

(iv) Systematic research on caries "risk assessment" is needed using population-based cohort techniques.

(v) Studies of "clinical practice" including effectiveness, quality of care, outcomes, health-related quality of life, and appropriateness of care are needed.

(vi) "Genetic" studies are necessary to identify genes and genetic markers of diagnostic, prognostic, and therapeutic value.

This Special Issue is a sample of the current research efforts addressing a subset of the topics described above. I would like to thank the authors for their excellent contributions, in addition to many colleagues who assisted me in the peer-review process. Finally, I would like to thank the support provided by my Guest Editors, Dr. Marilia Buzalaf, from the University of São Paulo, Brazil, and Dr. Figen Seymen, from the Istanbul University, Turkey.

On the topic of "epidemiology and caries risk assessment" F. J. S. Pieralisi et al., K. K. Mäkinen, J. Vanobbergen et al., M. Okada et al., M. Ferraro and A. K. Vieira, A. P. Dasanayake et al., and J. D. Ruby et al. provide different perspectives to the problem which are good examples of how difficult is to study this multifaceted disease in a more comprehensive fashion.

There is currently great interest in "diagnostic methods," and the use of technology to differentiate diseased and healthy tissue, as well as to provide care. Different aspects of diagnostic and treatment approaches are addressed by S. Umemori et al., M. Aspiras et al., J. Wu et al., L. Karlsson, M. Yildirim et al., and A. Huminicki et al.

The biggest challenge continues to be designing rigorous clinical trials that can provide conclusive answers related to approaches that more effectively control caries at the segments of the population with higher risk for developing the disease.

I invite you to read, evaluate, and share this collection of 13 papers that comprise this special issue. Furthermore, I hope the readers will be interested in participating more

actively in this debate of what approaches are more efficient to revert the current figures of caries prevalence and what aspects of this disease should be the focus of research in the coming years.

Alexandre R. Vieira

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Clinical Study

Genotypic Diversity of *Streptococcus mutans* in Caries-Free and Caries-Active Preschool Children

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Aim. The aim of the present paper was to evaluate the genotypic diversity of *S. mutans* in caries-free and caries-active preschool children in Brazil. *Design*. Twenty-eight preschool children were examined regarding caries experience by the dmft index. DNA from 280 isolates of *S. mutans* was extracted. *S. mutans* evaluated using to the PCR method, with primers for the glucosyltransferase gene. The genetic diversity of *S. mutans* isolates was analyzed by arbitrary primed-PCR (AP-PCR) reactions. The differences between the diversity genotypic and dmft/caries experience were evaluated by $\chi 2$ test and Spearman's correlation. *Results*. The Spearman correlation test showed a strong association between genotypic diversity and caries experience ($r = 0.72$; $P < .001$). There were more *S. mutans* genotypes in the group of preschool children with dental caries, compared with the caries-free group. Among the children with more than 1 genotype, 13 had dental caries (2 to 5 genotypes) and 4 were caries-free (only 2 genotypes). *Conclusion*. Our results support the previous findings of genetic diversity of *S. mutans* in preschool children being associated with dental caries. The investigation of such populations may be important for directing the development of programs for caries prevention worldwide.

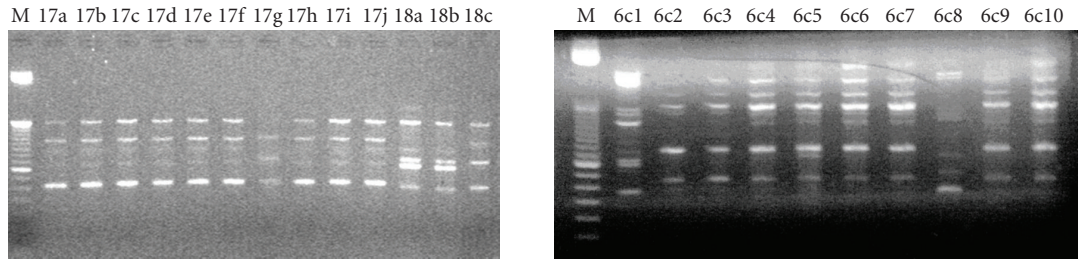
1. Introduction

Streptococcus mutans is generally considered to be the principal aetiological agent for dental caries [1, 2], which possesses a variety of mechanisms to colonize tooth surfaces. Clinical isolates of *S. mutans* exhibit considerable variations in their genomes or genes [3]. *S. mutans* species, under certain conditions, is numerically significant in cariogenic biofilms and forms biofilms with other organisms in the oral cavity [4] after the eruption and colonization of primary teeth [5]. Furthermore, epidemiologic surveys have confirmed that higher levels of *S. mutans* organisms in children are associated with a higher incidence of decayed, missing, and filled (dmf) teeth [2, 6]. Conversely, it can be found in populations with no caries or with low caries experience [7, 8]. One possible explanation for their presence in subjects with low caries experience is that *S. mutans* virulence factors can differ between populations with contrasting caries prevalence [9].

Bowden [10] pointed out the necessity for understanding the clonality patterns of *S. mutans* in the caries-free subjects where it is important to ascertain whether *S. mutans* populations in subjects free of caries exhibit the same clonal diversity of caries-active groups or not [10].

Several studies have showed genetic heterogeneity among *Streptococcus mutans* strains [11–16]; however, the relationship between caries activity and the genetic diversity of *S. mutans* is still controversial. Alaluusua et al. [17] suggested that caries-active children with high sucrose consumption carried greater ribotype diversity of S. *mutans* compared with caries-free children. Napimoga et al. [18] found that caries-active subjects have more genotypes than caries-free subjects. On the other hand, Kreulen et al. [19] showed a negative correlation between caries activity and genotypic diversity.

The aim of the present paper was to evaluate the genotypic diversity of *S. mutans* in caries-free and caries-active preschool children in Brazil.



(a) (b)

Figure 1: AP-PCR patterns of *S. mutans* isolated from caries-free and dental caries preschool children and detected with OPA-02 (lanes 2–14) and OPA-13 primers (lanes 16–25). Lanes 1 and 15 = size markers 100 bp Ladder (Invitrogen).

2. Material and Methods

2.1. Subjects. Study participants consisted of 28 preschool children aged between 4 and 5 years old from low socioeconomic level families. They had similar lifestyle, dietary, and oral hygiene habits. The subjects were selected from a group of children attending a nursery located in a medium-sized city from Southern Brazil. All of them were from the day nursery, staying in the nursery for 5 days per week, 8 hours per day. During the sample selection, subjects who had any chronic disease and were using antibiotic in the last 3 months were excluded. The aim and details of the experiments were explained, and the informed consent was obtained from parents and guardians prior to the beginning of the research procedures. Experimental procedures were approved by the Ethical Committee of the University of North of Parana School of Dentistry.

2.2. Clinical Examination. The children were examined while sitting on a chair under natural light. Diagnosis was visual, using a mouth mirror and cotton rolls to assist visibility and a periodontal probe to remove any plaque or debris when necessary.

Caries experience was measured by the dmft (decayed, missing, and filled teeth) index, according to the World Health Organization [20]. The caries experience was dichotomized into two groups: caries-free (dmft = 0) and dental caries children (dmft > 0). The clinical examination was performed by the same examiner (F.J.S.P.). The intraexaminer agreement was high ($\kappa = 0.92$).

2.3. Bacterial Strains and DNA Extraction. Streptococcus mutans clinical isolates were obtained from Mitis-Salivarius Agar with bacitracin and potassium telurite [21]. About 10 colonies resembling *S. mutans* from each child were transferred to brain heart infusion broth—BHI (Difco, Detroit, USA) and incubated at 37°C for 48 hours in an anaerobic jar. DNA from 280 isolates were extracted by using a simple DNA preparation in which the cells were washed and boiled for 10 minutes with TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) modified from Saarela et al. [13] and Welsh and McClelland [22]. The debris were pelleted and the supernatants were stored in a freezer at −20°C until use.

2.4. PCR Analyses. Isolates were confirmed for species identity in PCR reactions with primers specific for gtfB, encoding glucosyltransferase 5′ACTACACTTTCGGGTGGCTTGG3′ and 5′CAGTATAAGCGCCAGTTTCATC3′—(Invitrogen) [23], yielding an amplicon of 517 pb for *S. mutans gtfB* gene. Each reaction consisted of 5 μL template DNA, 1 μM of each primer, 200 μM of each dNTP, 5 μL 1x PCR buffer, 1.5 mM MgCl2, and 1 U Taq DNA polymerase (Invitrogen, São Paulo, Brazil) in a total volume of 25 μL. The amplification reaction was performed in 30 cycles as follows: denaturation 95°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 1 minute. One reference strain (ATCC 25175) was used as a positive control of *S. mutans* and distilled water was used as a negative control. Amplification products were analysed electrophoretically in 1% agarose gels using TBE buffer (89 mmol l^{-1} Tris borate, 89 mmol l^{-1} boric acid, 2 mmol l^{-1} EDTA; pH 8), stained with ethidium bromide and observed under UV light. A 100 bp DNA ladder served as molecular-size marker in each gel. All reactions were repeated at least twice.

2.5. AP-PCR Typing. Strains identified as *S. mutans* were genotyped. The genetic diversity of *S. mutans* isolates was analyzed by AP-PCR reactions. The sequences of the primers OPA 02 (5′TGCCGAGCTG3′) and OPA 13 (5′CAGCACCCAC3′) were used. The PCR reactions were performed as follows: 1X PCR buffer (200 mmol l^{-1} Tris-HCl pH 8.4; 500 mmol l^{-1} KCl) with 3.5 mM of $MgCl_2$, 0.2 mM of each dNTPs, 0.4 mM of primers, 2.5 U of Taq DNA polymerase, and 2.5 μL of DNA sample. The PCR conditions included 35 cycles of denaturation at 94°C for 1 minute, annealing at 36°C for 2 minutes, extension at 72°C for 2 minutes, with initial denaturation at 94°C for 5 minutes, and a final extension at 72°C for 5 minutes. The eletrophoresis was carried out as described previously; however amplification products were analysed in 2% agarose gel.

Individual AP-PCR amplicons were marked, and the individual bands were analyzed by using the Dice coefficient (>95%) following Mitchell et al. [24]. A dendrogram was constructed using the UPGMA cluster analysis with the aid of Numerical Taxonomy and Multivariate Analysis System (NTSYS) program (Exeter Software, Setauket, NY).

Table 1: Distribution of the preschool children with one or more *S. mutans* amplitypes by gender and caries experience ($N = 28$).

	Number of preschool children with	
	1 amplitype	>1 amplitype
Gender		
Boys ($n = 10$)	5 (50.0%)	5 (50.0%)
Girls ($n = 18$)	6 (33.3%)	12 (66.7%)
*Caries experience**		
Caries-free preschool children	10 (71.4%)	4 (28.6%)
Preschool children with caries	1 (7.1%)	13 (92.9%)

*Spearman correlation

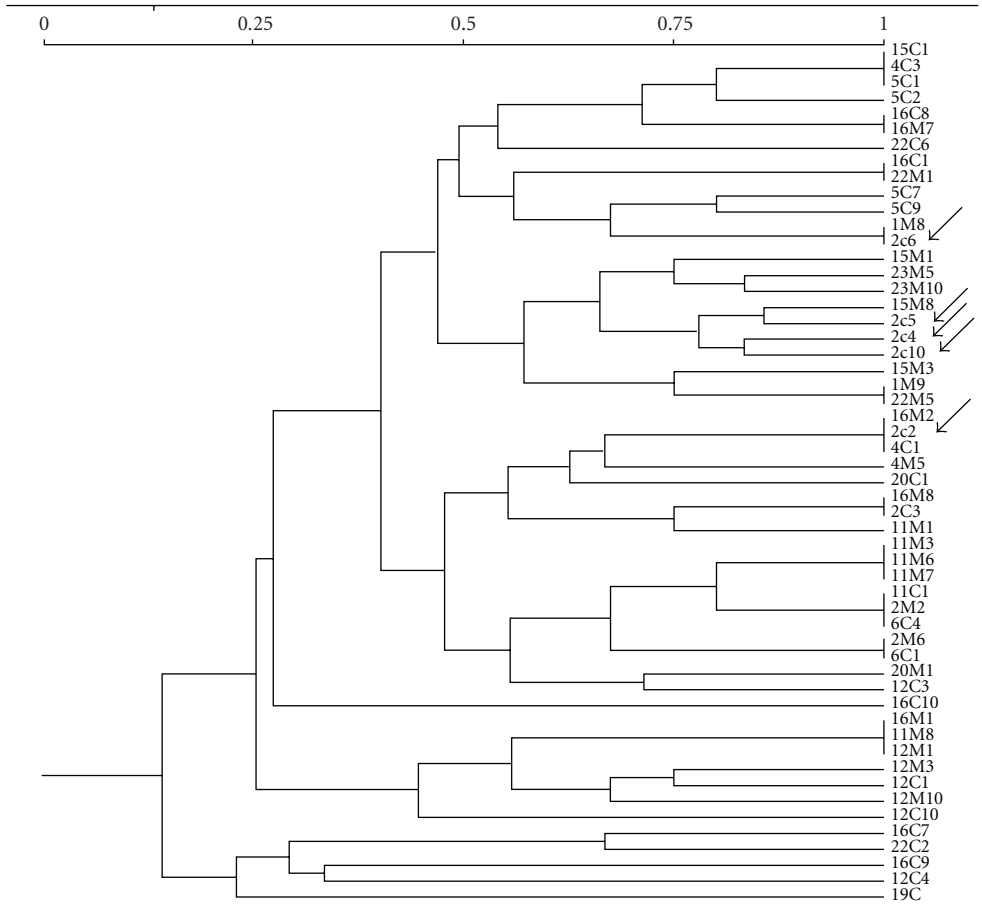


Figure 2: Dendrogram illustrating genotypic diversity between *S. mutans* strains isolated from caries-free and caries children. The Dice coefficient was generated from UPGMA clustering analysis based upon the comparison of the similarity matrices of all *S. mutans* strains type. The arrows indicate the preschool children no. 2c, who showed five distinct genotypes.

2.6. Statistical Analysis. The differences between the genotypic diversity and dmft/caries experience were evaluated by χ^2 test and the Spearman's coefficient of correlation. Statistical significance was considered to be at $\alpha < 0.05$. The Software Statistical Package for Social Science, v. 11.5 (SSPS, Chicago, IL, USA) was used for the data analysis.

3. Results

A total of 140 isolates of the preschool children with dental caries and 140 isolates of the caries-free preschool children were analyzed by AP-PCR, and 62 different amplitypes were identified. Figure 1 illustrates the AP-PCR patterns performed with OPA-02 and OPA-13, with each of these primers generating a different spectrum of amplicons, indicative of genetic polymorphism.

Characteristics of the children with colonization of *S. mutans* are presented in Table 1. No significant correlation of *S. mutans* was found between genotypic diversity of *S. mutans* and gender. The Spearman correlation test showed a strong association between genotypic diversity and caries experience ($r = 0.72$; $P < .001$). There were more *S. mutans* genotypes in the group of preschool children with dental caries, compared with the caries-free group. Among

the children with more than 1 genotype, 13 had dental caries (2 to 5 genotypes) and 4 were caries-free (only 2 genotypes).

Considering the whole population, some of the preschool children harbored just one genotype whereas others exhibited until five genotypes (Figure 2).

4. Discussion

The dental biofilm consists of a complex bacterial community, and the ability of specific strains of *Streptococcus mutans* to compete with other strains may be essential for colonization [25]. Studies of *S. mutans* virulence factors and their correlation with other species are fundamental to understand the role played by colonization of different genotypes in the same individual [26].

The knowledge of genotypic diversity of *S. mutans* may help in the development of new treatment strategies for caries, so as to prevent disease and promote health in addition to standard prevention treatments [26].

Although the findings of Kreulen et al. [19] have demonstrated a negative relationship between caries activity and genotype diversity and the results of Lembo et al. [27] have shown no significant differences in the number of genotypes detected in caries-free and caries-active children, the findings of the present study showed a positive relationship between caries activity and the genetic diversity of *S. mutans*. The preschool children with dental caries have more genotypes than the caries-free children, which is consistent with earlier reports [17, 18, 28, 29]. The existence of several genotypes in the biofilm could merely be a consequence of favorable circumstances for *S. mutans*. Moreover, it is possible that the simultaneous action of different genotypes, with distinct virulence potential, further increases the risk of caries [17].

In studies with young adults, Emanuelsson et al. [29] found a maximum of seven genotypes in subjects who had previously experienced dental caries. Napimoga et al. [18] also found a maximum of eight genotypes in caries-active subjects using AP-PCR. However, it has been observed that children harbor only one to five distinct genotypes of *S. mutans* [3, 11, 15–17, 19, 30]. The results of this research are consistent with previous studies reported in children. It was observed that in the caries-free group, 10 preschool children had only one genotype. On the other hand, in the dental caries group, 13 children had more than one genotype. Of these 13, only 2 harbored five distinct genotypes. This may be attributed to heavy colonization and growth of multiple genotypes in the same oral cavity is likely to be consequences of frequent consumption of fermentable carbohydrates [31]. Different clonal types of *S. mutans* detected within the oral cavity of one subject may exhibit different phenotypic and genetic properties [31]. In addition, the high clonal diversity of *S. mutans* can result in colonization by clones with different virulence attributes [32].

Our results support the previous findings of genetic diversity of *S. mutans* in preschool children being associated with dental caries. The investigation of such populations may be important for directing the development of programs for caries prevention worldwide.

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Review Article

Sugar Alcohols, Caries Incidence, and Remineralization of Caries Lesions: A Literature Review

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Remineralization of minor enamel defects is a normal physiological process that is well known to clinicians and researchers in dentistry and oral biology. This process can be facilitated by various dietary and oral hygiene procedures and may also concern dentin caries lesions. Dental caries is reversible if detected and treated sufficiently early. Habitual use of xylitol, a sugar alcohol of the pentitol type, can be associated with significant reduction in caries incidence and with tooth remineralization. Other dietary polyols that can remarkably lower the incidence of caries include erythritol which is a tetritol-type alditol. Based on known molecular parameters of simple dietary alditols, it is conceivable to predict that their efficacy in caries prevention will follow the homologous series, that is, that the number of OH-groups present in the alditol molecule will determine the efficacy as follows: erythritol ≥ xylitol > sorbitol. The possible difference between erythritol and xylitol must be confirmed in future clinical trials.

1. Introduction

The ability of human saliva to reharden acid-softened enamel was definitively first reported by Head in 1912 [1]. His report was not appreciated owing to the then-exiguous understanding of the chemical mechanisms that underlie innate host defence reactions, including the natural rehardening of caries lesions. Later, Koulourides, Pigman, and their contemporaries started to pay attention to remineralization of dental enamel by saliva [2–4]. Other earlier works are referred to in the latter references. More recent reviews and treatises were provided, for example, by the Demineralization/Remineralization Working Group [5] and by Kashket [6]. Several researchers, including Leach et al. [7] and Edgar [8] have emphasized the role that diet can play in tooth remineralization.

Rehardening of deep dentin caries lesions is not commonly encountered in most industrialized countries because dentists normally treat the teeth long before an initial lesion reaches the dentin caries level. It is well known, however, that also untreated, open dentin caries lesions can undergo a rehardening process, provided that the microbiological and chemical environment of the carious tooth changes remarkably. Such changes can be occasioned by a significant improvement of oral hygiene and remarkable changes in dietary habits. The common clinical rule is, of course, that dentin caries should always require operative treatment in order to save the tooth. The everyday reality can, however, be very different in many juvenile populations, especially in developing countries that lack the necessary dental resources. On the other hand, comment has also been made about remineralization of advanced dentinal lesions in nonindustrialized societies where restorations are not commonly completed by a dentist [9]. Furthermore, hardening, darkening, and continued service for many years by remineralized teeth have been noted [10].

Clinical studies and laboratory experiments have shown that the usage of certain dietary sugar alcohols (polyols), notably xylitol and sorbitol, can be associated with rehardening of artificial and genuine caries lesions. It is surprising that previous remineralization reviews have not mentioned even a single scientific study in this field, that is, sugar alcohol-associated remineralization. Earlier reviews have ignored the potential that sugar alcohols, especially xylitol, can provide in the planning of caries prevention strategies that may lead to remineralization of caries lesions. Consequently, the aim of this treatise is (1) to summarize the clinical caries trials on xylitol, (2) to examine those chemical and biologic features

of sugar alcohols that are assumed to play a role in caries prevention and tooth remineralization, (3) to summarize the roles that innate salivary factors can play in physiologic remineralization, and (4) to review results obtained in clinical and laboratory remineralization studies with sugar alcohols. The majority of remineralization-associated investigations have been carried out with xylitol and sorbitol. Several reviews have dealt with caries limitation and sugar alcohols (*vide infra*). Consequently, the present treatise focuses mainly on tooth remineralization. The cariostatic potential of erythritol, a sugar alcohol of the tetritol type, will also be discussed.

2. Clinical Caries Trials on Sugar Alcohols

The first dental caries studies on xylitol began at the Institute of Dentistry at the University of Turku in Finland in late 1969. The results of these studies showed that consumption of xylitol reduced the growth of dental plaque in participating dental students by up to 50% compared with use of sucrose, *D*-glucose, or *D*-fructose [11, 12]. Based on these observations, a two-year clinical caries study and a one-year chewing-gum trial (collectively called the Turku Sugar Studies) were set up in 1972-1973 [13]. The results showed that xylitol consumption was associated with an impressive caries reduction and prompted other researchers to repeat the Turku studies. Accordingly, a newly formed "xylitol concept" was presented in 1975 to the world scientific dental health community for purposes of expansion and verification. The confirmatory rounds of testing carried out during the next 30 years showed that the most important original claims of the dental efficacy of xylitol were verified by independent researchers in long-term clinical trials which were carried out under greatly varying and challenging conditions. Nineteen clinical trials and most of the nearly 300 short-term oral biologic laboratory studies on xylitol have been reviewed and commented [14–29].

The presently available information on clinical trials on the caries-limiting effects of xylitol (reviewed in the above publications) is summarized in Table 1. Several trials also investigated sorbitol. The accumulated data of these trials have in part constituted the rationale behind current public endorsements of xylitol worldwide [30, 31]. The clinical efficacy of xylitol in caries prevention was further discussed and reviewed at the U.S. National Institutes of Health Consensus Conference in 2001 [26, 32].

The two plaque studies [11, 12], mentioned above, deserve attention since they can justifiably be regarded as pioneers of the subsequent wealth of dental investigations on xylitol. Figure 1(a) shows a rendering of the "how-it-all-began" plaque study published in 1971. Four-day use of xylitol as a sweetener in subjects' diet resulted in about 50% reduction in plaque compared with the use of sucrose. The strong plaque mass-reducing effect of xylitol along with supportive biochemical effects observed in plaque in 1970-1971 [11, 12] generated first the Turku Sugar Studies [13] and next all other xylitol caries trials shown in Table 1. Figure 1(b) additionally reveals a particular oral biologic feature of dental plaque exposed to xylitol: the increase of

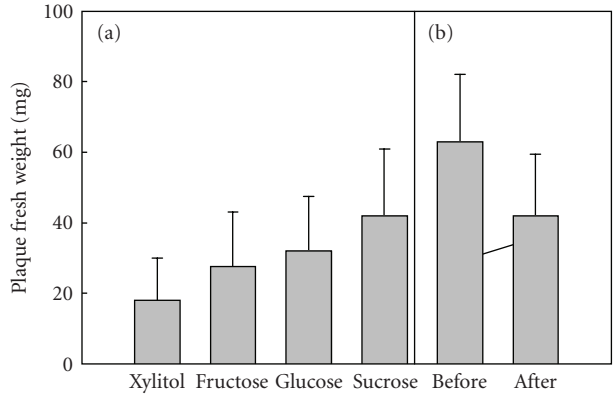

Figure 1: "How it all began": a pioneering plaque assessment study carried out in 1970 (a). Effect of dietary carbohydrates and xylitol on the growth of dental plaque after consumption of the shown sweeteners for four days (while the subjects refrained from oral hygiene), mainly in coffee or tea, and in the form of hard candies [11]. The consumption level of each sweetener was about 20 g per day and per subject. The values shown are means ± S.D. of fresh weight of plaque collected from all available tooth surfaces. (b) Inverse relationship between plaque fresh weight and its protein content. Twelve test subjects used xylitol chewing gum five times a day over a period of one month. Plaque from all available surfaces was collected following a 2-day no-oral-hygiene period. Consumption level of xylitol per day and per subject was 6.7 g. Xylitol consumption was associated with reduced plaque mass while the protein content of plaque simultaneously rose from 1.1 ± 0.2 mg to 1.4 ± 0.2 mg per mL of plaque suspension (straight line). Protein and nitrogen analyses should not be claimed to accurately determine the amount of dental plaque in clinical studies involving sugar alcohols.

nitrogen- and protein-associated metabolism in plaque—although the plaque volume, mass, adhesiveness, and cariogenicity simultaneously decrease, and its alkalinity increases. The reason for emphasizing this plaque feature stems from the exploitation of protein or nitrogen determination as a single plaque assessment procedure by some authors who have been unaware of the biochemical effects of xylitol on plaque. This situation has led to erroneous conclusions on the true changes in plaque virulence as affected by xylitol [20]. In other words, plaque protein analysis should not be used as the sole (and decisive) analytical method to measure the effect of sugar alcohols on plaque.

3. "Utility Value" of Polyol Strategies in Caries Prevention

Although the clinical efficacy of sugar alcohols in caries prevention is incontestable (Table 1), it is important to evaluate their true utility value assessed by independent meta-analyses and cost/benefit evaluations. A study by Deshpande and Jadad [33] on the impact of polyol-containing chewing gums on dental caries concluded that "research evidence supports using polyol-containing chewing gum as part of normal oral hygiene to prevent dental caries". The mean "preventive fraction" (PR; with 95% confidence interval) for the use

of xylitol, xylitol-sorbitol blend, and sorbitol was 58.66% (35.42–81.90), 52.82% (39.64–66.00), and 20.01% (12.74–27.27), respectively. For the sorbitol-mannitol blend, the value of PR was 10.71% (−20.50–41.93), which is statistically insignificant. The appraisal of Mickenautsch et al. [34] arrived at an almost identical conclusion. One of the studies evaluated was that of Machiulskiene et al. [35], carried out in Lithuania. It was not possible to deduce from the data of Deshpande and Jadad [33] and Mickenautsch et al. [34] whether the subsequent "rectifying" paper of Hayes [36] had been considered; Hayes determined that xylitol gum (among the several gums tested in the Lithuanian study) was the only gum that lowered the DMFS increment compared with the no-gum group after three years. The original authors [35] of the Lithuanian study had failed to recognize the potential of xylitol gum in their own clinical trial. Other public health evaluations [29, 37] have supported the above contentions on the potential that dental-protective chewing gums can provide in oral health interventions.

Further support for the utility value of xylitol interventions was provided by Milgrom et al. [38–40], who were among the first to determine the dose and frequency response of xylitol usage on mutans streptococci growth. A commendable effort was made by Milgrom et al. [39], who examined all xylitol products available on the United States market. Another practical achievement was the use of a xylitol-containing gummy bear snack as a means to reduce the growth of *S. mutans*/*sobrinus* levels [41]. The efficacy of xylitol in a dentifrice has been demonstrated in several studies [42–45], and in a pacifier as a slow-release mechanism in infants [46]. Further support for the practicality of xylitol usage has been obtained from its suggested synergistic effects with fluoride [47, 48], its use in combination with chlorhexidine [49, 50], as an alternative to fissure sealing [51], and as a sweetener in hard candies [52]. The practicality of the use of sorbitol may be somewhat affected by its low sweetness, the adaptation of oral streptococci to sorbitol [53, 54], and its customary support of the growth of dental plaque and mutans streptococci.

Use of xylitol in caries limitation has been investigated from the point of view of public health [55–57]. These surveys provided information on factual usage of xylitol as a caries-limiting agent. They reviewed public endorsements and use of xylitol for caries prevention programs worldwide [30]. It is significant that researchers have emphasized the role that xylitol-containing chewing gums could potentially have as a preventive measure in public health [58]. Basic research began to provide meaningful explanations for the observed xylitol effects about twenty years ago [15, 59, 60].

4. Literature Searches on Tooth Remineralization

Online literature searches provide interesting historical views into tooth remineralization research. Table 2 shows a typical PubMed search using the program's own MeSH vocabulary, whose term for the search was "tooth remineralization". The number of references per year presumed during the 1980s the current reference rate of more than 40 per year. These figures tend to change over time, however, since more detailed searches retroactively add new references to the database. The Science Citation Index gave slightly different figures. Selecting "enamel rehardening" as the key word resulted in 16 references through the ages, the oldest one from 1964, while simple "remineralization" yielded a total of 2268 references (as of December 17, 2008), the oldest one from 1910, but this figure includes a large number of nondental references. The increase in the number of dental remineralization papers reflects general acceptance within the relevant clinical and scientific circles that the remineralization of caries lesions is a normal physiologic repair process that can also be aided by various dietary measures.

5. Nonspecific Xylitol Effects versus Specific Ones

The use of xylitol in food and oral hygiene products has been found to result in significant caries reduction (Table 1 and the above references). Remineralization and rehardening are terms that have most often been employed to describe the type of caries arrest demonstrated in clinical xylitol programs and laboratory studies. Several studies suggest that xylitol can exert specific effects on dental caries not shown by hexitols. Authors who have opposed the existence of specific xylitol-associated effects in caries reduction have contended that caries reduction observed after xylitol use can simply be explained in terms of the following passive xylitol effects.

(i) Involvement of mere salivary effects, that is, the increase in salivation regularly associated with the consumption of sweet items, constitutes the only reason that explains the clinical observations made with xylitol in caries prevention studies.

(ii) Mere partial removal of a caries-inducive agent (sugar, notably sucrose) from the diet and substituting it with an essentially non-fermentable sweetener (xylitol) explains the observed caries reduction. In other words, in the presence of xylitol the cariogenic organisms are merely deprived of their normal growth substrate. The growth of dental plaque and the progression of caries will reduce only as a result of partial removal of the cariogenic challenge.

The above passive xylitol effects naturally constitute an important cornerstone in xylitol-associated caries limitation and would even as such fully justify the promotion of xylitol as a caries-reducing agent. The scientific review papers and professional evaluations thus far published have not denied this fact. Scientific literature is, however, replete with findings that also support the involvement of active, specific xylitol effects that operate even in the presence of fermentable hexose-based carbohydrates, that is, in situations where a strong cariogenic challenge is present. In the Turku Sugar Studies [13] sucrose and xylitol gums differed significantly from each other in their caries-limiting ability in a situation where the salivary involvement (i.e., the chewing effect) was regarded as similar in both study cohorts. The Belize studies [61] and the preceding animal experiments [62–64] also

support the idea of specific xylitol effects; xylitol was found to limit dental caries even in the presence of a strong cariogenic challenge and was more effective than sorbitol.

Shyu and Hsu [63] showed in rats that alditols differed significantly in their caries-reducing potential: xylitol caused the lowest caries scores (86% reduction). *D*-Mannitol reduced caries by 70%, sorbitol by 48%, and plain diet by 39% (compared with sucrose added to the plain diet; all sweeteners were tested at a 10% level). This study also reflects a problem that plagues most comparative dental trials on dietary sweeteners: the use of percentage levels. When 10% of either xylitol (mol. wt. 152.1), sorbitol (182.2), mannitol (182.2), or sucrose (342.3) is expressed in molarities (connoting the number of molecules present in solution), the true chemical concentrations differ significantly, that is, 0.657 M for xylitol, 0.549 M for the hexitols, and 0.292 M for sucrose. Comparing 10% erythritol (122.1) with 10% sucrose reveals even more remarkable differences in true chemical concentrations: 0.819 M versus 0.292 M. Critical appraisal of clinical studies should thus be conducted by considering the above discrepancy in expressing concentrations. However, the conclusions of Shyu and Hsu [63] were not remarkably affected by the above criticism.

Some of the physicochemical properties of xylitol discussed below will further elucidate the complex scientific background that is assumed to lie behind the clinical effects reported in literature. All dental xylitol studies have not, however, reached positive clinical and oral biologic findings. Long-term field experience has shown that in most cases failures in demonstrating such effects can be explained in terms of the following features of the studies in question.

(i) Use of caries-resistant study cohorts or cohorts with extremely low caries experience.

(ii) Use of too-small study cohorts.

(iii) Use of too-low concentrations of xylitol.

(iv) Use of too-short intervention.

(v) Use of too-short or too-infrequent exposure to xylitol.

(vi) Simultaneous use of other caries-limiting agents and strategies (such as fluorides).

(vii) Use of too-insensitive analytical procedures.

(viii) Use of a single analytical procedure to assess oral biologic parameters (such as plaque growth).

A recommended practice is to use 6 to 7 g of xylitol daily, preferably in 3 to 5 separate episodes. Regarding oral biologic measurements (such as plaque growth, microbiology, and the chemical composition of saliva and plaque), experiments lasting from a few days to several months or even years, have been implemented. Regarding dental caries outcomes, trials that last several years are recommended. A particular dilemma has indeed been occasioned by studies that have leaned toward a single plaque assessment procedure (such as protein determination, which can lead to erroneous conclusions). In plaque studies, it is advantageous to rely on simultaneous gravimetric, planimetric (before and after, using disclosing dyes and colour photography), clinical (plaque index), bacteriologic, chemical, and enzyme measurements.

Among enzyme determinations, an analysis of the combined invertase (EC 3.2.1.26) and sucrase (EC 3.2.1.48) activities have turned out promising. (The numbers shown refer to the Enzyme Commission's classification.) Consumption of a xylitol diet is normally associated with significantly decreased whole saliva and plaque invertase-sucrase activity levels [13], suggesting diminished sucrose-splitting capacity with concomitantly reduced acid production. Reduced dextranase (EC 3.2.1.11) activity of dental plaque may also result from xylitol consumption [13]; this enzyme causes endohydrolysis of 1,6-α-*D*-glucosidic linkages in dextran, whose levels in plaque are normally high after sugar consumption. Similarly, strongly reduced salivary α-amylase (EC 3.2.1.1) activity has been found in xylitol-consuming subjects [13]. The activity levels of α-*L*-fucosidase (EC 3.2.1.51) may in turn increase during xylitol consumption (possibly resulting from increased hydrolysis of the named fucoside linkages present in salivary glycoproteins; this process may be important in the formation of the acquired tooth pellicle). All of the above glycosidases can be regarded as markers of plaque metabolism. Similarly, certain proteinase and aminopeptidase activities (which are normally increased in plaque after xylitol use) can be regarded as suitable markers of plaque biochemistry. The above changes in enzyme activity can normally be encountered when analyzing plaque and plaque extracellular fluid (with the exception of α-amylase, which is derived from glandular saliva), indicating that the origin of those activities is predominantly in the oral microbiota. The proteinase, aminopeptidase, and α-fucosidase activities increased most likely because plaque microorganisms were deprived of their preferred growth substrates (hexose-based sugars) and converted their energy-yielding enzyme activities towards the proteins and glycoproteins present in saliva. This resulted in an overall increase in plaque and saliva nitrogen metabolism. The reduction in α-amylase, dextranase, and invertase/sucrase activities can in turn be interpreted as resulting from the lowering of the sucrose levels in the subjects' diet during xylitol regimen.

6. Chemical Features of Sugar Alcohols That Can be Associated with Remineralization

The term "sugar alcohol" refers in chemical colloquialism to the reduction products of "sugars", indicating that all oxygen atoms present in a simple sugar alcohol molecule are in the form of hydroxyl groups. The terms "polyol" and "polyhydric alcohol" in turn refer to chemical compounds that contain three or more hydroxyl groups. All sugar alcohols are polyols. The polyols can be divided into acyclic compounds (alditols or glycitols, which can be regarded as true sugar alcohols) and cyclic polyols. Examples of the former are erythritol, xylitol, and *D*-glucitol (sorbitol), while *myo*-inositol serves as an example of cyclic polyols. The most important dietary sugar alcohols that will be discussed in this treatise include the four-, five-, and six-carbon members

Table 1: Summary of human caries studies on xylitol that in part have constituted the justifications for public endorsements of xylitol. The percent-reductions are in comparison with a control group that received a normal diet, fluoride treatment, or sucrose products. Nondietary (dentifrice) studies and programs on multiple preventive measures that included the use of xylitol are also shown. CH = Chlorhexidine.

Study location	Product(s) tested	Duration (years)	Dose (g/day)	Caries reduction (%). Comments. (References).
Finland	Full diet	2	67	>85. Compared with sucrose diet [13, 65]. Mostly adults.
Finland	Chewing gum	1	6.7	>82. Compared with sugar gum. 1/10 of the above dosage [13, 65]. Young adults.
Soviet Union	Candies	2	30	Up to 73. Compared with sucrose candies [66].
French Polynesia	Chewing gum	3	About 20	58–62. Compared with normal diet [67].
Hungary	Gum, candies, dentifrice	2-3	14–20	37–45. Compared with fluoride [68, 69].
Canada	Chewing gum	1-2	1.0–3.9	52. [70].
Finland	Chewing gum	2	7–10	30–57. All subjects (no-gum as control)[(a)]. [71].
Finland	Chewing gum	3	7–10	59–84. High-risk subjects[(a)]. [71].
Costa Rica	Dentifrice + NaF	3	Twice/day	Up to 12.3. 10% xylitol in the product. [44].
Costa Rica	Dentifrice + Na_2FPO_3	3	Twice/day	Up to 10. 10% xylitol in the product. [45].
Belize	Chewing gum	3.3	<10.7	Up to 73. Permanent teeth[(b)]. [61].
Belize	Chewing gum	2	<10.7	Up to 63. Deciduous teeth[(c)]. [72].
USA	Gum, pastilles	1.8	8.5	80. Supragingival root surface caries. Elderly subjects [73].
Estonia	Gum, pastilles	2-3	5	50–60. Used on school days[(d)]. [51]. Pastille as effective as gum.
Finland	Chewing gum (by mothers)	ca. 1.75	6	70 (in children). NaF and CH as control [74].
Lithuania	Chewing gum	3	2.95	21–36. [35]. Rectification of initial results [36][(e)].
Sweden	Chewing gum (by mothers)	1	2	"Significant" or 40% (in children). [75, 76][(f)].
Kuwait	Hard caramels	1.5	2.3	50. Läkerol-type hard candies were used [57][(g)].
Finland	"Slow-release pacifier"	1	159 mg	No new dentinal lesions in infants [46]. A mixture of xylitol, sorbitol, and NaF was tested. The pacifier features a pocket for the sweetened tablet.
Finland	Multiple measures	About 3.4	4.6	Counselling and the use of fluoride- and xylitol products reduced caries ($P < .001$) compared with basic prevention [77][(i)].

[(a)] Long-term effects (after up to 5-year use) have been reported [78–80]. An independent analysis showed that the total number of new restored surfaces was 4.0 per child in the xylitol group and 9.3 in the controls during the decade after the onset of the trial. Participation in the xylitol gum trial thus resulted in significant reduction in the number of first restorations and hence in costs during the subsequent decade [81].

[(b)] 16-month use of xylitol gum following the 3.3-year use of sucrose gum reduced caries significantly [82]. "<10.7" indicates the maximum calculated, supervised use (at school) per day and subject.

[(c)] Two-year use of xylitol gum remarkably protected erupting permanent teeth against caries, that is, long-term effects were involved [83].

[(d)] Saliva stimulants were given only on school days (about 200 per school year). Gums were as effective as pastilles (hard candies of the "Läkerol-type").

[(e)] The original authors failed to recognize that, in their study, xylitol gum was the only gum that lowered the DMFS increment compared with the no-gum group after 3 years. "To still observe a significant caries-lowering effect of xylitol with such a small dosage is quite remarkable". The faulty conclusions were rectified by Hayes [36].

[(f)] In one literature source, the authors reported an 80% reduction between "test and control". Also, when the children were 18 months old, the authors reported that "maternal consumption of xylitol- and CH/xylitol-containing chewing gums significantly reduced the mother-child transmission of salivary mutans streptococci". This study actually compared a gum with high xylitol content with gums with lower xylitol content, supplemented with either CH or NaF.

[(g)] Xylitol hard candies were given only on school days (one piece of candy at a time, three times a day).

[(h)] The pacifier features a pocket from which the saliva stimulants dissolve.

[(i)] The Läkerol Dents brand (Leaf). The products were given to the subjects with instructions "to be used according to directions" (i.e., two pieces of candy three times a day). The calculated maximum consumption level of xylitol was about 4.6 g/day.

Table 2: PubMed literature search for "Tooth Remineralization".

Years	Number of references	References per year
1966–1975	1	0.1
1976–1984	10	1.3
1985–1990	180	36.0
1991–1995	156	39.0
1996–1999	136	45.3
2000–2004	222	44.4
2005–2008	228	57.0

of the homologous alditol series, that is, erythritol, xylitol, sorbitol, and related polyols. These molecules are based on a single monosaccharide skeleton. Among disaccharide sugar alcohols, maltitol (derived from maltose), lactitol (derived from lactose), and palatinit (equimolar mixture of *α-D*-glucopyranosyl-1,6-sorbitol and *α-D*-glucopyranosyl-1,6-*D*-mannitol) have received attention in nutritional and special medical uses. Complex, long-chain polyols have been manufactured for various food uses by hydrogenation of starch hydrolysates. Such products (hydrogenated starch syrups) often contain varying amounts of simpler polyols (such as sorbitol, maltitol, and trimeric and even higher homologues) as by-products.

According to carbohydrate nomenclature, it is permissible to use the name adonitol for ribitol, arabitol for arabinitol, sorbitol for *D*-glucitol, and dulcitol for galactitol. The name mannitol requires *D* or *L*. Names without *D* and *L* include erythritol, xylitol, ribitol, sorbitol, dulcitol, lactitol, and maltitol (only those polyhydric alcohols are mentioned that will be discussed in the present text). Erythritol (1,2,3,4-tetrahydroxybutane) has appeared in texts as *meso*-erythritol and *i*-erythritol; *meso* in this case stands for optical inactivity owing to internal compensation.

The simple alditols are crystalline substances varying in taste from faintly sweet (galactitol) to very sweet (erythritol and xylitol, which are almost isosweet with sucrose). Several alditols, notably erythritol and xylitol, exert relatively strong negative heat of solution, a physiochemical property that in practice is reflected in the perception of a "cooling effect" in the mouth as crystalline alditol dissolves in saliva; the energy which is required in the dissolution process is taken from the environment, producing a cooling effect. Of the hexitols, sorbitol and *D*-mannitol show specific optical rotation for sodium *D* line, $[\alpha_D]$, while galactitol is optically inactive. Xylitol, ribitol, and erythritol are also optically inactive. Historic and various evolutionary and chemical aspects of sugar alcohols have been discussed in other contexts [17, 18, 84–87].

For the purpose of the present discussion, it is necessary to review some of the common sugar alcohol properties as follows.

(a) *Absence of a reducing group*. The absence of a reducing carbonyl group in the alditol molecules makes them chemically somewhat less reactive than the corresponding aldoses and ketoses. Some sugar alcohols can thus avoid those chemical reactions that normally make many dietary hexose-based sugars acidogenic and cariogenic in dental plaque. Xylitol, for example, is not normally recognized by cariogenic organisms' transport mechanisms. In cases where transport occurs, as via a constitutive mechanism normally serving pentitols other than xylitol (cf. Scangos and Reiner [88] as a historic case with *E. coli*), the xylitol molecule is not directly involved in lactic acid production, nor is it directly involved in cariogenesis (*vide infra*). Some hexitols, such as sorbitol and *D*-mannitol can, however, be readily recognized by several strains of cariogenic mutans streptococci (Bergey's Manual of Determinative Bacteriology regards mutans streptococci as organisms that can be identified based on their positive hexitol fermentation). The absence of the reducing carbonyl group does make these hexitols less acidogenic than the corresponding aldose and ketose forms. However, sorbitol and *D*-mannitol normally support the growth of mutans streptococci and dental plaque.

(b) *The reducing power*. The sugar alcohol molecules contain an "extra" number of hydrogen atoms that can be deposited on other metabolites such as coenzymes (e.g., NADP or NAD) and other acceptors to generate chemically reduced products and intermediates of metabolism. The alditol molecules' two "extra" hydrogen atoms must thus be present in products that are formed from the alditols. The general alditol structure $(CH_2O)_n \cdot 2H$ may eventually give rise to some organic acids also in dental plaque. The regular sugars (such as glucose and fructose) have an elementary composition equalling $(CH_2O)_n$. The hexitols sorbitol and *D*-mannitol should normally yield one mole each of lactic acid, formic acid, and ethanol, while the pentitol xylitol should normally form one mole each of acetic acid, formic acid, and ethanol. For all practical purposes, the acidogenicity of dietary xylitol and erythritol, present in dental plaque, is normally insignificant.

(c) *Complexation*. Owing to the polyoxy structure of the sugar alcohol molecules they can form complex compounds with various metal cations and oxyacids. From the point of view of tooth remineralization, the complexes with Ca(II) are important. (In chemical literature, Ca(II) refers to the divalent calcium ion. In the text below, II is omitted.)

(d) *Hydrophilicity.* The presence of a large number of hydroxyl groups makes most sugar alcohols readily soluble in saliva. The most hydrophilic alditols can compete with water molecules for the hydration layer of bio-molecules (such as proteins and metal cations). Some consequences of the pronounced hydrophilicity can be seen in the strengthening of hydrophobic interactions between protein molecules (and within a protein molecule). This is in practice reflected in the protection of proteins against thermal and other denaturation or damaging processes. The protected protein configurations can include *α*-helix and *β*-structures. Related to hydrophilicity is the action of sugar alcohols as chaotropic agents under certain chemical conditions. Chaotropic agents break up organized water structures (such as the primary hydration layer of proteins and metal cations) and affect reactions that obtain their energy from the release of structured water. Another concept that will be used below, that is, the stabilization of salivary Ca phosphate systems, is a prerequisite in tooth remineralization, and

results from the combined effects of complexation of Ca and displacement of water molecules.

(e) *Osmoregulator's role*. Owing to the relatively low molecular weight and the hydrophilic nature of alditols, they can function as osmoregulators in various biological systems. Perhaps the best known and most commonly exploited case concerns the use of intravenous *D*-mannitol in lowering intracranial pressure in brain surgery, in renal function studies, as a diuretic, and so forth. Sorbitol has been used as an active principal in several cathartics preparations. Erythritol (at 40 mM) exerted a significant osmoprotector effect against stress activation of corneal epithelial cells [89].

(f) *Free radical scavenging*. Because of their polyol nature, some sugar alcohols, such as *D*-mannitol, xylitol, and erythritol can act as free radical scavengers in biological and experimental systems [90].

(g) *Nucleophilicity of sugar alcohols in some hydrolytic reactions*. Several studies have shown that polyhydric compounds accelerate the hydrolysis of β-lactam antibiotics, *p*-nitrophenyl esters, cephalosporins, and so forth. in aqueous solutions at neutral and alkaline pH values [91, 92]. Consequently, polyhydric alcohols have shown to be catalytically active. This effect is attributed to a nucleophilic reaction mechanism affecting the molecule under attack by an alkoxide ion derived from proton ionization of one the hydroxyl groups. It is necessary to recall that several common alditols are alcohols with pK_a values in the range of 12 to 13. The nucleophilicity described is also displayed by other polyhydric compounds such as *D*-glucose and sucrose [92].

(h) *General comparison between "glucose-polyols" and "non-glucose polyols"*. The successful use of xylitol in parenteral nutrition is directly associated with the molecule's "non-glucose-polyol" nature, that is, its pentitol structure [18]. Such effects cannot be observed when sorbitol or glucose is used as a source of energy in infusion therapy [18]. This decisive difference between the xylitol and sorbitol molecules is graphically presented in Figure 2. The "glucose-polyol" nature of common dietary hexitols actually constitutes an advantage in particular nutritional uses of those hexitols such as sorbitol, *D*-mannitol, and galactitol, whereas it naturally elicits questions about the latter's stimulating effect on the growth of plaque and certain strains of mutans streptococci.

(i) *Competition between water and alditol molecules for Ca*. When alditols such as xylitol and sorbitol are introduced into the oral cavity, they compete with water molecules for the primary hydration layer of Ca. The latter may comprise 4 to 12 water molecules that surround the metal ion. The partial displacement of water molecules in the hydration layer of Ca results in the formation of a new layer consisting of alditol and water, as shown schematically in Figure 3. This interaction between alditols and Ca contribute to the stabilizing effect of polyols in salivary Ca phosphate systems [14, 18].

7. Specific Alditol Properties

In addition to all alditols displaying the above-mentioned common polyol properties, laboratory observations and computer-based examination of various structural features of the alditol series have shown that tetritol, pentitol, and hexitol is each also characterized by properties specific to individual alditols or alditol groups only. These properties have been adequately described in the relevant organic and physicochemical literature and are thus old news to chemists and biochemists. Such structural and physicochemical differences between alditols are inevitably also reflected in human and microbial metabolism. Since most tooth remineralization studies on polyols have dealt with xylitol and sorbitol, it is necessary to examine these alditols more closely. The differences between xylitol and sorbitol become evident when examining the alditol literature shown below. Some properties believed to be important in the permeability of alditols and in their relationship with water molecules are shown in Table 3.

The scientific literature is indeed replete with descriptions on interesting differences between alditols. The very sweetening capacity of dietary alditols varies remarkably and presumes important differences between the detailed chemical structures of these homologues. Some alditols are optically inactive whereas for others a clear $[\alpha_D]$ value has been determined (*vide supra*). The pronounced differences between the gastrointestinal tolerances of alditols in humans speak for the existence of physicochemical specificities. Extreme examples in this sense are erythritol (well tolerated) and *D*-mannitol (poorly tolerated). The very chromatographic separation of alditols (in their analytics and manufacturing) indicates the existence of important differences between individual alditols. The early studies of Mills [87] showed how the conformation of alditol exerts pronounced effects on the physical properties of the molecule, such as the retention times in gas chromatography. Further evidence is shown below:

(1) *Historic remarks*. The epoch-making examination of Mills [87] divided alditols into several distinct series based on their stereoregular differences; the alditol series discussed show stereoregularities with short repeating units. The alditol series differ from each other, for example, with regard to the energy content of the individual homologues. Space does not allow a detailed account of the revelations that Mills and others have found between individual alditols. For example, the hydroxymethyl end-groups of galactitol can adopt their particular conformation (the so-called *A* conformation, designating the conformation round the C–C bond) more readily than the end-groups in *D*-mannitol can. This results in an important difference between galactitol and *D*-mannitol in terms of their chromatographic behaviour [87]. Analogous differences can exist also in the metabolic behaviour and clinical effects of those hexitols.

(2) *Complexation*. Although complexation can be regarded as a "common" polyol property, differences between individual alditols do exist. The hexitols, the pentitols, and the tetritols can form four, three, and two oxygen atom triangles, respectively. The exact nature of the O-triangles differs from alditol to alditol. Since these triangles can participate in complexation with Ca, complexes with different stabilities will form. The possible conformations by the interaction of alditols with partially hydrated Ca were

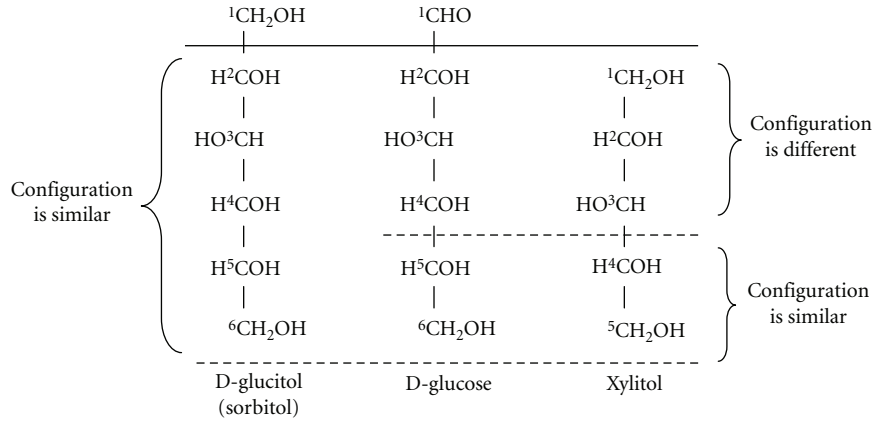

Figure 2: Relationship between the structural configurations of sorbitol (*D*-glucitol), *D*-glucose, and xylitol. The molecular configurations of sorbitol and glucose are relatively similar. Hence, sorbitol can be called a "glucose-polyol". The configuration of xylitol (a "non-glucose polyol") markedly differs from the two other configurations. The close similarity of sorbitol with glucose partly explains its plaque-promoting and mutans streptococci-stimulating effects.

Table 3: Physicochemical properties of alditols at 25°C.

Alditol	Molecular weight	Maxium van der Waals radius (Å)[(a)]	Partial molar volume ($cm^3\ mol^{-1}$)[(b)]	Permeability ($m\ s^{-1}$)[(c)]	"Water activity" constant K[(d)]
Glycerol	92.1	2.8	70.84	$1.49 \pm 0.40 \times 10^{-10}$	1.16
Erythritol	122.1	3.1–3.2	86.83	$4.92 \pm 0.27 \times 10^{-10}$	1.34
Xylitol	152.1	3.2–3.3	102.12	$9.9 \pm 3.4 \times 10^{-11}$	1.66
D-Arabitol	152.1	3.2			1.41
L-Arabitol	152.1	3.2			1.21
Ribitol	152.1	3.2	100.6		1.49
D-Glucitol	182.2	3.4	118.8		1.65
D-Mannitol	182.2	3.4	119.22	$7.6 \pm 4.8 \times 10^{-11}$	0.906

[(a)]The values for glycerol, erythritol, xylitol and *D*-mannitol are from Kiyosawa [93]. Other values represent estimates of the present author.
[(b)]At infinite dilution at 25°C [94]. Values for ribitol and sorbitol are from Back et al. [95].
[(c)]Using the giant alga *Chara* cell membrane [96].
[(d)]The values of K are those of a correlating constant from the equation $a_w = x_1 \exp(-Kx_2^2)$, where x_1 and x_2 are molar fractions of water and solute, respectively, and a_w is water activity [97].

Table 4: Concentration of calcium (determined by means of atomic absorption spectrophotometry) in dental plaque of subjects who used products containing xylitol.

Study	Xylitol	Control or sucrose	Remarks
Chewing of xylitol gums (paraffin as control)	1.22 ± 0.45	0.78 ± 0.30	In μg/mg fresh weight (n = 10–12; $P < .01$). Sorbitol gave similar results [98].
Chewing of xylitol gum (compared with sucrose gum and gum base)	3.7 ± 0.5	2.4 ± 0.2	In μg/mg dry weight ($n = 83$). Gum base: 3.4 ± 0.7. Significance of differences was not given [99].
Rinsing with 0.4 M xylitol or sucrose solutions	0.90	0.67	In μg/mg protein. Plaque pools from 11 subjects in both groups. 0.01 M Na cyclamate: 0.60 [100].
Xylitol or sorbitol chewing gum compared with no gum	1.77 ± 0.99	1.70 ± 1.33	In % dry weight in plaque. No gum: 1.24 ± 0.82%. For both polyols: $P < .03$ when compared with no gum. $n = 25$ [101].

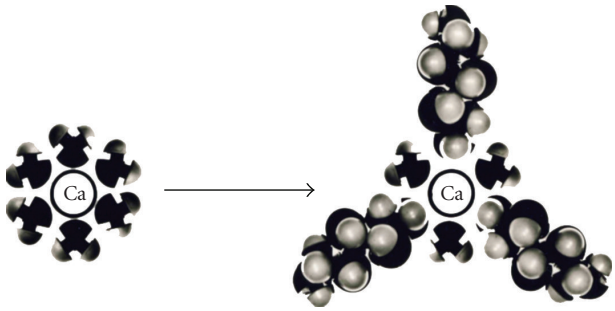

Figure 3: A simplified presentation of the competition between water and xylitol molecules for Ca, assumed to play a role in environments involving whole-mouth saliva and plaque fluid. Here, Ca has interacted with six water molecules which constitute the primary hydration layer of the metal ion (the actual number of water molecules surrounding the spherical Ca ion may vary from 4 to 12). The resulting new hydration layer consists of water molecules and xylitol molecules. This leads to stabilization of the salivary Ca phosphate systems [14, 18]. Reproduced with permission [14].

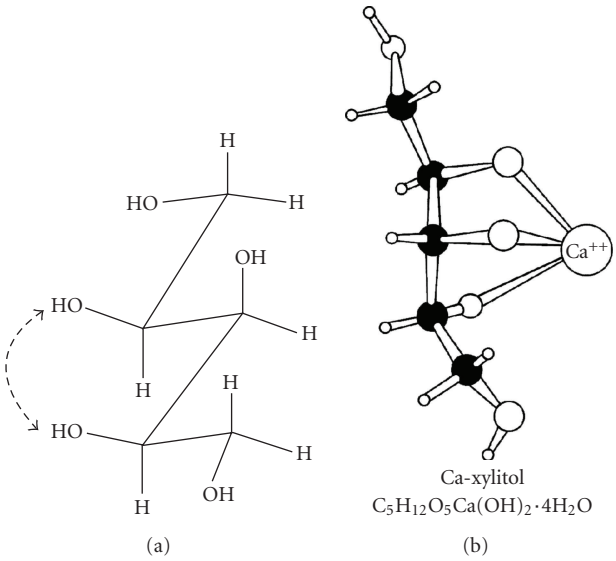

Figure 4: The zigzag structure of xylitol (a) and that of a xylitol-Ca complex (b) assumed to exist also in salivary environments and generally under physiologic conditions in the human body. The double-headed arrow in (a) reflects the special interaction between the oxygen atoms shown. The complex formation can facilitate the transport of Ca through membrane pores and also against weak Ca gradients. This structure may aid in the transport of Ca through the gut wall.

presented by Schöllner et al. [94]. The difference between pentitols and hexitols was striking. Complex formation between polyols and divalent metal cations is a well-studied bioinorganic research area. Some remineralization-related aspects of polyol-Ca complexes have been studied [7, 14, 18, 102–104]. The proposed structure of a xylitol-Ca complex is shown in Figure 4.

(3) *Molecular parameters*. The molecular volumes, the axial lengths, and the maximum molecular radii of alditols differ significantly. The apparent molar volumes and other molecular dimensions of several alditols were measured by Kiyosawa [93], who found marked differences. The differences between the molecular weights of alditols are further reflected in numerous practical situations, such as in the permeability of alditols through membranes [93, 96]. Alditols have turned out to be valuable tools in assessing the "radius" of water-filled pores in biological systems (Table 3).

(4) *Hydration and effect on water structure*. Even pentitols differ significantly from one another, for example, regarding hydrogen bonding within the molecule. Such differences are often reflected in pronounced differences in the hygroscopicity and other properties of the pentitols [104]. Remarkable differences between the hydration properties of alditols were observed by Carlevaro et al. [105]. In line with these studies, there is a relatively strong hydration of xylitol which should be classified as positively hydrated with an extended effect on the immediate environment. The xylitol molecule exhibits a perturbational effect on water structure. Other common alditols such as erythritol, sorbitol, and *D*-mannitol exhibit negative hydration. Accordingly, xylitol can be expected to protect, for example, foodstuffs against non-enzymatic browning and ascorbic acid destruction more effectively than the above hexitols do. Related to these effects are the observed differences between alditols in their "water activity" levels [97]. This property strongly depends on the chain length and the detailed molecular configuration of the alditol. The alditol-specific effects on water structure have been known since early 1970s [95]. Polyols in general interact with water to an extent which depends on their molecular structure. For example, *D*-mannitol behaves differently from sorbitol, and glucose behaves differently from sucrose. Polyol molecules induce structure in the water molecules, surrounding them if the orientation of OH groups is such that some of the O-O spacings correspond with the O-O distance of 0.486 nm of the water lattice [95]. This effect is not a colligative property of the polyol but a property related to the OH groups.

(5) *Selective enzyme inhibition; enzyme affinity characteristics*. Xylitol inhibits more potently various microbial *D*-glucose isomerase-catalyzed reactions than the hexitols do; even the pentitols differ in this sense decisively from each other. Previous reports have tabulated representative inhibition constants (or percentage degrees of inhibition) that have demonstrated significant differences between alditols; the affinity constants of polyol dehydrogenases for their alditol substrates also differ significantly [15, 18]. Some authors have emphasized the selectivity of bacterial growth inhibition and the degree of pentose isomerase inhibition by pentitols already in the 1970s [106]. The simple alditols show distinctly different affinities for dehydrogenases involved in oxidation reactions. For example, the apparent K_m values for *D*-glucitol and galactitol were 6.2 mM and 1.5 mM, respectively, in the *Rhodobacter sphaeroides* sorbitol dehydrogenase-catalyzed reactions [107]. The ribitol dehydrogenase of the same organism showed the following significantly different affinities: ribitol, 6.3 mM; xylitol, 77 mM [108]. Differences of the above magnitude, and even higher, are customarily found between alditols in enzyme kinetic studies of dehydrogenases. The pronounced differences, above, in enzyme

affinities suggest that decisive differences between alditol effects can be encountered also in other biological reactions.

(6) *Kinetics of oxidation of alditols.* In addition to the above dehydrogenase-associated instances, kinetic oxidation studies of alditols offer a further point of comparison between alditols. This is a well-researched area and research findings have shown significant differences between pentitols and hexitols. For example, kinetic measurements of the rate of oxidation of xylitol and galactitol by alkaline hexacyanoferrate(III) ion showed that both oxidation reactions followed first-order kinetics with respect to hydroxide ion, but with xylitol this was true only for lower hydroxyl ion concentrations, whereas with galactitol the first-order kinetics rule applied even up to manyfold variation [109]. At lower NaOH concentrations the rate constants of oxidation of xylitol and galactitol were 0.0128 and 0.0170 $mol^{-2}l^2s^{-1}$, respectively. The reaction progresses via an alkoxide ion, the end products being dicarboxylic acids. The difference between xylitol and galactitol was remarkable.

8. Alditols Are Not Tooth-Demineralizing and Calculus-Promoting Agents

The complexation between alditols and polyvalent cations may raise concern about the possible Ca-chelating (demineralizing) effect in the oral cavity. Readers unfamiliar with the true nature of complexation prevailing in the oral cavity may be misled by the terminology that is customarily used in chemistry. For example, $CaSO_4$ is a relatively water-insoluble compound (at 18.75°C about 0.2 parts dissolves in 100 parts water). Yet the solubility is enhanced in the presence of, for example, sorbitol. It is important to observe, however, that the stabilizing effect of polyols on the Ca phosphate systems of the oral cavity is predominantly directed to the solubility of salivary Ca and phosphate, rendering their prolonged, dissolved, supersaturated state possible, compared with the presence of, say, sucrose, which tends to initiate instantaneous precipitation of Ca and phosphate in saliva (thus eliminating a part of those substances from remineralization). The polyols' role in saliva and plaque fluid is one of stabilization; Ca and phosphate salts are stabilized in the presence of polyols and will remain in solution even at supersaturated concentrations [14, 15, 102–104].

The polyol-Ca complexes are weak compared with those formed with common food acidulants such as adipic acid, glutaric acid, ascorbic acid, succinic acid, malic acid, tartaric acid, fumaric acid, oxalic acid, and other related carboxylic acids. Research has shown that the ability of the above food acids to chelate Ca (demineralize enamel) is directly proportional to their acidity. When present in a sorbitol candy, the amount of enamel dissolution was correlated with the potential of the acids to chelate Ca. In other words, sorbitol did not chelate enamel calcium, whereas the above acids did [110]. The same notion concerns other dietary alditols.

Various techniques such as ultrasonic absorption, conductometry, solubility measurements, electrophoresis, and chromatography, have been used to determine that the Ca-alditol complexes are indeed relatively weak [98]. Relatively low Ca-polyol stability constants were measured for xylitol [97]. Therefore, the role normally given to alditols in the salivary environment is that of stabilization of the salivary Ca phosphate system [7, 14, 104]. This role is supposed to mimic that displayed by natural salivary peptides, such as statherin. Although the alditol-Ca complexes have thus been found to be relatively weak, they may still contribute, as Ca-carriers, to tooth remineralization and enhanced Ca absorption [18]. The consumption of xylitol has been suggested to be associated with increased Ca levels of dental plaque (Table 4). Instead of plaque hardening, (calculus formation), the extra plaque calcium is believed to enhance tooth remineralization. It is also necessary to point out that the Ca-alditol stability constants depend on temperature (stronger complexes are normally formed at lower temperatures) and that it is possible to calculate equilibrium constants for each of alditol's carbon atoms.

Although chemical analyses of dental plaque have thus shown increased Ca levels after xylitol consumption (Table 4), clinical studies on habitual xylitol users have not shown any increase in plaque mineralization; none of the clinical studies shown in Table 1 have reported on periodontal or calculus-forming problems. On the contrary, there are reports on the inflammation-dampening effects of xylitol in clinical and laboratory studies. In addition to xylitol generally reducing the growth and adhesiveness of dental plaque, xylitol formed plaque that was less inflammatory in a hamster cheek pouch microcirculation test than plaque grown in the presence of sucrose or fructose [111, 112]. Similar results were obtained in a bone culture study [113]. 5-Day-old "xylitol plaque" was less irritating to macrophages and bones than plaque grown in the presence of sucrose [114]. A study involving experimental gingivitis suggested that xylitol mouth rinses were periodontally less harmful than sucrose rinses (and equal to sodium cyclamate rinses) [100, 115]. Two clinical experiments on children indicated that the use of xylitol-containing chewable tablets and candies was associated with reduced plaque growth and gingival bleeding [116, 117]. More recently, xylitol was shown to inhibit cytokine expression by a lipopolysaccharide from *Porphyromonas gingivalis*, one of the suspected periodontopathic bacteria [118].

The above observations indicate that xylitol can be regarded as a periodontally safe dietary sweetener It is possible that xylitol's use can be augmented to comprise prevention of periodontal disease and gingival inflammation.

9. Salivary Factors Associated with Tooth Remineralization

The chemical conditions for tooth remineralization to occur can be summarized in the following five points.

(1) Sufficiently high salivary (and plaque extracellular phase) pH value.

(2) Sufficiently high salivary (and plaque extracellular phase) Ca level.

(3) Sufficiently high salivary (and plaque extracellular phase) phosphate level.

(4) Presence of natural salivary peptides that govern nucleation of hydroxyapatite crystals.

(5) Presence of the required organic and inorganic matrix (i.e., the mineral-deficient enamel or dentin sites are automatically present).

Under normal conditions human saliva meets all of the above chemical prerequisites of remineralization. Consequently, saliva is normally supersaturated with regard to Ca and phosphate, and the pH value of secreted saliva normally rises spontaneously owing to the release of carbon dioxide. These conditions will automatically facilitate the precipitation of calcium phosphate. The availability of the fluoride ion within the normal, physiologic, salivary concentration range will facilitate remineralization. The phosphate component may also be partly derived from a dietary organic source, such as casein and other protein phosphates. Typical dietary phosphate sources include milk, other dairy products (cheese), and various nuts. Dietary regimens that do not elicit too-frequent acid attacks on enamel normally guarantee that the plaque extracellular phase (plaque fluid) will not become too acidic. The nucleating and crystal growth-governing salivary factors are normally present in the saliva of all normal individuals. The concentration of Ca and inorganic phosphate (P_i) in mixed saliva, that is, 1-2 mmole/L and 2–10 mmole/L, respectively, are normally sufficient to create the required supersaturated state (of Ca phosphate) in human saliva. The overall effect of the salivary buffers gives a range of 6.2–7.4 in the saliva of most adults. These chemical conditions can be regarded as normal for tooth remineralization. Most of the buffering capacity of saliva at neutral pH values is attributed to the bicarbonate and the phosphate systems.

Saliva forms spontaneously an organic integument (the so-called acquired pellicle) on tooth surfaces. In this process extracellular enzymes secreted from plaque bacteria liberate the carbohydrate components from salivary glycoproteins (mucins), causing the residual protein structures to precipitate out of solution. The protein structures may eventually precipitate as part of the acquired pellicle film on tooth enamel. Certain salivary peptides are involved in the maintenance of the supersaturated state of Ca in saliva. Evidence has been presented on the importance of diet on the composition of the acquired pellicle; a distinct model of protein deposition on artificial hydroxyapatite discs was observed after rinses with sucrose, sorbitol, xylitol, and phosphate-buffered saline [119]. It is interesting that in the above study xylitol and sorbitol differed very significantly in Western blot tests of proteins extracted from the discs carried in the mouth for various periods of time (from 30 seconds to 20 minutes). Consequently, the alditol molecules behaved differently in the *in situ* studies of pellicle formation. The discs became saturated with protein very rapidly after each rinse, although clearly less salivary protein was adsorbed on to the discs after the sorbitol rinse than was adsorbed after xylitol rinse.

The important role salivary mucins and proteins may play in remineralization receives support from the studies of Kielbassa's group [120–122]. Mucin-based salivary substitutes ("artificial saliva") were considered effective remineralization-inducing adjuvants that could especially benefit hyposalivation patients. Some manufacturers have marketed mucin-based saliva substitutes that also contain xylitol.

10. Ca-Binding in Dental Plaque

As shown above, xylitol consumption has been found to be associated with an increase of plaque Ca levels (Table 4). The extra Ca (compared with control situations) present in dental plaque in its entirety and in plaque extracellular fluid in particular may participate in the remineralization of mineral-deficient enamel sites. Approximately half of the concentration of plaque Ca may be ionized. It is not yet known how much of the Ca present in "xylitol plaque" is in an ionized form. It is known, however, that polyols can facilitate the solubilization of the insoluble portion of plaque Ca. It is possible that the increased Ca inhibits demineralization through a common ion effect, and may additionally facilitate remineralization during periods of high pH values.

It is necessary to recall that Ca-binding by various Gram-positive plaque bacterial strains is a common phenomenon [123, 124]. Ca-binding by various surface components of oral bacteria may indeed exert significant effects on remineralization/demineralization processes. Various oral-care products such as mouth rinses and chewing gum have been used to increase the plaque levels of a combination of casein phosphopeptides and amorphous Ca phosphate (CPP-ACP), and to facilitate remineralization of enamel [125, 126]. Incorporation of CCP-ACP into plaque naturally also increases the plaque levels of Ca and P_i. In one mouth rinse study, the plaque Ca and P_i levels increased by 118% and 57%, respectively [126]. These authors strongly believe that the extra plaque Ca can significantly contribute to tooth remineralization.

The xylitol-associated increase of plaque Ca levels (Table 4) is most likely a general polyol-associated reaction; xylitol and sorbitol may not differ remarkably in this sense. The extra Ca is believed to contribute to tooth mineralization during a polyol regimen. Sorbitol, however, normally supports the growth of dental plaque and mutans streptococci.

Enamel permeability naturally plays a role in rendering Ca available for remineralization. The permeability of common salivary and dietary inorganic ions (such as Ca and P_i) has been discussed in several contexts, but less attention may have been paid to the permeability of common dietary carbohydrates. In this sense it is necessary recall that according to some permeability studies twice as much xylitol goes through the enamel as sucrose [127].

Polyols generally stabilize the calcium phosphate solutions in saliva [97]. This phenomenon results from the formation of complexes between Ca and the polyol. Although ketoses, aldoses, disaccharides, and other carbohydrates also

form complexes, those formed between xylitol and Ca are of particular interest owing to the general nonacidogenicity of xylitol in the human oral cavity. The stabilizing effect of xylitol on the salivary Ca-phosphate system can easily be demonstrated by letting acellular (Millipore-treated) whole-mouth saliva stand at room temperature in the presence or either sucrose or xylitol. Sucrose allows almost instantaneous precipitation of Ca-proteinates, whereas the turbidity formation is significantly delayed in the presence of xylitol [102, 103]. In other words, xylitol mimics some of the salivary peptides (such as statherin) which control crystal formation. It has been assumed that sucrose effectively eliminates from the solution the "extra" calcium and phosphate ions that in the presence of xylitol can maintain their supersaturated, natural concentration level, a prerequisite of remineralization. In one study, saliva precipitates contained a crystalline phase that had the structure of apatite [104]. Xylitol maintains a higher pH value in saliva and plaque fluid and simultaneously maintains a supersaturated Ca-level in saliva. The combined effect can manifest as remineralization that is governed in the same way as salivary peptides.

11. Formation of Basic Equivalents

Because the rate of tooth remineralization generally increases already in slightly alkaline conditions, it is pertinent to recall the increase in dental plaque and whole saliva of various basic substances during xylitol regimen. The first clue of this type biochemical effects was obtained in the Turku Sugar Studies [13] which showed that the consumption of a xylitol diet was associated with a general increase of nitrogen- and protein-containing substances in saliva. Among such substances were amino acids (which in turn can generate ammonia), and the ammonium ion itself [13, 14]. The pie chart in Figure 5 demonstrates the general increase in ninhydrin-positive substances (mostly amino acids) present in the whole-mouth saliva of subjects who habitually consumed a xylitol diet for 12 to 16.5 months. Other studies have also shown that the stimulation of saliva with xylitol products increases the ammonia [116] and the bicarbonate [13] content of whole-mouth saliva.

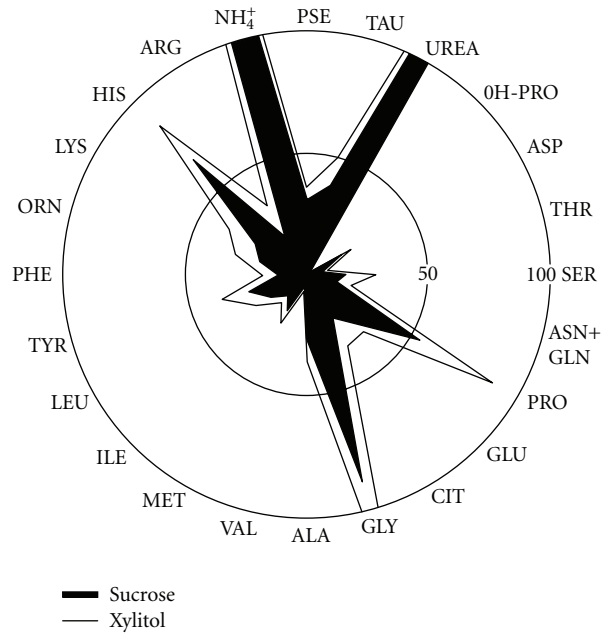

Figure 5: An important sialochemical effect of xylitol diet. Increase of the free amino acid content (in μmol/L, thin line) of whole-mouth saliva after long-term consumption of a xylitol diet, shown in a polar co-ordination diagram [13]. The analysis was carried out on pooled saliva of subjects who had consumed the xylitol diet for 12 to 16.5 months (average consumption level of xylitol: about 65 g/day). The solid black area shows the free amino acid levels in saliva of subjects who consumed a regular sugar-containing diet. The high levels of ammonia and most amino acids (which can in turn serve as sources of further ammonia production) speak for reduced plaque acidity and increased nitrogen metabolism in dental plaque from which a large part of the free amino acid pool of whole-mouth saliva is derived. Non-standard abbreviations: CIT = citrulline; TAU = taurine; PSE = phosphoserine. Reproduced with permission [14].

12. Description of Individual Remineralization Studies

The list below summarizes in chronological order 27 separate remineralization-associated clinical, basic science, and animal studies on xylitol and other sugar alcohols (two studies, #19 and #26, investigated enamel erosion). The list begins with the Turku Sugar Studies [13] whose authors used the phrase "remineralizing and therapeutic effect of xylitol" in their first clinical reports on xylitol-associated limitation of dental caries [128].

(1) Remineralization of Caries Lesions During a Two-Year Xylitol Feeding Trial and in a One-Year Chewing Gum Study. The final report of the two-year Turku feeding trial showed that the consumption of a xylitol diet was associated with the reversal of the caries process [65]. Because essentially similar observations were also made in a simultaneously conducted one-year chewing gum study [129], the authors of the trials were led to conclude that the usage of xylitol was indeed associated with remineralization of caries lesions [13]. Habitual use of xylitol gum resulted in a negative development of the DMFS index scores, an indication of remineralization. Subsequent planimetric evaluations of the lesion size reductions after xylitol usage in both studies supported the original conclusions made [130, 131]. An interesting, partly accidental outcome of these trials was that while the daily consumption level of xylitol in the two-year feeding trial was estimated to be 67 g per subject, the intake of xylitol in the one-year gum study amounted to one tenth of the above value, that is, 6.7 g. Partly owing to these consumption levels, the Finnish health authorities have recommended the use of 6 to 7 g xylitol daily in the prevention of caries. This recommendation has been widely followed in the instructions issued by national dental associations in several Asian countries [30].

(2) Effect of Xylitol-Supplemented Diet on the Regression of Fissure Caries in Rats. Dentinal molar fissure caries in rats, produced by initial exposure to dietary sucrose, were significantly reversed by subsequent exposure to a xylitol-supplemented (3% and 6%; about 0.2 M and 0.4 M, resp.) starch diet. Continued exposure to a xylitol-starch diet produced a successive regression of caries rates, which the authors interpreted as remineralization and as a therapeutic effect [7, 62].

(3) Remineralization of Artificial Caries-Like Lesions by a Xylitol-Containing Mouth Rinse. One of the mouth rinses tested by Featherstone et al. [132] contained 2.5% (0.164 M) xylitol "to mask the mineral taste and produce a palatable rinse". Other ingredients included 0.6 mM NaF, and K, Ca, Sr, and Zn salts. Human enamel slabs with demineralized lesions were embedded in intra-oral appliances. Complete rehardening of the inner 40–50 μm and twofold rehardening of the remaining body of the lesion occurred in 160-μm deep lesions with a 1-min mouth rinse on each of 14 consecutive days. Saliva re-hardened the inner 20 μm only. The authors did not pay attention to the possible role of xylitol as a remineralization-contributing factor.

(4) Remineralizing Properties of Xylitol in Combination with Sucrose in Rats. Rats, inoculated with *Streptococcus mutans*, were fed a diet containing 20% sucrose, 5% glucose, and 5% xylitol (SX diet), or solely a sucrose-glucose diet (S). (In molar terms, the above concentrations are about 0.58 M, 0.28 M, and 0.33 M, resp.) SX induced significantly fewer fissure lesions than S. Initial lesions induced by S were significantly reduced (remineralized) by subsequent exposure to SX. Change from SX to S resulted in substantial caries progression [64].

(5) Rehardening Properties of Xylitol-CMC-Containing Saliva Substitute. The rehardening of artificially softened human enamel by nine different saliva substitutes was investigated by micro-hardness measurements [133]. All saliva substitutes exhibited a rehardening potential. The largest reduction of the rehardening potential was observed after addition of high concentrations of carboxymethylcellulose (CMC) or mucins and the addition of sorbitol (3% in the substitute; 0.165 M). The best rehardening properties were observed for low-viscous mucin- or CMC-containing saliva substitutes with xylitol (2% or 0.13 M; Saliva Orthana, Copenhagen, Denmark). The difference between xylitol and sorbitol was significant ($P < .01$).

(6) Influence of Xylitol and Sucrose on Enamel Demineralization In Vivo. Fissure-like plaque retention grooves were created in human enamel blocks and demineralized. The blocks were mounted in prostheses of 11 subjects who used a 2.5% (0.164 M) xylitol, 2.5% (0.073 M) sucrose, or a water solution in a randomized cross-over design. During a period of 16 days, the subjects submerged the prosthesis twice a day in the test solutions for 5 minutes. Mineral loss and lesion depth were measured before and after the experiment by means of quantitative microradiography and polarized light microscopy. At the surface enamel, a significant reduction of enamel demineralization was found after the xylitol treatment. The lesion depth at the surface enamel increased 17 μm after sucrose and 7 μm after xylitol ($P < .05$) [134]. Similar enamel blocks were also treated for 28 days with 35% (2.3 M) xylitol toothpaste [135]. The results were in line with the above study.

(7) Effect of Xylitol, Lactitol, and Other Sweeteners on Tooth Demineralization. Mixed cultures of dental plaque organisms were incubated for 24 hours in media containing different sweeteners. The attack of acids so generated was measured by Ca and phosphorus analyses. Demineralization was most severe with glucose and sucrose. Lactitol and xylitol showed extremely low enamel demineralization figures [136].

(8) Effect of Fluoride-Polyol Gum on Remineralization. Maxillary acrylic appliances carrying carious enamel sections were worn by subjects who used Fluogum (0.113 mg F/stick) that also contains xylitol and sorbitol. After three days of chewing 15 sticks there was a significant reduction in both lesion depth and in the size of the body of the lesions by an average of 5% ($P < .05$) [137]. It is possible that both polyols contributed to remineralization.

(9) Combined Effect of Xylitol and Fluoride on Enamel Demineralization In Vitro. Bovine enamel was exposed to a buffered lactic acid solution at pH 4.5 at +37°C. The selected enamel windows were treated with 2.63 M xylitol, 0.3 mM NaF, and their combination. Control enamel was not treated. The lesion depth was assayed by means of transversal microradiography. Xylitol and NaF had comparable effects on lesion reduction. The effects of F^- and xylitol were additive [138].

(10) Effect of Sorbitol Chewing Gum on Enamel Lesion Remineralization. Subjects wore *in situ* appliances on which were mounted enamel sections containing artificial caries lesions [139]. Sorbitol-containing gum (the U.S. brand Orbit Extra) was chewed by volunteers for 20 minutes 5 times a day over a period of 7 weeks according to a cross-over design. Microradiography showed that gum chewing caused 18.2% remineralization compared with the control's 12.1% remineralization ($P = .07$). However, a regular sucrose gum, used in an identical protocol, also caused significant remineralization (18.3% versus 10.8% in the control). The latter observation and the long chewing time used in this study suggest that any possible, specific polyol effect was possibly masked by a common "salivary effect". Hence, no proof was obtained for a specific remineralization-enhancing effect of sorbitol itself.

(11) Effects of Sucralose, Xylitol, and Sorbitol on Remineralization of Rat Caries. Rats infected with *Streptococcus sobrinus* were first given drinking water containing 10% sucrose. A group of animals were thereafter fed either the same sucrose water, or received their nutrition by gavage and drank water containing 0.03% sucralose, 10% xylitol, or 17%

sorbitol (added at a sweetness equivalent to 10% sucrose). The authors [140] reported that removal of the cariogenic challenge (sucrose) allowed remineralization to occur and that no sweetening agent was superior to another in this respect. The following details of the study deserve attention. (1) The intervention lasted only three weeks. (2) The rats were mono-infected with one bacterial species. (3) The authors' attempt to create almost isosweet drinking water for the animals resulted in significant differences in the chemical concentrations of the sweeteners used: sucrose 0.292 M, sorbitol 0.934 M, xylitol 0.658 M, and sucralose 0.876 mM. These characteristics of the study make it impossible to draw conclusions on the relative ability of the named sweeteners to facilitate remineralization or to reverse the process of "natural" caries.

(12) Effects of Sorbitol and Sorbitol/Xylitol Chewing Gums on Human Enamel Remineralization. Intraoral remineralization of experimental caries-like lesions in human enamel was studied by Manning et al. [141]. Polarized light microscopy and quantitative microradiography showed that 20-min chewing of a sorbitol gum and a 3 : 1 sorbitol/xylitol gum 5 times per day over a period of 21 days promoted remineralization to a similar extent. The following features of the study deserve attention: (1) The long chewing time used may have abolished any possible, specific polyol effects. (2) Both chewing gums contained sorbitol as the only or as the clearly predominating polyol sweetener. Hence, it may be difficult to compare the remineralization-enhancing effect of xylitol and sorbitol in this study.

(13) Xylitol-Induced Changes of Enamel Microhardness after Consumption of Xylitol Candy. Slabs of bovine enamel were inserted in cavities of children with rampant caries. The negative control subjects did not receive sweets whereas the treated subjects received 20 g xylitol daily in the form of candies. Predemineralized and non-demineralized enamel showed pronounced, statistically significant rehardening at exposure to xylitol ($P < .001$). Microradiography confirmed these findings [142].

(14) Effect of Xylitol and Sorbitol in Chewing-Gums on Mineral Loss of Enamel. Human subjects with $>3 \times 10^5$ mutans streptococci per ml of saliva participated in a cross-over study involving the use of four different chewing-gums containing: (1) 70% xylitol, (2) 35% xylitol + 35% sorbitol, (3) 17.5% xylitol + 52.5% sorbitol, and (4) 70% sorbitol [143]. The subjects used 12 pieces of each gum (1.6 g) per day for 25 days. The wash-out periods lasted about 10 weeks. The subjects wore a removable palatinal plate containing two demineralized human enamel samples. The authors reported that the lesion depth and the mineral loss values, assessed microradiographically, did not differ significantly between groups. Increased concentration of xylitol in the gum resulted in a lower number of mutans streptococci in saliva and dental plaque. However, the pH drop in plaque measured in vivo after a 1-minute mouthrinse with a 10% sorbitol solution was least pronounced after the 70% xylitol gum and most pronounced after the 70% sorbitol gum period ($P < .01$). It is possible that the cross-over study design, the shorter wash-out periods, and shortness of the treatment affected the microradiographical data [144].

(15) Stabilization (Remineralization) of Rampant Caries by the Use of Polyol Chewing Gums. The results from two cohort studies on arrest and non-progression of dentin caries after long-term usage of xylitol- and sorbitol-containing chewing gums by young subjects are summarized [145, 146]. The original research papers [61, 72] suggested that 40- and 24-month consumption of xylitol-containing chewing gum was superior to sorbitol-containing gum in the arrest of dentin and enamel caries. A re-examination 5 years after the 24-month study lent further support to this finding [83].

(16) Remineralization of Dentin Caries Lesions of Primary Teeth by Means of Physical, Chemical, and Histologic Procedures. The Stann Creek study in Belize showed that habitual use of xylitol gum was associated with arrest of dental caries in young subjects [72]. After a 20–22-month intervention (when the children were 8 years old), a total of 23 primary teeth with extensive dentin caries lesions, whose surface in clinical examination was found to be totally remineralized, could be removed because the teeth were near their physiologic exfoliation time. The majority of the specimens had been remineralized from the surface by a non-cellular-mediated process. The topmost 20-μm layer of the lesions exhibited the highest Ca : P ratio. The rehardened surface layer (normally <0.1 mm in thickness) was significantly ($P < .001$) harder than sound dentin and nearly as hard as sound enamel. The extracellular remineralization was most likely mediated by odontoblasts [147]. The bioinorganic and physicochemical mechanisms behind these effects are outlined [14, 15, 102, 104].

(17) Timing of First Restorations in Relation to a Preventive Xylitol Trial. Re-examination 3 and 5 years after a 2-to 3-year xylitol gum program suggested that xylitol had "permanently prevented caries" [80]. An independent retrospective analysis showed that the need for restorations was significantly postponed in xylitol-receiving subjects [81].

(18) Rehardening of Enamel Caries Lesions after a 16-Month Xylitol Gum Program That was Preceded by a 40-month Sucrose Gum Program. The 40-month Belize chewing gum program showed that the use of sucrose gum by subjects with high dietary sugar consumption was associated with high caries activity. After the termination of the 40-month trial, subjects of the sucrose gum group were recruited to an intense xylitol gum program (xylitol consumption level: 14 g per day and subject) for 16 months. The intensified xylitol gum usage was associated with a significant reduction in the mean DMFS score ($P = .0013$). The reduction of the score most likely resulted from the change of the *D* component of the index and possibly reflected rehardening of some caries lesions to a non-progressive carious state [82].

(19) Influence of Xylitol and NaF on Dental Erosion In Vitro. Sectioned bovine incisors were treated with orange juice only or with juice containing 25% (1.64 M) xylitol and/or 0.5 ppm F. The samples were immersed in the test solutions six times daily for 5 min on each occasion for 24 days (total exposure: 12 hours). Mineral loss measurements showed that xylitol and F had an additive effect on the reduction of dental erosion by orange juice in vitro [148]. Using a similar study design, the same authors concluded that "tolerable levels of xylitol alone may not show significant caries inhibiting and remineralizing effect, but may act as a caries inhibitor additively with fluoride" [149].

(20) Japanese Remineralization Experiments with Xylitol and Ca Hydrogenphosphate. Yanagisawa and his co-workers [150, 151] have provided crystallographic evidence on the restorative process of xylitol-associated remineralization. In their studies human enamel specimens were demineralized in acetate buffer at pH 4.0 and subsequently immersed at 37°C for two weeks in a remineralizing solution containing Ca, phosphate, F, and 20% xylitol (control solutions did not contain xylitol). Contact microradiography showed that, in the absence of xylitol, remineralization was mainly observed in the surface layers of the samples. In the presence of xylitol, somewhat less remineralization was observed in the outermost surface layers (up to 10 μm), whereas the middle and deeper layers exhibited pronounced remineralization. Multipurpose image processor studies confirmed this observation. It was concluded that xylitol acted as a carrier of Ca, maintaining a constant Ca level by introducing more mineral from the surface layers into the middle and deeper zones. Eventually, in the presence of xylitol, remineralization occurred over the entire demineralized zone [152, 153]. Further studies demonstrated that a combination of xylitol and Ca hydrogenphosphate was more effective than this same Ca salt supplemented with erythritol, sorbitol, maltitol, or palatinit [154]. When funoran (a sulphated polysaccharide of the seaweed *Gloipeltis furcata*) was added to the xylitol-Ca salt mixture, an increase in the extent of remineralization was observed [155]. Another study showed that the use of a chewing gum containing xylitol, Ca hydrogenphosphate, and funoran was more effective than a similar gum containing maltitol [156]. The inhibitory effect of funoran on the adherence and colonization of oral bacteria was further clarified by Saeki [157–159]. Recently, Japanese and Thai researchers teamed up for studies which supported the above concept, that is, the remineralization-associated influence of xylitol chewing gum containing funoran and calcium hydrogenphosphate [160].

(21) Remineralization of Enamel Lesions by Various Forms of Ca in Mouth Rinse or Chewing Gum. Casein phosphopeptide and amorphous Ca phosphate nanocomplexes (CPP-ACP) have been intensively tested by Reynolds's research team [126, 161]. The CPP-ACP complex was tested in two different Lotte (Tokyo, Japan) xylitol gum formulas (one containing xylitol and $CaCO_3$, the other xylitol, $CaHPO_4/CaCO_3$, and funoran). Several CPP-ACP mouth rinse preparations were also tested. The xylitol-CPP-ACP and the sorbitol-CPP-ACP combinations prevented demineralization and promoted remineralization [126]. Xylitol and sorbitol gums did not differ [161]. These studies have employed a cross-over design with wash-out periods of only one week or somewhat longer between interventions. Owing to the fact that xylitol can exert long-term effects on dental caries [144], it is impossible to draw firm conclusions from these studies as to the relative ability of polyols to enhance remineralization.

(22) Effect of Polyol-Containing Saliva Substitutes on Demineralized Dentin Specimens. Bovine dentin specimens were demineralized for 14 days at pH 5.5 and subsequently treated for 14 days with mucin- and CMC-based saliva substitutes that also contained 2% xylitol or sorbitol. Loss of mineral was assayed by means of transversal microradiography. For the dentin specimens, significant ($P < .05$) differences were observed between xylitol and sorbitol, the xylitol-containing saliva substitutes displaying lower mineral loss values. For enamel specimens, no significant differences were observed [162].

(23) Effect of Xylitol + Ca Lactate on Remineralization. Artificial caries-like lesions were created in human enamel slabs which were subsequently worn by volunteers who used, in a three-leg study, over a period of two weeks, chewing gum containing either xylitol or xylitol + Ca lactate, or did not receive any gum [163]. X-ray spectrometry showed that the xylitol-Ca combination remineralized the lesions more effectively than the xylitol and the no-gum periods.

(24) Caries Prevention with Xylitol-Containing Hard Candies among Disabled Pupils. A field study of 18 months' intervention was carried out in physically disabled school children, who received about 2.3 g xylitol in the form of hard candies in three daily episodes. The control subjects did not receive xylitol candies. The authors concluded that xylitol had "a strong and a clear remineralizing effect on caries" [57].

(25) Effect of Xylitol + F Toothpaste on the Remineralization of Enamel In Vitro. Human enamel specimens, demineralized at pH 4.5, were treated twice a day for 14 days in a silica-based toothpaste slurry containing 500 ppm F, 500 ppm F + 5% xylitol, or no added F and xylitol. Quantitative light-induced fluorescence studies showed that the combination of xylitol and F toothpaste was superior to the other treatments in occasioning remineralization of demineralized enamel [164].

(26) Effect of Xylitol and Fluoride on Enamel Erosion In Vitro. Since enamel erosion and tooth demineralization are chemically partly similar processes, one human enamel erosion study will be mentioned below. Human third molars were immersed for one min or five min in various test solutions four times a day for 14 days. Mineral loss was determined from lesion depth and surface hardness. Addition of xylitol, NaF, or a xylitol/NaF combination to an acidic drink (orange juice) reduced (but did not prevent) enamel erosion [165].

(27) Effect of Isomalt on Enamel De- and Remineralization. In an in vitro study, subsurface bovine enamel lesions were subjected to 3-week pH-cycling involving 5-minute rinses with 10% isomalt (*α-D*-glucopyranosyl-1,6-sorbitol) solutions daily and 10% isomalt additions to re- or demineralizing solutions [166]. A 0.2 ppm fluoride "background" was used during the remineralization phase. In an *in situ* study, subsurface lesions were exposed for 2 months in vivo and brushed 3 times a day with a tooth paste containing 10% isomalt. In the in vitro study, 5-min rinses resulted in slightly increased remineralization, while continuous presence of 10% isomalt (in re- or demineralizing solutions) inhibited de- and/or demineralization. The *in situ* test confirmed enhancement of remineralization. It is possible that isomalt stabilizes the calcium phosphate system present in enamel surface.

13. Erythritol

The noncariogenicity of erythritol has been investigated in rats [167] and, based on the presence of various risk factors of caries (such as mutans streptococci and dental plaque), erythritol has been regarded as noncariogenic in humans [168–170]. All studies strongly support the idea of erythritol as a caries-reducing dietary polyol. Especially the way erythritol inhibited the growth of certain mutans streptococci isolates is interesting [170]; the results indicate that the mechanism of growth inhibition differs from that caused by xylitol. Partly based on such experiments, it is tempting to postulate that certain combinations of erythritol and xylitol will turn out to exert promising caries-limiting effects in humans. The combined effects may exceed or at least equal the separate effects of these polyols.

Also studies on erythritol have re-emphasized the inevitability of the existence of important differences between individual alditols; the alditols cannot be regarded as an entity with exactly identical molecular parameters and similar biological effects. Such a contention would be incongruent with accepted physical and chemical laws. It would be space-consuming to list all separate studies that have demonstrated the existence of selective alditol effects. In the case of erythritol, however, the following examples may serve a purpose: Sugar alcohols, especially erythritol, enhanced the fungicidal effect of benzethonium chloride toward in vitro candidal biofilms; the difference with xylitol and *D*-glucitol (sorbitol) was significant [171]. De Cock and Bechert [90] emphasized the free-radical-quenching effect of erythritol, a property which may play a role in the "functionality" of erythritol-containing food products.

Several erythritol caries trials have been initiated in various parts of the world. Although there is still no valid clinical proof, the known molecular parameters of simple dietary alditols suggest that their caries-limiting efficacy may follow the simple homologous alditol series, will depend on the number of hydroxyl groups present in the alditol molecule, and will decrease as follows: erythritol ≥ xylitol > sorbitol. Combinations of erythritol and xylitol may have an edge over either of the alditols used separately: their microbiologic mechanism of action in caries prevention seems to differ and they are believed to exert a concerted and additive effect. However, no long-term human caries trial on erythritol has been completed. Hence, the possible difference between erythritol and xylitol in terms of caries-limiting ability will hopefully be elucidated in the near future. It will be essential to carry out trials where all of the above polyols (one tetritol, one pentitol, and one hexitol) will be simultaneously tested. The excellent gastrointestinal tolerability of peroral erythritol even in infants may promote its combination with xylitol in caries-limiting strategies.

14. Addendum

The following research papers and statements have been published after the above text was completed.

The effect of xylitol chewing gum on the acquisition pattern of 39 bacterial species (including mutans streptococci) was investigated in infants [172]. Mothers used xylitol or sorbitol gum (4.2 g/day) or no gum; gums were used 3 times a day for 9 months. The authors concluded that maternal use of xylitol gum did not result in statistically significant differences in the microbial plaque composition of 9- to 14-month-old infants. These results partly contradict those obtained in Finnish and Swedish mother-child studies (Table 1) where the counts of mutans streptococci decreased in children whose mothers had used xylitol. Perhaps the treatment period should have been extended beyond 14 months since the "window of infectivity" for mutans streptococci does remain open longer than 14 months. It is also possible that the polyol level in the tested gums was too low.

The following study completes caries trial information of Table 1: Pediatric topical oral xylitol syrup was administered in a group of 94 children aged 9 to 15 months for about 10.5 months. Parents administered syrup twice a day (2 xylitol 4 g doses and 1 sorbitol dose) (Xyl-2x group) or three times per day (3 xylitol 2.67 g doses) (Xyl-3x group) versus a control syrup (1 xylitol 2.67 g dose and 2 sorbitol doses). The data showed that oral xylitol syrup administered topically 2 or 3 times daily at a total daily dose of 8 g was effective in preventing early childhood caries [173]. These findings seem to be in congruence with previous field experience (Table 1) and also with the theory of the pharmacologic mode of action of xylitol in caries limitation [18].

The concept of the formation of oral biofilm has emerged during the last decades to facilitate the understanding of physiological oral processes. A recent study suggested that xylitol not only effectively inhibits acid production of cariogenic bacteria, but also prevents the formation of a multi-species bio-film [174]. In many instances oral bio-film is tantamount to dental plaque.

It is noteworthy that recently published anonymous dental journal editorials have emphasized the role of xylitol in caries prevention [175–177]. In an article in The Journal of American Dental Association [175] the potential role of xylitol-containing oral syrup in the prevention of childhood caries was discussed. The European Union also finally cleared xylitol for certain anti-caries claims [176]. The American Academy of Pediatric Dentistry's Council on Clinical Affairs issued its policy on the use of xylitol in caries prevention

[177]. The resolutions and endorsements contained in the above texts are reflected in recent conclusions of leading experts on the use of polyol gums to prevent dental caries in general [178] and especially in early childhood [179, 180].

Finally, a recent research paper has shown that xylitol can indeed modify dental plaque, resulting in marked reduction in plaque acidogenicity (which was not detected using *D*-glucitol) [181], a finding that confirms similar results obtained almost 20 years earlier [182, 183]. Previously observed morphologic effects of xylitol on *S. mutans* [184] received partial support from a recent study [185] which showed that chewing xylitol gum over one year may negatively affect the synthesis of extracellular polysaccharides by *S. mutans* (because the adherence of colonies in xylitol-receiving subjects decreased). Related to these findings is the observation that both erythritol and xylitol can decrease polysaccharide-mediated cell adherence that contributes to plaque accumulation [186]. Since cooked starch can be regarded as potentially cariogenic, it is noteworthy that xylitol, along with maltotriitol ("gluco-gluco-sorbitol"; derived from maltotriose) and acarbose (a pseudotetrasaccharide containing an unsaturated cyclitol moiety; an α-glucosidase inhibitor) decreased the production of acid from starch by *S. mutans* and *S. sobrinus* [187].

15. Conclusions

Dental caries is a multi-factorial, diet-associated infectious disease that initiates as minor calcium-deficient lesions in tooth enamel. The repair (remineralization) of minor enamel defects is a normal physiological process that is well known to clinicians and researchers in dentistry and oral biology. This process can be facilitated by various dietary and oral hygiene procedures. The remineralization (rehardening) process may also concern dentin caries lesions. Consequently, the disease (dental caries) is reversible, if detected and treated sufficiently early. The scientific and clinical information available today indicates that habitual use of xylitol, a sugar alcohol of the pentitol type, can be associated with significant reduction in the incidence of dental caries and with remineralization of both enamel and dentin caries lesions. Suitable xylitol-containing "vehicles" include chewing gum, hard candies, gum arabic-based saliva stimulants, and dentifrice. Mouth rinses ("artificial saliva") and fluoride gels aimed at hyposalivation and at special dental caries patients, respectively, also serve as clinically effective oral health adjuvants. Other dietary polyols that can remarkably lower the incidence of caries include erythritol which is a tetritol-type alditol. Based on the molecular parameters of simple dietary alditols, it is conceivable to predict that their efficacy in caries reduction will follow the homologous series, that is, that the number of hydroxyl groups present in the alditol molecule will determine the efficacy as follows: erythritol $\geq$ xylitol > sorbitol (the possible difference between erythritol and xylitol has not yet been adequately established). Most caries-related information available today is focused on the effects of xylitol and sorbitol. The present review examines the physical, bioinorganic, and biological chemistry of alditols from points of view that are believed to play a role in oral biology and caries prevention. The review also provides an account of tooth remineralization studies carried out with xylitol and sorbitol, as well as reports on recent caries-associated findings on erythritol.

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Research Article

Sociodemographic Determinants for Oral Health Risk Profiles

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The present study aimed to explore the association between caries risk profiles and different sociodemographic factors . The study sample ($n = 104$) was randomly selected within an urban population in Flanders, Belgium. Caries risk was assessed by anamnesis, clinical examination, salivary tests, and a questionnaire. Age, gender, and socio-economic status were extracted from social insurance data files. Social indicators were "occupational status," "being entitled to the increased allowance for health care interventions" and having access to the "Maximum Bill" (MAF), initiatives undertaken to protect deprived families. In the bivariate analysis there were significant differences in risk profiles between occupational groups ($P < .001$), between entitled and non-entitled individuals to the increased allowance ($P = .02$), and between access or no-access to the MAF ($P < .01$). The multiple logistic model showed a significantly higher chance of being in the low risk group for individuals with no-access to the MAF compared to those with access (OR:14.33–95% C.I. 2.14–95.84).

1. Introduction

Taking into account new insights in the management of diseases, a patient-centred holistic approach is recommended. This involves that care providers should respect patients' prospects, concerns, preferences, wants and needs, and solicit patients' input into decisions [1].

This person-centred approach is very important in preventive care and is directed to increase patients' knowledge and beliefs, self-regulation skills and abilities, and social facilitation [2]. An initial assessment of these factors together with biological predictors of a potential disease will be part of new preventive health management strategies.

In order to plan appropriate, patient-centred caries management in oral health care, frameworks are elaborated which the dental team can use to bring together key elements of information about patients and patients' teeth. Recently "risk assessment" and "early detection" were focused [3–5]. "Risk assessment" aims to detect unfavourable factors before the initiation of the disease. It is the process of quantifying the probability of a harmful effect to individuals or populations from certain human activities or from unfavourable environmental factors. "Early detection" aims to detect any disease process in a very early stage.

Risk assessment is part of a primary prevention strategy, early detection is part of the secondary prevention.

Caries management by risk assessment (CAMBRA) coupled with early detection and a quick and effective response can be seen as one of the best and cost-efficient ways of dealing with one of the most prevalent oral health problems, caries. This "medical model," where the etiologic disease-driving agents are balanced against protective factors, and integrated in a risk assessment model, offers the possibility of patient-centred disease prevention and management before there is irreversible damage done to the teeth [6].

The rational for a caries risk assessment management in industrialized Western countries is as follows.

(i) A rather low incidence of the disease in the general population justifying the efforts and costs to identify high-risk groups. In the late 70s the incidence of caries was very high and omnipresent in all age groups. In contrast, today caries prevalence and incidence decreased and are concentrated in 20% of the population. An attempt to identify individuals and groups expected to be at high-risk seems sensible.

(ii) Risk assessment as a screening activity without followup and an adapted targeted prevention is useless.

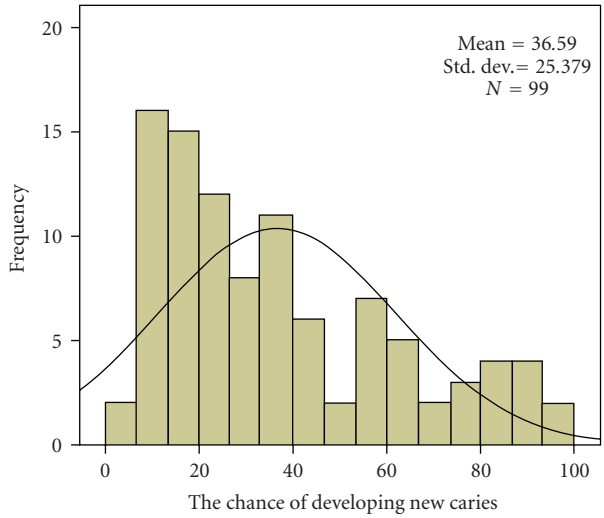

Figure 1: The chance of developing new caries expressed in a percentage.

On the other hand, when linked to an explicit strategy of targeted preventive care to well-defined and -identified risk groups, it becomes very useful, even compelling.

(iii) The match of preventive care to the individual risk profile of specific individuals or groups avoids wastage of already scarce resources.

Caries risk assessment combines an assessment of disease indicators and risk factors. A small number of key disease indicators and risk factors determine whether the individual is at low, moderate, or high-risk.

Risk factors can be biological, behavioural, or socioeconomic contributors to the caries disease process that can be modified as part of the treatment plan. If the disease is currently active, or if there is the future risk of progression of dental caries, intervention appropriate to the risk status is required to correct the caries imbalance before cavitation occurs [6].

The most difficult parameters to be modified are the socioeconomic contributors. Oral health risk profiles may be unevenly spread over the various social groups in the population. Insight of the risk profile of social vulnerable groups is an interesting item to pay attention to in the implementation of caries management by risk assessment.

Risk-based prevention programmes can be effective in further reducing dental caries in a low-caries community; this is demonstrated in previous research, especially targeting very young children [7]. Further research, exploring the perspectives of public health and targeting socially deprived groups, can contribute to this further reduction.

2. Objective

The aim of the present study was to explore the association between risk profile for dental caries and different sociodemographic factors on an individual level.

3. Material and Methods

The study sample (n = 1000) was randomly selected, after stratification by age, within the population of a metropolitan area in Flanders, Belgium: Ghent and surroundings. Five age groups were defined. Invitations to participate were sent in four consecutive quarters, starting in November 2007 and ending in April 2008.

Data from clinical examination, salivary tests, health anamnesis, and an oral health habits questionnaire were used to assess oral health risk. In particular, caries risk was assessed including

(a) past caries experience (clinical examination),

(b) assessment of the general health (mainly diabetes, epilepsy, polypharmacy, and smoking habits) (health anamnesis),

(c) diet: intake of nutrients with high sugar concentration and frequency (number of meals and between-meals) (questionnaire),

(d) oral hygiene: frequency of tooth brushing (questionnaire),

(e) quantity of clinical observable dental plaque (clinical examination),

(f) fluoride programme (questionnaire),

(g) saliva: flow and buffer capacity (salivary tests),

(h) risk enhancing dental patterns: crowding, exposed root surfaces, and ill-fitting restorations (clinical examination).

Three examiners participated in the oral examinations. A calibration for the diagnostic criteria of caries was performed on 43 teeth, registered within 21 clinical cases. The inter-examiner reliability was high, with weighted kappa values being 0,97, 0,93 and 0,92 for the respective examiners.

The oral health habits questionnaire was previously validated (content validity) and tested for reliability in a test-retest procedure with 12 participants.

Analyses were performed taking the risk profile as a dependent variable. Risk profile was calculated as a percentage and reduced to a categorical variable in the inferential analyses. Three risk levels have been defined: low (25% or less), moderate (between 25% and 75%) and high (75% and higher).

Age, gender, and socioeconomic status were used as independent variables. They were extracted from social insurance data files. Social indicators were "occupational status", "being entitled to the increased allowance for health care interventions" and "having access to the mechanism known as the Maximum Bill (MAF)". The two last mentioned initiatives were undertaken to improve access to the health care system and to protect deprived families from large expenses for health care.

Nonparametric bivariate analyses by means of nonparametric tests (Mann-Whitney U and Kruskal Wallis for 2 or more independent groups, resp.), and multiple logistic regression were performed to estimate the contribution of

Table 1: Cross-tabulation for different sociodemographic variables and the three caries risk levels (Mann-Whitney U and Kruskal Wallis for 2 or more independent groups, resp.).

	Low risk	Moderate risk	High risk	*P*-value
Age				NS
<12 years ($n = 6$)	33,3%	66,7%	0%	
Young ($n = 19$)	47,4%	26,3%	26,3%	
Adults ($n = 54$)	46,3%	46,3%	7,4%	
60+ ($n = 20$)	45%	50%	5%	
Gender				NS
Male ($n = 42$)	40,5%	52,4%	7,1%	
Female ($n = 57$)	49,1%	38,6%	12,3%	
SES				<, 0001
Worker ($n = 21$)	23,8%	61,9%	14,3%	
Employee ($n = 30$)	66,7%	26,7%	6,7%	
Managerial ($n = 11$)	81,8%	18,2%	0%	
Self-employed ($n = 7$)	14,3%	57,1%	28,6%	
Others ($n = 2$)	0%	0%	100%	
Increased allowance				0,02
No ($n = 80$)	52,5%	40%	7,5%	
Yes ($n = 12$)	25%	41,7%	33,3%	
MAF Family (Maximum Bill)				<, 01
No ($n = 82$)	52,4%	40,2%	7,3%	
Yes ($n = 10$)	20%	40%	40%	

Table 2: Differences in mean risk profiles and components for different social groups.

	Chance of developing new caries	Fluoride programme	Amount of dental plaque	Diet
Access to MAF*	54.20%	22.40%	15.20%	10.50%
No access to MAF	33.27%	8.50%	10.50%	8.50%
P-value	.01	.003	.05	.5

*MAF: Maximum bill, a mechanism to protect deprived families from large expenses for health care.

the independent risk indicators. The analyses were carried out using SAS statistical program. The level of significance was set at 0.05.

4. Results

The response rate was low with 104 out of 1000 invited participants accepting the invitation and presenting themselves at the dental clinic for the oral examination. The third quarter presented the lowest response rate while for the first call the response rate was the highest (12%). There was little difference between responders and non-responders in terms of gender, age and social indicators. There was a small over representation of participants of forty and a small under representation of self-employed in the responder group.

4.1. Explorative Data Analysis. The average chance of developing new caries, calculated on the basis of the risk profile as it was described in Section 3 is shown in Figure 1.

The overall average chance of developing new caries was 36,6%. A rather equal spread was found between the different risk factors diet (9.3%), oral hygiene and plaque amount (11.3%), fluoride program and saliva properties (10.1%), and past caries experience and related diseases (5.9%).

The distribution of the chance of developing new caries was left-skewed. 45,5% of participants belonged to the "low risk" group, meaning that they have less than 25% chance of developing new caries, 44,4% belonged to the "moderate risk" group and 10,1% belonged to the "high risk" group, which has a mean chance of 87% of developing new caries.

4.2. Inferential Analysis. In the bivariate analysis (Table 1) risk profiles were not significantly different between age groups and between males and females. All social variables showed strong and significant links with the risk profile. There were significant differences between occupational groups ($P < .001$), between entitled and non-entitled individuals to the increased allowance ($P = .02$), and between access or no-access to the MAF ($P < .01$). Participants from lower social classes showed a significantly higher mean risk profile for developing new caries.

The most important factors related to dental caries in this group were an inadequate fluoride program (mainly frequency of tooth brushing with a fluoride toothpaste), and insufficient oral hygiene (plaque amount) (Table 2).

Table 3: Odds ratio for the chance of being in the low caries risk group (adjusted for age, gender, and occupational status).

	Odds ratio	95% CI	*P*-value
Access to the Maximum Bill	1		
No access to the Maximum Bill	14.33	2.14–95.84	.006

The multiple model (Table 3) showed that the chance of being in the low risk group for individuals with no-access to the MAF was 14 times higher compared to the individuals with access to the MAF (OR:14.33–95% C.I. 2.14–95.84).

5. Discussion

The findings indicate that risk-based prevention can be correctly targeted to socially vulnerable groups within the community. A stepwise use of risk assessment tools can be very helpful to further decrease caries prevalence in different age groups. This complements the findings reported in earlier research targeting the group of very young children [7]. A first step will be to identify risk groups within the community, the second to identify high-risk individuals within these high-risk groups and finally, to identify risk profiles.

Within the present study lifestyle related factors have been identified as important risk factors for caries in high-risk groups, in particular in socially vulnerable high-risk groups. Fluoride programmes, assessed by the frequency of tooth brushing with a fluoride toothpaste and intake of fluoride supplements, have an important impact on the risk profile of these groups. Oral hygiene, expressed as the amount of dental plaque, seems to have an important negative impact on caries risk profiles of socially vulnerable groups.

In risk-based prevention targeting social vulnerable high-risk groups, these lifestyle related factors will be an important feature. Effectiveness of health education, dealing with lifestyle related factors, has been demonstrated in low socioeconomic families [8], but extra efforts will have to be done to implement strategies for changing oral health behaviour in order to have a long-term impact on risk profiles. Patient-dentist communication will be extremely important. The usefulness of additional therapeutic contacts via a combination of telephone coaching, mobile phone Short Message Service or even electronic mail, as introduced in other health care settings [8], has to be considered.

Of course it should be noted that these data are based on a rather small sample.

The response rate was low. This is a weakness of the study. This is typical for this kind of surveys with people randomly invited to participate and relying only on their own initiative to make an appointment in the dental clinic. Since the profile of responders and non-responders did not differ significantly the effect of the low response rate can be considered limited. Further longitudinal research will be opened to explore the clinical and economic effectiveness of risk-based prevention programmes, particularly in identified high-risk groups, including extra communication tools to increase patient adherence.

6. Conclusion

All social variables showed strong and significant links with the caries risk profile. For each social category a gradation has been observed between the three different oral health risk levels. Stepwise risk-based prevention opens opportunities to further decrease caries prevalence in low-prevalence communities.

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Research Article

Late Established Mutans Streptococci in Children over 3 Years Old

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Acquisition of mutans streptococci has been reported to most commonly occur at approximately 26 months of age. In the present study, we detected *Streptococcus mutans* and *S. sobrinus* using polymerase chain reaction (PCR) assays in children, then re-examined the subjects to determine the time of acquisition of these bacteria over a 1-year period. The subjects were 57 children ranging in age from 3 to 5 years old, each with primary dentition. Plaque samples were collected from all erupted tooth sites using a sterile toothbrush. PCR assays were performed to detect the targeted mutans streptococci at the beginning of the study (baseline) and after 1 year. At the baseline examination, the prevalence of *S. mutans* and *S. sobrinus* was 61.4% and 54.4%, respectively, in all subjects, of whom 14 (24.6%) were positive for *S. mutans* alone, 10 (17.5%) for *S. sobrinus* alone, and 21 (36.8%) for both *S. mutans* and *S. sobrinus*, with 12 (21.1%) negative for both. After 1 year, 4 of 22 (18.2%) subjects newly had acquired *S. mutans* and 15 of 26 (57.7%) had aquired *S. sobrinus*, while 5 (8.8%) remained negative for both bacteria. The age of the first positive *S. mutans* finding ranged from 49 to 71 months, while that for *S. sobrinus* ranged from 49 to 81 months old. Our results suggest that *S. sobrinus* becomes established later than *S. mutans* in the oral cavities of children over the age of 3 years old.

1. Introduction

Mutans streptococci, comprised of *Streptococcus mutans* and *S. sobrinus*, are considered to be the principal etiologic agents of dental caries in humans [1–3]. Preschool children harboring both *S. mutans* and *S. sobrinus* have a significantly higher incidence of dental caries than those with *S. mutans* alone [4]. Some studies have suggested that there is a "window of infectivity" for mutans streptococci at an early age, after which colonization is not likely to occur [5–7], and others have reported its predentate presence in infants as young as 3 months of age [8–10]. It was reported that 10 of 15 children acquired mutans streptococci during a 7-year period, and a second "window of infectivity" after the age of 5 when the permanent dentition erupts has been postulated [11]. Although several studies have attempted to determine the time of initial mutans streptococci acquisition, it remains controversial.

In several epidemiologic studies, identification of *S. mutans* and *S. sobrinus* on such selective media as mitis-salivarius (MS) or MS-bacitracin (MSB) agar has been performed using colonial morphology methods [12–14]. However, accurate differentiation between *S. mutans* and *S. sobrinus* is not easy, as well as time-consuming and laborious [6], and it has been reported that *S. sobrinus* from dental plaque samples is especially difficult to culture directly on MSB selective medium [15, 16]. Thus, it is of great importance to distinguish the presence of these 2 species separately in children for accurate prediction and effective prevention of dental caries.

Thus far, several methods used for detecting and identifying mutans streptococci, including direct microscopy, cultivation, enzyme tests, monoclonal antibodies, enzyme-linked immunosorbent assays, and species-specific DNA probes, have been reported [17–20]. Several investigators have also developed polymerase chain reaction (PCR)

methods and found them to be more sensitive for detection, when compared to conventional culture techniques [21–23], as they have been shown able to detect low numbers of bacterial species with a detection limit of as few as 25–100 cells [22–24], while being quick and relatively simple to perform. Further, PCR assays have been reported suitable for the specific detection and identification of human cariogenic bacteria, such as *S. mutans* and *S. sobrinus* [22–25].

In the present study, we detected *S. mutans* and *S. sobrinus* using polymerase chain reaction (PCR) assays in preschool children, then re-examined the subjects to determine acquisition of those bacteria over a 1-year period.

2. Materials and Methods

Fifty-seven Japanese preschool children, aged 3 to 5 years old with primary dentition, who were visitors to Hiroshima University Hospital, were enrolled. Consent for participation was obtained from at least one of their parents prior to the study according to the ethical guidelines of the Declaration of Helsinki (1975). Those who had received antibiotics within the previous 3 months or with systemic diseases were excluded.

2.1. Plaque Sampling. Dental plaque samples at the beginning (baseline) of the study and after 1 year were collected from all erupted teeth by professionally brushing with a sterile toothbrush for 1 minute, using a previously described method [26]. Plaque adhering to the toothbrush was removed by washing several times in a tube of sterile distilled water. The plaque samples were immediately transported to our research laboratory and stored at −20°C, prior to extraction of genomic DNA. One year later, plaque samples were again obtained from the same subjects in the same manner.

2.2. Genomic DNA Preparation. *Streptococcus mutans* JCM5175 and *S. sobrinus* ATCC27607 were used as control organisms. PCR detection of the tested species was performed using primers, as previously described [22, 24], while that of 16S ribosomal RNA encoding gene (GenBank accession number M75035) was used as previously described [27].

Plaque samples were first harvested by centrifugation at 1,600× g for 20 minutes. The supernatants were discarded, and the individual cell pellets were stored at −20°C until DNA isolation. A genomic DNA preparation from each plaque sample was obtained using a standard miniprep procedure [28], to which we added an RNase treatment [29]. DNA concentrations in the dental plaque samples were calculated by measuring A260 and the quality was estimated by the A260/A280 ratio [30].

2.3. Conditions of PCR Amplification. PCR amplification was performed in a reaction mixture (25 μL) consisting of PCR beads (GE Healthcare UK Limited, UK) that contained an enzyme (Taq DNA polymerase), along with the required reagents, 25 pmol of each primer, and 20 to 50 ng of template DNA solution in a thermal cycler (PC-700 program temp control system, ASTEC Co. Ltd., Fukuoka, Japan). Each set of PCR analyses included a negative control (water blank) in addition to the positive control. The reaction mixture was denatured at 95°C for 3 minutes, followed by a series of amplification-denaturation steps at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute, which was repeated for 26 cycles, with a final cycle at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 5 minutes [22]. Following amplification, 15 μL of the PCR products were analyzed by electrophoresis on a 1.2% agarose gel. After staining with ethidium bromide, the newly synthesized DNA fragments were visualized under a 302-nm ultraviolet light. The size of the PCR products was estimated from the electrophoretic migration of products relative to a 100 base-ladder marker (Amersham Pharmacia Biotech, AB, Uppsala, Sweden).

Table 1: Distribution of mutans streptococci at the base line.

S. mutans	*S. sobrinus*	Number of subjects (%)
+	−	14 (24.6)
+	+	21 (36.8)
−	+	10 (17.5)
−	−	12 (21.1)
Total		57 (100)

3. Results

Table 1 shows the distribution of mutans streptococci at the baseline examination. The prevalence of *S. mutans* and *S. sobrinus* was 61.4% and 54.3%, respectively, in all subjects, of whom 14 (24.6%) were positive for *S. mutans* alone, 10 (17.5%) for *S. sobrinus* alone, and 21 (36.8%) for both *S. mutans* and *S. sobrinus*, with 12 (21.1%) negative for both *S. mutans* and *S. sobrinus*.

Table 2 shows the children that were found to have newly acquired mutans streptococci after 1 year and their ages. Four of 22 (18.2%) subjects negative for *S. mutans* at the baseline had acquired *S. mutans* and 15 of 26 (57.7%) *S. sobrinus*. The mean age for the first positive *S. mutans* and *S. sobrinus* result was 60.8 ± 11.4 months and 60.2 ± 8.6 months, respectively, while the age for the first positive *S. mutans* detection ranged from 49 to 71 months old and that for *S. sobrinus* ranged from 49 to 81 months old.

Figure 1(a) shows the distribution of *S. mutans* in different age groups at the baseline and after 1 year. Overall, the prevalence of *S. mutans* at the baseline and after 1 year was 61.4% and 64.9%, respectively. In the 3-year-old group, 12 (66.7%) of 18 subjects were positive at the baseline and 13 (72.2%) of those were positive for *S. mutans* after 1 year, while the same 13 (59.1%) of 22 subjects in the 4-year old group were found positive at both the baseline and after 1 year. In the 5-year old group, 10 (58.8%) of 17 subjects were found positive for *S. mutans* at the baseline and 11 (64.7%) were positive after 1 year.

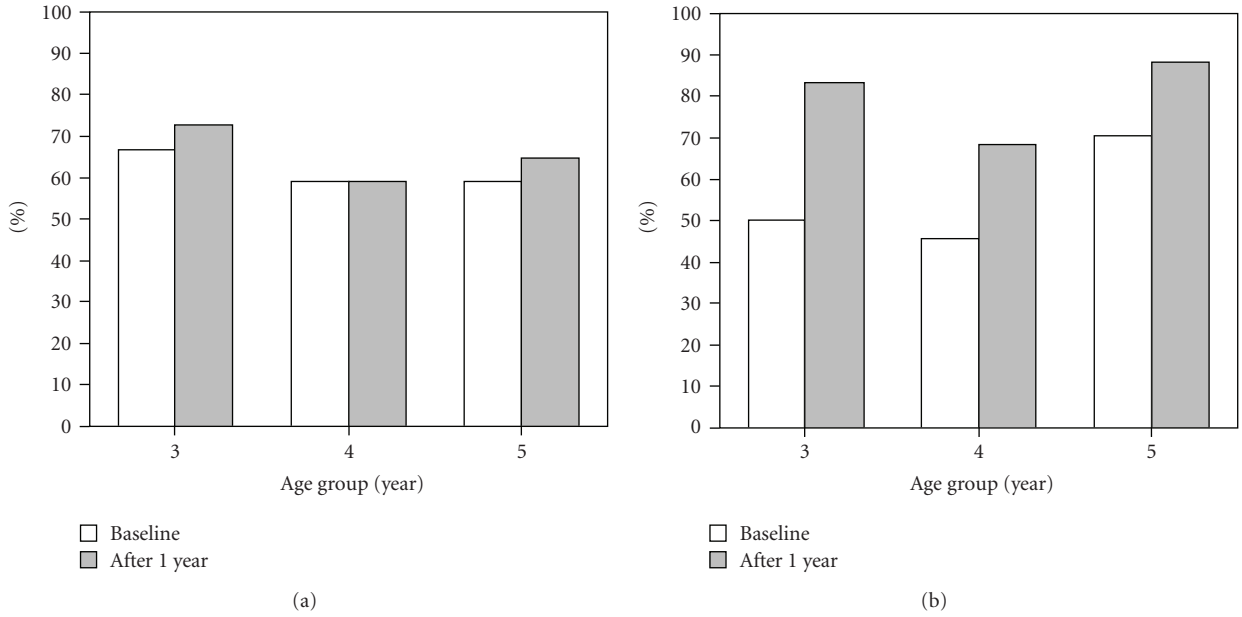

Figure 1: Distribution of *S. mutans* (a) and *S. sobrinus* (b) in different age groups at the baseline and after 1 year.

Table 2: Children and their ages with mutans streptococci acquired after 1 year.

Organisms present	Age[(a)] (months)	Age range (months)	Number (%) of subjects
S. mutans (− → +)	60.8 ± 11.4	49–71	4/22 (18.2)
S. sobrinus (− → +)	60.2 ± 8.6	49–81	15/26 (57.7)

[(a)]: Mean age ± Standard deviation.

Figure 1(b) shows the distribution of *S. sobrinus* in different age groups at the baseline and after 1 year. Overall, the prevalence of *S. sobrinus* at the baseline and after 1 year was 54.4% and 78.9%, respectively. In the 3-year old group, 9 (50.0%) of 18 subjects were found positive at the baseline and 15 (83.3%) were positive for *S. sobrinus* after 1 year. In the 4-year old group, 10 (45.5%) of 22 and 15 (68.2%) of 22 were shown to be positive at the baseline and after 1 year, respectively, while 12 (70.6%) of 17 and 15 (88.2%), respectively, were positive in the 5-year old group. The increased ratios (number of positive subjects after 1 year/number of those at the baseline) for *S. mutans* were 1.1, 1.0, and 1.1, at 3, 4, and 5 years of age, respectively, while the ratios for *S. sobrinus* were 1.7, 1.5, and 1.3, respectively.

4. Discussion

We performed a longitudinal study to determine whether mutans streptococci are frequently established in the oral cavities of children during a discrete-time period, known as the "window of infectivity," that is considered to range from 19 to 31 months of age, with a median age of 26 months [5], and the time of emergence of the primary molars [7, 31]. The majority of studies have suggested that mutans streptococci are not found until teeth erupt and become attachment sites for permanent oral bacterial colonization [5–7], though some authors have mentioned its predentate presence [8–10].

In the present longitudinal study, the PCR method used to detect *S. mutans* and *S. sobrinus* with 16S rRNA primers confirmed the presence of bacteria in all plaque samples (data not shown). This tool provides a more sensitive means of detection of cariogenic bacterial species, as compared with conventional cultural techniques [22, 24, 32]. Further, it has been reported that mitis-salivarius bacitracin inhibits the growth of *S. sobrinus* more than that of *S. mutans* [15, 16], and the recovery of *S. sobrinus* can be significantly underestimated using conventional cultural techniques [33]. We conclude that results of the present study show that this PCR method is suitable for investigation of the intra-oral distribution of *S. sobrinus* as well as *S. mutans*.

The prevalence of *S. mutans* and *S. sobrinus* at the baseline investigation conducted at 3 years old was 66.7% and 50.0%, respectively, and 27.8% for both, which is in agreement with other reports of preschool children [34–36]. The prevalence of children positive for *S. mutans* was constant in each age group at both the baseline and 1 year examinations. It has been suggested that *S. mutans* generally becomes established in the oral cavity of children before the age of 3 years old. However, the prevalence of children positive for *S. sobrinus* after 1 year was higher than that at the baseline, with increase ratios of 1.7, 1.5, and 1.3 in the 3-, 4-, and 5-year-old groups, respectively, indicating that the rate of increase gradually slowed. Further, 57.7% of all the subjects eventually acquired *S. sobrinus* and their mean age for the first positive detection was 60 months, of whom 18.2% had *S. mutans* detected first. It was also noted that *S. sobrinus* was frequently found in the oral cavities of children without

S. mutans after 3 years of age. Therefore, it is suggested that *S. sobrinus* is established later in the oral cavity of children as compared to *S. mutans* and that acquisition of *S. sobrinus* is still possible after the so-called "window of infectivity."

In the present study, 7 (50.0%) of the 14 subjects with *S. mutans* became colonized with *S. sobrinus*, while 2 (20.0%) of 10 subjects with *S. sobrinus* were first found positive for *S. mutans*. Considering the means of colonization by mutans streptococci, we speculate that *S. sobrinus* might easily colonize after the colonization of *S. mutans*, allowing it to become established in the oral cavity of *S. mutans* positive children. It is also suggested that the colonization of *S. sanguinis* may have an influence on subsequent colonization by mutans streptococci [37]. Further studies are required to understand the timing of initial infection with *S. mutans* and *S. sobrinus*.

The transmission to and colonization of mutans streptococci in the oral cavity are important factors for the prevention of dental caries. Previous studies have suggested that earlier colonization of *S. mutans* is related to a higher caries risk [34], and that children harboring both *S. mutans* and *S. sobrinus* showed a significantly higher incidence of dental caries than those with *S. mutans* alone in studies that used a conventional cultural [38], indirect immunofluorescence [39], and PCR methods [32]. In addition, the level of mutans streptococci in saliva has been shown to correlate both with past caries experience [40, 41] and future caries activity [42, 43]. We previously reported that incremental caries increases were significantly greater in children with both *S. mutans* and *S. sobrinus*, as compared to those with *S. mutans* alone [4]. Based on the present results, it is predicted that caries risk will increase in 3-year-old children with *S. mutans* already established, since *S. sobrinus* can become established after 3 years of age. We considered that the present results are highly relevant for development of prevention strategies for caries in childhood, as delayed acquisition of mutans streptococci might reduce the number of caries experienced in primary and permanent dentition at later ages [44].

In conclusion, our results suggest that *S. sobrinus* becomes established later in the oral cavity of children over the age of 3 years old as compared to *S. mutans*.

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Review Article

Explaining Gender Differences in Caries: A Multifactorial Approach to a Multifactorial Disease

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Many studies have demonstrated that caries rates are higher in women than in men. This review attempts to provide an explanation for this trend by examining each factor which contributes to caries and how the factor differs in men and women. Evidence has been provided to demonstrate that caries risk factors for women include a different salivary composition and flow rate, hormonal fluctuations, dietary habits, genetic variations, and particular social roles among their family. Systemic diseases that have been found to be associated with caries have also been found to have an association with the female gender. An extended exposure to the oral cavity or a more cariogenic oral microflora has not been proven to contribute to higher caries in women. Further research in these areas could be done in the future to explain their contribution, or lack thereof, to a higher caries rate in women.

1. Introduction

The significant impact of caries on the world's population makes the disease an important topic of understanding. The development of caries is multifactorial, depending on many interacting variables to promote its development. In particular, the presence of bacteria, a substrate for the bacteria (food/sugars), the host's oral environment, as well as the passing of time are the main contributing factors in the formation of caries. Epidemiological and clinical studies, through the use of tools such as DMFT and DMFS scores, have revealed a consistent trend in caries development, with females having higher prevalence than males [1]. The mechanisms underlying the reasoning for this trend can possibly be explained by an investigation of the suggested factors involved in caries development.

2. Genetic Contributions: *AMELX*

The underlying mechanisms of any genetic contributions to the increased prevalence of caries in females versus males can be speculated to reside in the sex chromosomes, exhibiting sex-linked modes of inheritance. Genes present on the X or Y chromosome whose function affects those factors which contribute to the development of caries can be investigated. Variations in these genes would alter the host's oral environment and the host's response to the initiation of caries.

The Amelogenin (AMELX) gene resides on the p arm of the X chromosome. Its locus is Xp22.31-p22.1 [2]. This gene and its protein product contribute to enamel formation in the dentition. The amelogenin protein constitutes 90% of the enamel matrix [3]. A mutation/deletion in the AMELX gene results in X-linked amelogenesis imperfecta [2]. There is a possibility that a deficient amelogenin gene or a decreased amount of amelogenin protein leads to disruption of formation of enamel matrix and therefore increased caries susceptibility [3].

The possibility of such a "caries susceptibility amelogenin variant" has been investigated. These studies explain that in females, it is possible for this kind of variation in AMELX to occur through the mechanisms of X inactivation and mosaicism. Normally, the inactivation of one X chromosome is random, with 1 : 1 distribution of the two AMELX genes inherited in females on the X chromosomes (mosaicism in regards to the X chromosome, since one comes from one parent and the other comes from the other parent). If a variant allele were present, the normal alleles that make up

50% of the genes could compensate and override any disease caused by the variant allele. However, if the X-inactivation is biased to favor those chromosomes with the deleterious variant AMELX allele, and the normal allele is nonrandomly inactivated, it would be expected that an increase in caries would be observed [3].

One study of 110 individuals used single-nucleotide polymorphism markers to genotype ameloblastin alleles. Individuals with "higher caries experience" (DMFT ≥ 3) were found to have an association with a variant allele marker for Amelogenin [3]. Another study using similar methods to target specific ameloblastin allele variants investigated the relationship in children. Overrepresentation of a particular allele marker (C allele) was seen in cases with DMFT scores higher than 8 [2]. These two studies have supported a link between the AMELX gene and caries rate.

If an AMELX gene variant affects females who exhibit mosaicism for the X chromosome, it would be expected that this variant would also increase caries incidence in males since they too have only one copy of the gene on the X chromosome. To explain why this is not observed, the homologous AMELY gene on the Y chromosome (locus Yp11.2) has been suggested to be involved. A male with a normal and active AMELY gene may display compensation for a caries susceptibility AMELX allele on his X chromosome [3]. Another way to explain the role of AMELY in caries susceptibility is to consider its production of the amelogenin protein. AMELY gene only expresses 10% of amelogenin that is expressed by AMELX [2]. However, this additional 10% is not attained by females exhibiting X inactivation. Therefore, males may be expressing a greater amount of amelogenin, contributing to the strength of the tooth and less caries susceptibility of the host [2]. These proposed mechanisms of AMELY may be one way to explain why when exploring the role of amelogenin on caries formation, females exhibit greater prevalence than males.

3. Saliva

The composition and flow rate of saliva in the host oral environment seem to be another source of susceptibility of caries formation in women. Saliva plays a protective role in the oral cavity through its buffering, mechanical washing, antimicrobial, and remineralization activities. However, the flow rates of saliva and compositional analysis have been shown to be generally less protective in women than in men. Additionally, the hormonal fluctuations in women tend to play a role in the less protective composition and flow rate of saliva.

When the flow rates of resting whole saliva and stimulated parotid saliva in participants aged 20 and older were measured using timed expectoration and a Lashley cup respectively, differences were found between genders. In all age groups, females were found to have a lower mean flow rate of whole saliva than males, with significant differences in the 80+ age group ($P < .02$). In addition, the overall mean parotid salivary flow rate in females (0.45 mL/min) was significantly lower than the mean parotid salivary flow rate in males (0.59 mL/min $P < .05$) [4]. These findings have been supported in a more recent study on minor salivary gland secretion rates. By measuring the salivary rate from 142 individuals using The Periotron 6000 model 2, investigators found that the salivary flow rates from the buccal and labial glands in women were lower than those in men, especially in the elderly participants [5]. A lower salivary flow rate in females puts them at a higher risk for caries because they lack more of saliva's mechanical washing, buffering, and remineralization benefits.

In addition, the report by Ellaison et al. (2006) investigated the salivary IgA concentrations of the men and women participants. Salivary IgA is an immunoglobulin found in the oral cavity which is protective against caries. Results of this study show a difference in IgA concentration between men and women, with women having a lower concentration of the protective IgA from minor glands, (buccal, palatal, and labial) [5]. Information was obtained using enzyme-linked immunosorbent assay (ELISA) technique. Samples from the buccal glands showed mean IgA concentrations of $95.2 \pm 76.9\,\mu$/mL in women as compared to $155 \pm 160\,\mu$/mL in men, in the palatal glands, $121.3 \pm 139.5\,\mu$/mL in women and $210.4 \pm 249.7\,\mu$/mL in men, and in the labial glands, $46.2 \pm 31.0\,\mu$/mL in women and $58.8 \pm 40.2\,\mu$/mL in men [5]. It appears that males have inherently higher concentrations of IgA immunoglobulin to defend their oral surfaces against carious activity.

4. Pregnancy

A compelling reasoning why women have greater caries activity than men argues that pregnancies have several negative effects on the oral cavity environment. In general, the experience of pregnancy includes immune suppression, cravings, hormonal fluctuations, salivary alterations, and other physiological changes that would be expected to adversely affect the host resistance to caries.

Hormonal fluctuations of estrogen occur in females during pregnancy, menstruation, and puberty. These elevated estrogen levels can lead to significant changes in the environment of the oral cavity. Clinical studies have investigated perhaps the most severe of these hormonal fluctuation events, pregnancy. Lukacs and Largaespada (2006) discussed rat studies in which a causal link was found between caries rate and estrogen levels, but similar androgen level fluctuations experienced by males did not show this same kind of link or even a correlation [1].

Pregnancy can also have negative effects on salivary flow, impairing the protective washing and buffering mechanisms of saliva against caries development. The study discussed earlier by Eliasson et al. (2006) compared resting whole saliva rates of pregnant women to control (nonpregnant) women and found that the pregnant women had a mean secretion rate less than their control counterparts (0.21 ± 0.13 versus 0.30 ± 0.16 mL/min).

An anthropologic argument by Lukacs (2008) suggests that the development of agriculture may be indirectly responsible for an increased caries rate in females. Lukacs argues that the transition to agriculture is associated with an increase in fertility due to a more sedentary lifestyle

and need for division of labor (as opposed to foraging and hunting). According to Lukacs, this would account for a decline in women's oral health related to the adverse effects of pregnancy on the oral environment [6].

5. Substrate/Diet

The presence of sucrose for *Streptococcus mutans*' metabolism is an additional factor in the establishment of a cariogenic environment. Dietary habits can have a major impact based on the form and frequency of the food. In many cultures historically, women have been the family member with the responsibility of food preparation. This would allow easier access to foods and snacks outside of mealtime, which provide bacteria in their oral flora with more substrate for caries development [7]. Particular dietary routines have been shown to increase the incidence of caries. In particular, vegetarian diets have been investigated in this aspect. Indian subjects are of particular interest in dietary habits because many have been vegetarian their whole life, offering a specific population. In a study of caries prevalence among 104 Indian participants ages of 5–72, patients with a vegetarian diet were found to have the highest numbers of caries when compared to those with a "vegetarian + tobacco" diet and a "nonvegetarian" diet. The investigators attribute this to a lack of putrefaction, a result of protein consumption, which contributes to the formation of a less acidic oral environment [8].

With the discovery that a vegetarian diet among Indian participants contributes to caries formation, it might be expected that more women are vegetarian than men, and this can be one way to explain their higher prevalence of caries. An epidemiological study by Shah (2003) included 1240 participants in the Southern Delhi area of India. Using survey techniques, investigators reported that more of the women participants were vegetarian than men (71% versus 56%) [9]. With the earlier described findings that vegetarian diets contribute to caries formation, this information can be added to the list of factors causing the gender disparity in caries prevalence.

6. Psychosocial and Economic Factors

Although clinical studies have been revealing in the reasons behind gender prevalence in females, epidemiological studies can also contribute to the cause. Women's role in society has been suggested as a causative factor in caries development. A study by Shah investigated sociodemographic gender differences in subjects and related them to health and disease. Shah's study included 1240 participants aged 60 and older in India and used survey techniques to evaluate the status of women in this region. In his findings, Shah includes a higher percentage of women being low SES (35% versus 2.8% in males), widowed (24.3% versus 13.9% in males), and economically dependent (79% versus 5% in males). Women also reported a lower level of literacy (35.5% versus 57.6% in males) and a lower sense of being loved and respected by their family (7% versus 92% in males) [9]. All of these factors contribute to a greater difficulty in an understanding of the disease process, ability to prevent the caries development process, and access to care for women.

Shah also includes information on the general population of women, pointing out that more women are single parents subject to the stress of care giving and also at an economic disadvantage. Women are more subject to domestic violence and eating disorders. They also live longer, increasing their prevalence of systemic issues and their use of medications. All of these factors can have an effect on host defense responses which would fight disease in the oral cavity.

7. Time

In the pattern of tooth eruption, females tend to acquire their teeth at an earlier age than males. A female's teeth are therefore exposed to the oral environment, bacteria, and bacterial substrates for a longer time than the teeth of a male the same age, providing more opportunity for the caries developing process to take place. Because of this trend, one might expect that the gender disparity in caries prevalence would be evident at an early age, but some recent investigations have found contradicting support of this expectation.

When investigating the prevalence and causes of Nursing Caries, it was discovered that of 544 children, the prevalence of Nursing Caries was greater in girls than in boys, with 23.5% prevalence in girls compared to 16.5% prevalence in boys. The children included in this study were 18 to 60 months old [10]. This study coincides with the idea that girls should be demonstrating more caries than boys early in life. However, Nursing Caries is a specific form of carious disease. Data from this investigation only reports the presence or absence of Nursing Caries, and does not report the gender comparative results in the form of DMFT or other more complete caries experience indicator.

An examination of 771 children who were 2 years old in Zurich identified the number of initial or cavitated lesions in children and reports male gender as a risk factor for caries [11]. However, a study of prevalence of dental health problems in children of Kerala examined 1068 children from ages 12–15 years old. They reported boys and girls being almost equally affected by caries, or with females slightly more affected (49% male versus 51% female) [12].

In a cross sectional examination of 10 and 11 years olds in southern Italy, a significant difference was found between the young boys and girls. The mean DFT for boys was 3.20 versus a mean DFT of 1.96 in girls. This difference was statistically significant, in finding that in this group, it was more common for the male children to have caries than the female children [13]. In India a similar investigation was done which demonstrates a window of time where prevalence switches from male to female. The study population was limited to 5 and 12 year old children (1009, 5-year-olds and 1013, 12 year olds). In the 5 year old age group, 47.4% of those children with caries were male, while 41.1% of those children were female. However, in the 12-year-old age group a greater percentage of those children with caries were female (24.1% female versus 20.6% male). Implications

made from this study can suggest that there is a time between 5 and 12 years old when higher caries prevalence in children switches from male to female [14].

Although, intuitively, the earlier exposure of female teeth to the oral cavity should provide explanation for the higher incidence of caries in females, contradictory information has been found to support this idea in children.

8. Bacteria

The primary initiator of dental caries is *Streptococcus mutans.* The presence of *S. mutans* can greatly increase the risk of caries if the host's defense mechanisms do not override the bacteria. It can be proposed that females are found with more caries because they harbor more of the caries causing bacteria (i.e., *S. mutans*). A study of twins (both monozygotic and dizygotic) tested the amount of *S. mutans* in participants using the Stripmutans test. Results of this study found no statistically significant gender differences in the amount of *S. mutans*. These results included those from 28 pairs of dizygotic opposite gender twins [15]. According to this study, it appears that there is no gender bias in the amount of *S. mutans* of the individual to contribute to caries formation. Additionally, an investigation by Loyola-Rodriguez et al. found no statistically significant differences in *S. mutans or S. sobrinus* (another caries causing microbe) between genders after inoculating saliva of caries-free and caries active children [16].

9. Systemic Correlations

The investigation in gender preference in caries rates can look at correlations with systemic diseases. If we know that there is a positive correlation between the female gender and caries rates and if positive correlations are found between caries and systemic disease, it could be expected that more women would be seen with the same systemic disease. This expectation was found to be true in a recent study aiming to link systemic diseases and oral conditions. With the use of the Dental Registry and DNA Repository at the University of Pittsburgh School of Dental Medicine, the medical history and DMFT/DMFS scores of 318 subjects were examined. An association was found between asthma and those individuals with DMFT above 15 and DMFS above 50. A similar association was found between high DMFT/DMFS scores and epilepsy [17]. According to our expectations, we would therefore anticipate that more women than men would report having asthma or epilepsy. Upon further inspection of those with these systemic diseases, another association was found in the prevalence of asthma, with females experiencing a higher occurrence ($P = .04$) [17].

10. Conclusions

In explaining the consistent trend of caries rates being higher in females than males, all contributing factors must be considered. There is no one reason for this disparity. Women's roles in their community (caretaker, meal preparation, etc.), along with other social factors, such as differing salivary flow rates and compositions, dietary habits, hormonal changes during pregnancy, and particular variants of the *AMELX* gene must all be included in the assessment of an individual woman's caries risk assessment. Substantial evidence has not been found concerning the contributions of the longer time female teeth are exposed to the oral cavity or concerning a differing microbial oral flora which might enhance the caries developing process. More research is needed to define the role of these two possible contributors more clearly in order for us to more completely understand the development of caries in women and to anticipate the disease process before it begins.

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Research Article

Tooth Decay in Alcohol Abusers Compared to Alcohol and Drug Abusers

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Alcohol and drug abuse are detrimental to general and oral health. Though we know the effects of these harmful habits on oral mucosa, their independent and combined effect on the dental caries experience is unknown and worthy of investigation. We compared 363 "alcohol only" abusers to 300 "alcohol and drug" abusers to test the hypothesis that various components of their dental caries experience are significantly different due to plausible sociobiological explanations. After controlling for the potential confounders, we observe that the "alcohol and drug" group had a 38% higher risk of having decayed teeth compared to the "alcohol only" group ($P < .05$). As expected, those who belonged to a higher social class (OR = 1.98; 95% CI = 1.43–2.75) and drank wine (OR = 1.85; 95% CI = 1.16–2.96) had a higher risk of having more filled teeth. We conclude that the risk of tooth decay among "alcohol only" abusers is significantly lower compared to "alcohol and drug" abusers.

1. Introduction

Alcohol and drug dependence are conditions characterized by psychological, physiological, and pathological changes, all of which are directly relevant to dentistry [1]. The psychological effects and the personality changes in the abuser may affect the patient/dentist relationship as they take a reduced interest in seeking and paying for dental care. The physiological effect of alcohol intoxication may lead to the inability to understand and accept advice given by health care workers that may result in noncompliance. Pathological aspects of alcohol and drug abuse on dental and oral tissues have not been examined in detail except for its effects on the oral mucosa [2].

We hypothesize that "alcohol only" abusers have a significantly different caries experience compared to "alcohol and drug" abusers due to a variety of biological reasons. We propose the following biological model to explain the potential association between alcohol and drug abuse and dental caries. Microbial oxidation of ethanol in saliva in alcohol abusers will result in the formation of acetaldehyde [3] that may further alter the cariogenic oral flora by reducing their levels [4]. Warnakulasuriya et al. have shown that certain alcoholic beverages in the UK contain high levels of fluoride and those who consume three cans of beer a day in the UK would receive the recommended daily upper limit of fluoride through beer alone [5]. As most alcoholics may consume more than three cans, their exposure to higher levels of fluoride via alcoholic beverages may reduce their caries susceptibility. Alcoholic beverages may also enhance the fluoride release in restorative materials such as compomers [6]. On the other hand, alcohol and drug abusers might experience dry mouth at night [7] and neglect both personal and professional oral health care [8]. They may

also consume higher levels of refined carbohydrates [9] to satisfy their "munchies." All of these might increase their risk of caries.

However, it is unclear how the alcohol and drug abuse may affect different components of their overall caries experience. Figure 1 explains the scenario described above as an attempt to provide the basis for our hypothesis. It is important to evaluate the effect of these exposures independently, and in combination, to better understand the association between alcohol and drug abuse and different components of the dental caries experience.

Globally, reliable epidemiological data on dental caries of alcohol and drug abusers are scarce. The objective of this study, therefore, was to test the effect of "alcohol only" abuse and "alcohol and drug" abuse on selected components of the caries experience in abusers who are residents in South London. It would have been ideal to have another similar-sized comparison group of those who abuse "drugs only," but we only had a limited sample of that group, and therefore, we will only describe the findings from that group as an adjunct to the main discussion.

2. Materials and Methods

The study group comprised of persons who attended the following clinical care facilities in south London between 1994 and 1999: A weekly out-patients' alcohol intervention clinic at King's College Hospital, the Drink Crises Centre (Voluntary Sector Residential Centre), Detoxification Units at The Maudsley and the Royal Bethlem Hospitals, the Community Drink/Drug Project Unit, a Rehabilitation Centre at St. Luke's Mission, and several local half-way housing units for chronic alcoholics. The study protocol was approved by the Research Ethics Committee of the King's Healthcare NHS Trust. Each volunteer was given an information sheet and a verbal explanation before being asked for written consent to participate in the study. All clinical care facilities were visited by one author (C.K.Harris) monthly/bimonthly subject to their availability. Using a feasibility sampling scheme all newly admitted subjects in residence or in attendance on the day of the visit were approached and invited to a dental and oral examination, except in situations where a Nurse Manager thought the person was too ill or would be unfit for an interview.

A questionnaire was used to record the type of alcohol beverage used, its frequency and duration of use, smoking habits, and standard demographic data (see the appendix in the Supplementary Material available online at doi:10.1155/2010/786503). Any drug abuse, its duration and the type of drug used were also recorded. Any prescribed or self-administered medication for the patient was also recorded. The examiner administered the questionnaire to each subject at the interview. The questionnaire was pilot tested [10] using 107 subjects drawn from three of the centers listed earlier. The subjects included in the pilot study were not included in the present analyses. We had no means of testing the validity of self-reported data but our experience is that UK study subjects are less likely to under-report even harmful habits.

Standard demographic data including ethnicity were recorded. Patients were classified according to the Registrar General's socioeconomic classification [11, 12]. A comprehensive clinical oral examination was performed on each subject. The standard World Health Organization protocol for dental caries examination and categorization was used [13]. No radiographs were taken. Oral examination lasted approximately fifteen minutes and the questionnaire administration took about 30 minutes on average. Every attempt was made to mask the interviewer to the examination data and vice-versa. Detail examination methods are given elsewhere [10].

Any abnormal findings and treatments required were reported to the patient on examination, the head of the unit at the institution where the patient was seen, and to the patient's General Medical Practitioner (GP), and the General Dental Practitioner (GDP). Patients not registered with a GDP were referred to the Primary Care Unit of the King's Dental School or to the St. Giles Trust for the Homeless in Camberwell, South London.

Data collected were managed and analyzed using SPSS Version 16. Univariate comparisons between the two groups were made using the independent samples t-test for quantitative variables and the chi-square test for categorical variables. Binary logistic regression models were developed for exploring both univariate and multivariate relationships; included in the latter were all variables with significance of 0.1 or less in the univariate analysis. Two-sided Type I Error probability $\leq .05$ was used as the level of significance.

3. Results

There were 388 subjects who identified themselves as "alcohol only" abusers and 305 subjects who admitted to abusing both "alcohol and drugs." We decided to exclude those who were edentulous. When the edentulous subjects were excluded from both groups, there were 363 "alcohol only" abusers and 300 "alcohol and drugs" abusers. Subjects were on average in their 3rd and 4th decades of life and predominantly White (over 90%) and male (over 75%). The "alcohol only" group was significantly older (43.5 ± 8.8 versus 35.4 ± 7.3 years; $P < .001$) and had abused alcohol for a longer period (22.9 ± 10.3 versus 16.6 ± 8.5 years; $P < .001$). However, their self-reported current smoking was significantly lower (84%) compared to the "alcohol and drugs" group (95%; $P < .001$; Table 1). There was no significant difference in mean weekly alcohol consumption (units per week) between the two groups ($P = .60$).

Types of alcohol and drugs used by men and women in each group are given in Figure 2. Significantly higher proportion of men in "alcohol only" group drank spirits and a lower proportion drank wine compared to the "alcohol and drugs" group (Figure 2(a)). Contrastingly, significantly lower proportion of women in the "alcohol only" group drank less cider (Figure 2(b)). Gender differences in the types of drugs used within "alcohol and drugs" group were not significant (Figure 2(c)).

The dental status and the caries experience are shown in Table 2. The "alcohol only" group had significantly fewer

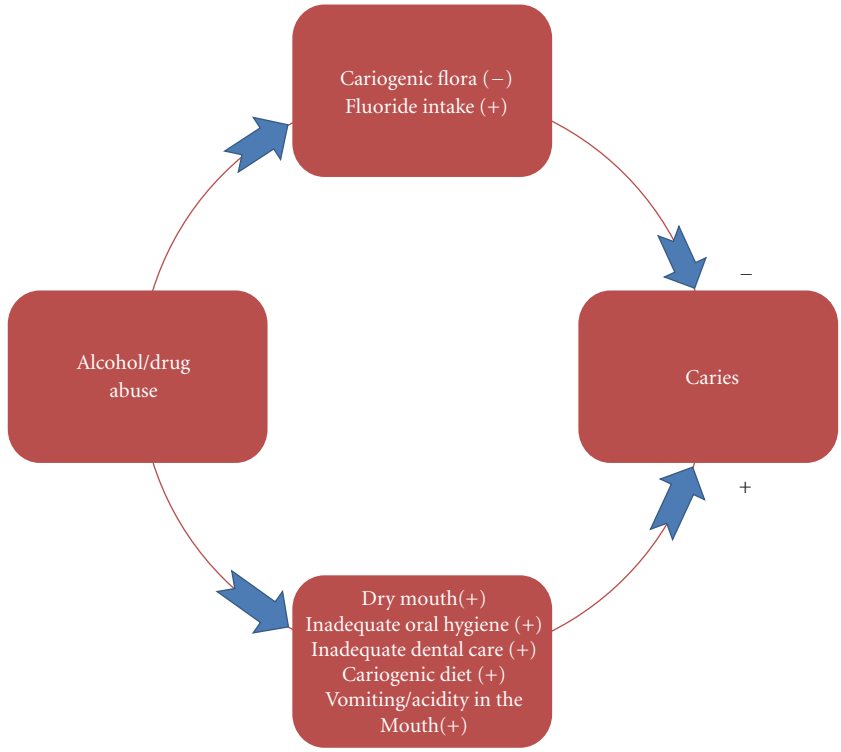


Figure 1: Hypothetical biological model to explain the association between alcohol and drug abuse and dental caries.

Table 1: Demographic and behavioural characteristics of the study subjects.

Variable	Alcohol only ($N = 363$)	Alcohol + drug use ($N = 300$)	P
Age (y): mean (SD)	43.51 (8.81)	35.41 (7.30)	<.001
Gender (%): M/F			.26
Male	288 (79.3%)	226 (75.3%)	
Female	75 (20.7%)	74 (24.7%)	
Race (%): W/B/A			.15
White	339 (93.4%)	274 (91.3%)	
Black	11 (3.0%)	18 (6.0%)	
Asian	13 (3.6%)	8 (2.7%)	
Duration of abuse (years)	22.93 (10.34)	16.63 (8.54)	<.001
Alcohol units (per week)	286.02 (126.23)	280.91 (119.38)	.60
Current smoking (%)	306 (84.3%)	285 (95.0%)	<.001

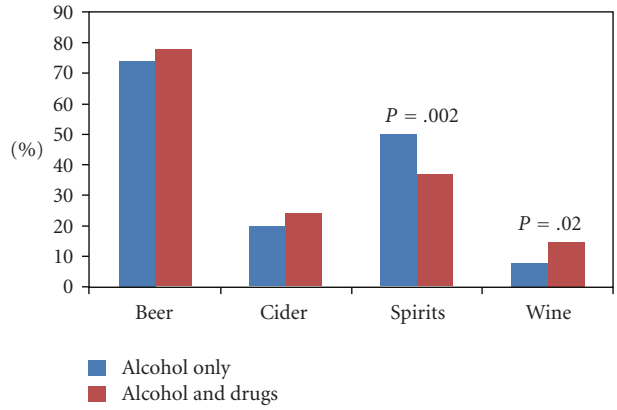
teeth, more missing teeth, and a higher DMFT value. Their D and F components however, were lower compared to the "alcohol and drug" group (though the F component failed to achieve statistical significance).

In order to test if the lower D and F values in the "alcohol only" group are confounded due to other variables, we performed multivariate binary logistic regression analysis. Variables included in the multivariate model were the ones that were significant at the 10% level in the bivariate analysis (Table 3). We dichotomized the D component using the median of 0 versus 1+ and the F component using less than or equal to the median of 8 versus >8 to define the respective outcome variables for the D and the F components. Table 3 shows the variables that were associated with either the higher D or the higher F component of the caries experience. White race, "Alcohol and drug" abuse, and the amount of alcohol consumed per week were positively associated with a higher D component (at 10% level of significance). Higher social class and wine drinking were positively associated with a higher F component. Male gender and beer drinking reduced the risk of having a higher F component.

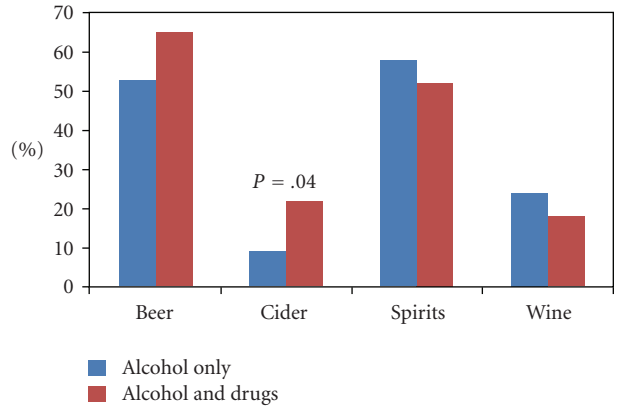
In the final multivariate model (Table 3), Whites (OR = 2.26; 95% CI = 1.15–4.45; $P = .018$) and "alcohol and drug" abusers (OR = 1.38; 95% CI = 1.01–1.89; $P = .049$) had a significantly higher D component. Those who belonged to a higher social class and drank wine had a significantly higher risk of having more filled teeth ($P < .05$). Beer drinkers had a lower risk (OR = 0.83) as we hypothesized, but

Table 2: Remaining teeth and caries status by group.

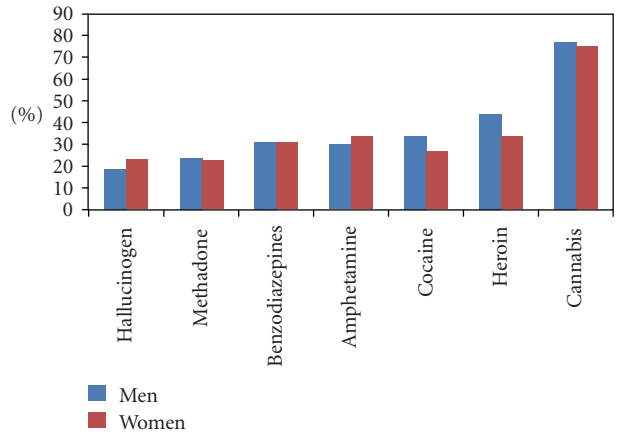
Variable Mean (SD)	Alcohol Only ($N = 363$)	Alcohol + Drug Use ($N = 300$)	P
Total Teeth Present	23.24 (6.63)	26.17 (4.59)	<.001
Decayed	0.95 (1.71)	1.31 (2.50)	.032
Missing	8.75 (6.64)	5.81 (4.59)	<.001
Filled	8.09 (5.52)	8.53 (5.30)	.30
DMFT	17.79 (6.87)	15.67 (6.65)	<.001



(a) Type of alcoholic beverage used by men in the two groups



(b) Type of alcoholic beverage used by women in the two groups



(c) Type of drugs used by men and women in the alcohol and drug use group (differences are not significant)

Figure 2: Alcohol and drug type used (%) by men and women in each group.

the difference between beer drinkers and nonbeer drinkers was not statistically significant (95% CI = 0.58–1.19; $P = .31$).

4. Discussion

Using over 600 alcohol and drug abusers, we observed that their total DMFT is around 16–18. However, the D component of the caries experience among alcoholics was significantly lower compared to those who abused both alcohol and drugs. Our multivariate analysis also confirmed that the alcohol and drug abusers in south London had a higher risk of having decayed teeth compared to "alcohol only" group.

Alcoholics and substance abusers are known to have poor oral health in other populations. In a survey of hospitalized alcoholic patients in Wyoming, USA, alcoholics had a three times higher permanent tooth loss than the national average for corresponding ages [14]. A smaller group of alcoholics in Maryland also had a higher number of missing teeth [15]. In a case-control study of 85 volunteer Finnish alcoholics, there were significantly fewer teeth and more remaining teeth with caries [16].

Among drug abusers, higher rates of caries have been reported in Australia [17], Poland [18], Sweden [19], Holland [20], and Denmark [21]. Methadone users are also known to have a higher caries experience [22], which is now known as "meth mouth".

Table 3: Factors associated with decayed teeth and filled teeth (bivariate analyses at 10% level of significance and multivariate logistic regression analysis.

Bivariate analyses [a]	OR	95% CI	*P*
Decayed teeth (0 versus >0):			
White versus other	2.38	1.22–4.64	.01
"Alcohol and drugs" abuse versus "alcohol only"	1.34	0.98–1.82	.07
Units of alcohol per week	1.001	1.000–1.003	.04
Filled teeth (0–8 versus >8):			
Male	0.63	0.44–0.91	.013
Higher social class	2.16	1.57–2.98	<.001
Beer drinking versus no beer drinking	0.69	0.49–0.97	.031
Wine drinking versus no wine drinking	2.11	1.34–3.33	<.001
Multivariate analyses[a]			
Decayed teeth (0 versus >0):			
White versus other	2.26	1.15–4.45	.018
"Alcohol and drugs" abuse versus "alcohol Only"	1.38	1.01–1.89	.049
Filled teeth (0–8 versus >8):			
Male	1.30	0.89–1.91	.18
Higher social class	1.98	1.43–2.75	<.001
Beer drinking versus no beer drinking	0.83	0.58–1.19	.31
Wine drinking versus no wine drinking	1.85	1.16–2.96	<.05

[a]Decayed teeth cut-off median of 0 versus >0 and filled teeth cut-off median of 8 versus > 8.

Before we interpret our findings, we need to examine the strengths and limitations of our study. This study is unique as we had over 600 predominantly adult White males included in the study from south London, minimizing the heterogeneity of the findings. However, among the study limitations are our feasibility sampling due to logistics, potential under or over reporting of self-reported data, and the inherent limitations in the field dental examinations. We however would argue that these limitations were randomly distributed (i.e., non-differentially) among both "alcohol only" and "alcohol and drug" abuse groups, thus biasing our estimates towards the null value.

It is not "earth-shattering" to state that alcohol and drug abusers have poor oral health. That was not the intention of this study. We wanted to further evaluate the effect of alcohol and drug abuse either alone or in combination on various components of the dental caries experience. As noted, our "alcohol only" group had fewer teeth and a higher DMFT. Alcohol is currently considered an independent risk factor for periodontal disease [23], and therefore, one can expect fewer teeth among alcohol users. Our "alcohol and drug" abuse group was significantly younger though they too consumed alcohol, but the significant age difference might also explain why the "alcohol only" group had significantly fewer teeth. What is interesting is that the amount of alcohol (units per week) consumed by subjects in each group was very high (over 280 units per week), but that was not statistically significantly different. This challenges our hypothesis that alcohol consumption reduces the decayed and filled component of DMFT. One possible explanation for this is that the potential higher consumption of refined carbohydrates by the "alcohol and drug" abuse group can override the "caries reducing" effect of "alcohol alone". To support this notion, we looked at data from 76 subjects who only abused drugs without alcohol (as a part of the dissertation of one of the authors-C.K.Harris). These subjects came from the same clinics that gave rise to the study subjects included in this. Harris [24] reported that "drugs only" group had significantly higher decayed teeth (mean = 3.0; SD = 4.4) compared to the other two groups ($P < .05$) that are reported in this—"alcohol only" group = 0.95 (1.7) and "alcohol and drugs" group = 1.3 (2.5).

As we argued in the introduction, it is possible that the "alcohol only" group has fewer decayed teeth due to fluoride in alcohol and/or the inhibitory effect of alcohol on their cariogenic flora. Alcohol may also enhance the release of fluoride from certain restorative materials. This, and the possibility that they probably sought and received dental care less frequently, may explain why they have fewer filled teeth. Unfortunately, we did not have data on the frequency of their dental visits.

Our multivariate analysis that took into account several potential confounders confirmed that the "alcohol and drug" abuse group had a 38% higher risk of having decayed teeth compared to the "alcohol only" group. Our attempt to see if beer drinking alone (which contains higher levels of fluoride) would explain this lower risk for decayed teeth among "alcohol only" group failed to yield statistical significance but was in the anticipated direction (OR = 0.92; $P = .66$). The fact that the units of alcohol consumed per week within each group did not make a significant difference in the risk for decayed teeth (OR = 1.001; 95% CI = 1.0–1.002), perhaps indirectly supports that it is the beer drinking that reduces the risk for the D component of caries.

Finally, when we explored the risk factors for the higher F component of caries while controlling for the known confounders, we saw that males who belonged to a higher social class and drank wine were the ones who had more filled teeth (Table 3). This is what one would expect. Beer drinkers in this model also had a lower risk of having a higher F component (OR = 0.83) but that association failed to reach statistical significance ($P = .31$).

As we have stated, there are limitations in this study. However, it addresses an important scientific question that has not been addressed sufficiently before. Even though both alcohol and drug abuse, either independently or in combination, are deleterious to overall health, understanding the true nature of the effect of these harmful exposures on various components of dental caries experience is worthy of further scientific investigation.

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Review Article

The Caries Phenomenon: A Timeline from Witchcraft and Superstition to Opinions of the 1500s to Today's Science

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This historical treatise follows the documented timeline of tooth decay into today's understanding, treatment, and teaching of caries biology. Caries has been attributed to many different causes for several millennia, however, only since the late 1900s has research revealed its complex multifactorial nature. European writers of the 1600s to 1700s held views that general health, mechanical injuries, trauma, and sudden temperature changes all caused caries—holding a common belief that decay was due to chemical agents, faulty saliva, and food particles. Until the early 1800s most writers believed that caries was due to inflammation from surrounding diseased alveolar bone. Today's science has demonstrated that caries is caused by indigenous oral microorganisms becoming a dynamic biofilm, that in the presence of fermentable sugars produce organic acids capable of dissolving inorganic enamel and dentin followed by the proteolytic destruction of collagen leaving soft infected dentin. As bacteria enter the pulp, infection follows.

1. The Human Tooth

The human tooth is a unique tissue composite of soft and mineralized tissues. Enamel is the hardest nonvital mineralized tissue, dentin is the hardest vital tissue and the pulp is a specialized connective tissue lined by dedicated end-stage odontoblasts that produce dentin throughout the life of the tooth, in which the pulp chamber becomes smaller over time. Each tooth is composed of unique regional diversity of anatomy, chemistry, sensory physiology, and mineral and organic components that constantly change throughout life. The interested reader is referred to Ten Cate's text for a comprehensive review of oral facial development, maturation, and growth [1].

Caries is a common human disease that only attacks vital teeth in an environment under certain oral conditions—conversely—caries does not infect a tooth once the host is dead. Studies by 19th century clinicians such as Drs. Abbot, Black, Leon Williams, Webb, Miller, and Dexter suggested a bacterial etiology to dental caries [2–11]. This paper considers the caries literature and analyzes its timeline (Table 1); erudite articles by Mandel, Newbrun, Nikiforuk, Tanzer and Zero have discussed human caries from antiquity to today [12–16]. Twentieth century scientists have clarified the intriguing complexity of the caries mosaic as an infectious disease [17–19]. The dental community realizes that the failure of the patient to remove or disrupt dental plaque biofilms or minimize frequent consumption of dietary sugars permits cariogenic bacteria to establish a dominant parasitic community.

2. The Antiquity of Tooth Decay

Skeletal remains are an excellent historical kymograph of human conditions. Lufkin reported that a 500,000 year-old Pleistocene skull from a human ancestor (*Pithecanthropus erectus*) from Java had severely worn teeth, however no decay

was evident. He also showed a Neanderthal skull from the Paleothic era (40,000 to 25,000 year ago) with major alveolar bone loss, missing teeth, and various levels of decay in the remaining teeth; decay was recognized as a widespread disease, revealing that periodontal disease existed in almost every prehistoric race—more prevalent than decay [20].

Guerini wrote that during the reign of Hammurabi (circa 2100 B.C.) a "Code of Laws", was left on clay tablets with judicial dictates defining fees and demanding skillful medical treatment of patients against unscrupulous mystics [21]. Before then, Ruffer discussed that most disease was attributed to the presence of unseen demons in the body or to an insult that was caused against a particular god [22]. Cuneiform tablets from that age served as the medical reference that defined special incantations to request the Babylonian god, Ea to "get hold of the worm and pull it from the offending tooth?" [21, 23].

Breasted wrote of ancient writings that provided accounts that healing of disease was linked to magic and superstitions, but had not been challenged beyond mystical thinking, until Hippocrates (460–357 BC) proclaimed that disease was due to natural causes and should be treated by means of human reason [24]. Hippocrates suggested that medicine should be dissociated from magic and witchcraft—his doctrine of disease based on humoral pathology exerted its influence on medical thought for many centuries. Stagnation of depraved juices in teeth caused dental pain [21]. "He considered affections of the teeth to depend (in part) on natural predisposition and accumulated filth and corroding action of same" [25]. Moreover, Aristotle (384–322 BC) observed a relationship of eating sweets with dental caries and proposed the question, "Why do figs, when they are soft and sweet, produce damage to teeth" [26].

Joris wrote of Galen (131 AD) who considered that lack of proper nutrition caused "weak, thin and brittle teeth ... excessive nutrition caused inflammation to produce soft tissues and that loose teeth were the result of excess moisture that impaired the nerves" and that caries is the result of the internal accumulation of corroding humors [14, 27].

From his research of Roman cemeteries, Bressia wrote that caries was a common observation in cultures that had learned of luxury [28]. The early Roman society had elevated the Druid priesthood as a guiding influence over the health of the general population—including treatment of diseases like toothache. Ancient folklore thought that the tooth worm caused tooth decay and continued into the 1300s as seen in the writings of de Chauliac [29].

Did a tooth worm really exist? Pliny the Elder wrote of the Greek, Agatharchidas, that "people of the Red Sea suffered many strange and unheard attacks ... worms and little snakes came out upon them, gnawed away their legs and arms and when touched, retracted ... giving rise to unsupportable pains". He also described the death of Pherecydes of Syros who "died of a great quantitie of creepers that came crawling out of his bodie" [30]. In 1674, Velschius described the winding of a worm on a small stick to gently remove it from the person's body [31]. In 1870, Fedechenko published the first scientific report of a 12-cm Guinea worm nematode, which he removed from a person's body, naming it *Drancunculus medinensis* [31]. The Caduceus serpent staff of Asclepius was adopted by the American Medical Association as their symbol in 1912, and could in fact represent the removal of a guinea worm with a stick by the ancients [31].

Ancient folklore described a tooth worm in holes of decay and tissues around the teeth, which caused toothache—many worldwide cultures left oral and written accounts of a tooth worm. Veracity—the truthfulness or agreement with reported facts—allows us to judge early writings. It is recorded that van Leeuwenhoek, the father of microscopy, had received three worms in a just extracted tooth—two were dead and one was alive—noting the worms were the same as ones frequenting cheese shops. When he compared live cheese-shop worms to his three, he could "not descry the least difference either in the Head or the whole Body ... many old rotten cheeses had a great many little Worms in it ... that upon chewing, the cheese worms insinuates themselves into the substance of the Teeth that gnawed the sensible parts, and so occasioned great pain". Van Leeuwenhoek reported that his "wife ate heartly of old Cheese, which was seized with rottenness, and had a great many little worms in it" [32]. One of the common treatments for the tooth worm at that era was to place a few drops of *Oil of Vitriol* (sulphuric acid) into the cavity [33]. It is not surprising the ancient tooth worm theory as reported by Guy de Chauliac (1300–1368) continued into so many cultures [12].

Perhaps the Guinea worm, *Druncunculus medinensis,* that came from infected drinking water is the tooth worm. In *Dracunculiasis*, the gravid female can expel over 500,000 juvenile worms in the presence of cool water, which facilitates the release process [31]. Could it be that exposed vital pulps, which are periodically exposed to cool drinking water attract gravid females with their release of thousands of Guinea worms? This could have occurred in the ancient world where drinking water was often obtained from deep cool wells—the natural reservoir for the intermediate host of *Druncunculus medinensis*, a cyclopoid crustacean [31].

3. The Internal Theory of Caries: Inflammation from the Tooth Pulp

The Frenchman Paré (1510 to 1590) is credited to have almost singlehandedly elevated the respect of the dentist to a position of valued recognition in the public eyes. Paré moved away from the tooth worm theory, declaring that a toothache was due to internal forces of hot or cold humors that resulted in caries, he stated that "teeth organs alter the manner of bones, suffer inflammation and quickly suppurate to become rotten"—hence the concept of inflammation from within the tooth [34]. Kirk wrote that Pierre Fauchard (1678–1761) discredited the tooth worm theory, and was one of the first to prefer the more technical term of caries, which he thought was caused by a tumor of osseous fibers that displaced parts of the teeth causing its destruction [35].

Lufkin discussed the writings of Bondett and Jourdain who preferred the term of dental gangrene to caries [20]. Lufkin wrote that the common thought of many in the 1700s

was that tooth decay was caused by death of bone and soft tissues from around or within the teeth [20]. Hunter of London expressed dissatisfaction with the term caries and preferred the term mortification, and held to the concept of the inflammation theory from internal decay, but he did not offer an alternative opinion of any substance [36].

In 1806, Fox was among the first of his contemporaries to use the term dental caries [37]. The common thought was that caries was the result of inflammation of the lining membrane (*membrane eboris*) along the pulp-dentin wall, which penetrated from the inner pulp outwards. The collective theory of many writers of that time was that nutritive factors from surrounding tissues and the pulp and were simply withheld—the pulp died and decomposed and caries proceeded through the dentin to the outer enamel surface.

In 1831, Bell of England adhered to the concept of inner inflammation, but he felt caries had a hereditary factor; he preferred the term dental gangrene to decay or caries; thinking that gangrene was a consequence of thermal changes (cold to hot), which immediately penetrated to the enamel-dentin junction, resulting in decay. Bell wrote that when dental gangrene first occurred in the bone surrounding the tooth, necrosis resulted in gangrene of the pulp resulting in its destruction and then penetrated through the dentin, and eventually to the enamel [38].

By 1825 Köecker emigrated from Germany to America and became a prominent practicing clinician in New York, he then moved to England in 1832 where he assembled his clinical observations and published his own theory of decay [39]. Köecker held similar opinions to Hunter and Fox who felt that decay was due to changes in the tooth temperature that caused inflammation. However, Köecker differed sharply with them noting from his clinical observations that decay first began on the outer enamel surface and then penetrated to the enamel-dentin junction and invaded the tubules to eventually infect the pulp tissues [39].

4. The External Chemical Theory of Caries Replaces the Internal Inflammation Theory

In the late 1700s into the early 1800s, a number of colleagues from different counties—using histological preparation and stain technologies—made parallel observations that caries was caused by external chemical agents. Professor Harris of Baltimore Maryland [40], Robertson of England [41], Hope of Edinburgh [42], and Drs. Wescott and Dalyrymple [43] had collectively studied histological preparations of extracted human teeth and noted that caries could not have been caused by the mechanism of internal inflammation or from physiological changes inside the tooth. Their collective observations reported that decay was caused from outside the tooth. Robertson opined in 1835 that caries was caused by chemical disintegration of the tooth denouncing the theory of inflammation from inside the tooth. He postulated that gastric acids acted upon particles of food lodged in pits and fissures and began their destruction.

A parallel publication by Rognard of Paris in 1838 noted that caries began on the tooth surface where its effects were first seen. Rognard's clinical observations demonstrated that when extracted noncarious teeth were fixed in place of missing human teeth, caries occurred in the pits and fissures of the fixed tooth—within a few weeks [44]. Abbott described enamel caries in its earliest stage as a chemical process that dissolved the minerals that caused the breaking apart of crystals, followed by the organization of a protoplasmic mass that invaded the dentin. Abbot wrote that caries consisted of chemical demineralization and the dissolution of dentin into a "glue-giving basis-substance" around and between the tubules that breaks apart into medullary elements associated with secondary formations of micrococci and leptothrix [2–4].

Dèsirabode, the Surgeon Dentist to the King, differed with the period's collective writings on inflammation. He designated seven varieties of decay that were based on age, color, texture, damage, and other effects [45]. During those years, a great deal of confusion surrounded the idea that caries was the cause of mingling of gastric acids with mouth fluids; consequently, many simply preferred to adhere to "the chemical theory".

Dr. Black was one of the first academics to assemble the complete pieces of the puzzle regarding the cause of caries. Several factors played to Black's favor; he had access to the current literature, plus his personal research and clinical observations gave him a unique perspective on the available written data of that day. Black wrote that tooth caries could occur when mouth fluids were habitually acidic or alkaline, and that initiation of caries was directly dependent upon lodging of food particles and gelatinous debris (plaque) at irregular pits and fissures of the tooth, followed by the fermentation of the debris with the production of acids that began the demineralization process [5]. It should be noted that for centuries, vintners had used fermentation technology to make wine, but the science of fermentation was unknown regarding the cause of dental caries. It seems Harris, Robertson, Rognard, and others had simply failed to grasp the full meaning of the relationship of caries to fermentation.

5. Answers Arrive from an Unlikely Source: Agricultural Chemistry

In 1840, the theory of fermentation had been fully explained by Von Liebig—an unlikely nondental scientist whose chemistry research was first presented as an oral report to the British Association for the Advancement of Science, with their full acceptance [46]. The mechanics of fermentation had been used for centuries, but it required the genius of Professor Von Liebig to present it to the scientific world in a meaningful form. Until Von Liebig, there was no understanding of fermentation in terms of chemical processes. In that era, an acceptable theory of dental caries required something more than the simple hypothesis of chemical dissolution of enamel by an acid. The acid theory was close to the true cause of caries, but the level of science

of the preceding decades simply failed to understand the missing equation—bacteria. In retrospect, due to the absence of available fermentation science before Von Liebig, it is easy to understand that until the work of Louis Pasteur from 1857 to 1876 demonstrating the necessity of microbes in fermentation [47], just why the scientific understanding of bacterial fermentation causing caries was never completely understood.

When we project a few decades ahead in our scientific understanding of bacterial fermentation, we can see that Miller presented the chemo-parasitic nature of bacteria within the oral cavity and their importance in the initial cause of acid demineralization of enamel and invasion through the enamel-dentin junction to infect the tubule complex leading to destruction of collagen and other proteins [10]. It seems the actual person who might be credited with actually "FIRST" describing the exact science of caries may be left to other writers. It simply appears that its "discovery" was a collective effort by several individuals.

6. Defensive Capacity of Dentin against Caries

The dentist microscopist Tomes had written in 1848 from his clinical observation "the beginnings of caries, the dentine at the point of incipient disintegration becomes hypersensitive ... and not just a few patients complain when parts are disturbed by the contact of foreign bodies—the dentinal tubule complex contained a life force by which the dentin was able to build a barrier against the process of disintegration and that dentine is possessed of vitality ... and that vitality must have been lost before caries began and once the dentin vitality was lost in a specific area or localized point, gelatin was left to undergo gradual decomposition favored by the heat and moisture of the mouth" [48]. When Tomes applied litmus paper to the cavity of a carious tooth, it always gave a strong acid reaction that demonstrated the destruction of the mineral portions of enamel and dentin.

Professor Black wrote [49] that the 1878 studies of Leber and Rottenstein discussed that decay was a consequence of bacteria and their capacity to promote fermentation. Black showed that by treating decayed human dentin with iodine solutions, the underlying tubules showed a violet color, indicative of bacterial glycogen; he concluded that the tubules were filled with bacteria [49]. In their haste to report their observations, Leber and Rottenstein indicated that the fungus *Leptothrix buccalis* was constant in the production of caries [50]. Their observations were important to Miller as he understood the difficulties others had to contend with, but were of little use to understand the fermentation of bacteria and the cause of caries. In the late 1870s, Leber and Rottenstein showed the presence of bacteria in the tubules causing carious dentin, making a profound impact on the dental profession [50]. Milles and Underwood of London used the techniques of Koch, to verify the work of Leber and Rottenstein. A series of sterile flask experiments showed that tooth demineralization was due to acids secreted by bacteria. However, they could not accept the chemical theory of caries from acid demineralization of dentin under aseptic situations, as they placed a tooth in a closed flask with malic and butyric acid with human saliva in a meat suspension under aseptic conditions and no caries developed, finding uniform demineralization on all tooth surfaces, which did not resemble naturally occurring human caries, which was known to be more localized [51].

7. Science Prevails: Caries Is No Longer an Enigma

In his small Berlin laboratory that he shared with Robert Koch, Miller observed certain bacteria could convert starch by ptyalin (amylase) to form sugar that was fermented to lactic acid [10]. Miller cited the work of Milles and Underwood who wrote that caries most likely caused decalcification as a consequence of acids secreted by oral bacteria [51]. Miller's experiments supported studies that implicated caries due to the corrosive action of lactic acid from bacteria that demineralized the mineral of enamel and dentin [10]. In hindsight, it seems that Miller's failure to recognize the true relationship of plaque bacteria to localized dental caries may have been due to his lack of clinical experience compared to that of Black [5].

Professor Black strikes an important point in his discussion that must have come to him in a "eureka" moment. He wrote in his 1884 paper *Formations of Poisons by Micro-organisms* "That fermentation is the result of the life-processes of certain forms of micro-organisms may now be accepted as a truism, and will not be argued". He realized that fermentation was a chemical process and that a number of substances may be formed naturally by "true processes". Having read Miller's publications and studies Black wrote "what is called fermentation by an organized fermentable agent is but the first step in true fermentation [5]". Until that time, Miller's observations of fermentation had been mainly to study the digested agent (dentin) by lactic acid [10]. Miller had asked of the microorganisms of decay "what is its food, and in what chemical form is it delivered back after having served the purposes of the organism". It now seems that Black was able to piece together the complex puzzle of the cause of human caries by his own and other colleague's research data.

8. The Final Unraveling of the Caries Phenomena

Professor Davis wrote in his textbook "the most rapid caries was of a light or white color and that the hypersensitive nature of this substrate is very high ... Whereas moderately colored yellow and brown varieties are less sensitive and that the darker brown to black that represents the slow progressing form is much less sensitive when compared to normal." Davis identified two levels of carious dentin—a superficial zone—located towards the oral surface and called infected dentin was caused by the action of lactic acid and proteases from certain bacteria that left a soft leathery substrate. The deeper zone, located towards the pulp, was

Table 1: The caries phenomenon timeline.

Date	Clinical/Scientist	Observations
40.000–25,000 BC		Decay and alveolar bone loss is evident in the jaws of Neanderthal skulls from the Paleolithic Era [20].
22,000 BC		Decay of teeth and bone loss on Cro-Magnon jaws from the Paleolithic Period showed most lesions were located at or along the cement-enamel junction [20].
2,100 BC		Clay tablets from Assyria asked the goddess Ea to place the tooth worm between the teeth and jaw bone to destroy the blood and strength of the teeth [21, 23].
1,500 BC		Oracle bones of the Shang Dynasty of China showed characters that mentioned a tooth worm that invaded the mouth and teeth [21].
460–377 BC	Hippocrates	Greek Father of Medicine whose doctrine of disease was based on humoral pathology: stagnation of depraved juices in teeth caused pain. He discredited disease being caused by magic or mythology [21, 24].
384-322 BC	Aristotle	Greek philosopher who observed that sweet foods such as soft figs and dates caused a sticky film on the tooth that led to putrification and tooth decay [26].
200 BC	Agatharchidas	People of the Red Sea suffered and died from small worms that gnawed away on many body tissues [30].
62 AD	Pliny the Elder	Wrote that his friend Pherercydes of Syros died from creepers that crawled from his mouth and body [30].
129–200/217 AD	Galen of Pergamum	A Greek physician who believed that poor nutrition caused weak, thin, and brittle teeth; accumulation of internal corroding humors caused caries [14, 27].
1300–1368 AD	Guy de Chauliac	Believed the tooth worm existed and was responsible for tooth decay. He suggested fumigation with leek, onion, and Henbane to cure the persons tooth pain [29].
1525 AD	Ambroise Paré	Internal life forces from within the body and teeth caused decay. He discredited the tooth worm idea [34].
1684 AD	Antonie van Leeuwenhoek	Observed many small spinning microorganisms from mouth spittle,which he called animalcules [47].
1700 AD	Bondette and Jourdain	They called caries a dental gangrene that was caused by tissue inflammation and death of the bone around the tooth neck [20].
1700 AD	Antonie van Leeuwenhoek	Wrote to the Royal London Society that he took live tooth worms from corrupt teeth of his wife, noting they were the same as living cheese-worms that were found from a cheese shop [32].
1728 AD	Pierre Fauchard	Considered to be The Father of Modern Dentistry, discredited the tooth worm theory, and thought dental caries was caused by a tumor of osseous fibers [20, 35].
1780 AD	John Hunter	Preferred the term mortification to caries, and believed the source of decay was due to an imbalance of internal forces that caused inflamation and pulp disease [36].
1798 AD	T. Charles Hope	He believed caries was due to external forces, and dismissed the internal tooth inflammation theory [42].
1806 AD	Joseph Fox	Preferred the term caries. He believed tooth inflammation was due to internal injury of the lining membrane along the pulp-dentin wall [37].
1831 AD	Thomas Bell	Believed that caries had a hereditary component [38].
1835 AD	William Robertson	Caries was due to the chemical disintegration on the outside of the tooth. He denounced internal factors [41].
1838 AD	M. Rognard	Believed that caries began in pits and fissures of the crown on the outside of the tooth [44].
1841 AD	M. A. Dèsirabode	Designated seven stages of tooth decay [45].
1841 AD	Levi Spear Parmly	The first advocate of oral hygiene for the patient [52].

Table 1: Continued.

Date	Clinical/Scientist	Observations
1842 AD	Leonard Köecker	Believed that tooth caries was due to internal inflammation from rapid temperature changes [39].
1843 AD	A. Wescott and J. W. Dalyrymple	English clinicians who believed tooth decay was caused by external forces of the oral environment [43].
1847 AD	Justis von Liebig	Described fermentation as a chemical process [46].
1848 AD	John Tomes	Believed that incipient caries caused mineral disintegration that led to tooth hypersensitivity [48].
1855 AD	Chapin A. Harris	Early American educator who believed that caries was due to external factors of the oral environment [40].
1861 AD	Louis Pasteur	Demonstrated that fermentations are "vital processes" requiring microorganisms [47].
1878 AD	T. Leber and J. W. Rottenstein	Believed that caries was due to bacterial fermentation of food debris, and oral fluids that led to the presence of bacteria in dentin tubules [50].
1879 AD	Frank Abbott	Believed that caries was due to a chemical process that dissolved tooth minerals, followed by the formation and organization of a protoplasmic gelatinous mass [2–4].
1881 AD	G. A. Milles and A. S. Underwood	Caries was most likely due to demineralization by organic acids produced by bacteria [51].
1884 AD	Greene Vardiman Black	First to assemble the caries puzzle that involved food debris, gelatinous debris, and acids, which caused demineralization leading to the initial caries lesion [5].
1890 AD	Willoughby D. Miller	Caries was due to corrosive actions of lactic acid from bacteria that caused enamel lesions [10].
1897 AD	John Leon Williams	Decayed human teeth showed a dense felt-like mass of acid-forming microorganisms, dental plaque, that exerted its chemical influence upon calcified tissues [6–8].
1923 AD	W. Clyde Davis	Identified a soft superficial carious zone with many bacteria and deeper caries zone with fewer bacteria and some demineralization [53].
1940 AD	R. M. Stephan	*In situ* changes in dental plaque biofilm pH in the presence of sugar [54].
1954 AD	B. E. Gustafsson	Frequency of sugar consumption in institutionalized children (Vipeholm) related to caries experience [55].
1955 AD	Frank J. Orland	Demonstrated that caries did not develop in germ-free rats [15].
1960 AD	Ron Fitzgerald and Paul Keyes	They demonstrated the etiological role of specific streptococci in the caries process making it an infectious and transmissible disease [15].
1965 AD	Sam Kakehashi	Demonstrated bacteria are necessary for pulpal inflammation or necrosis using germ-free animals [56].
1972 AD	Takao Fusayama and S. Terachima	Showed clinical discrimination of two layers of carious dentin with a biological stain that provided distinct visual differentiation of infected and affected layers [57].
1975 AD	A. Scheinin and K. K. Makinen	Turku study indicated that replacement of sugar with xylitol decreased caries experience [58].
1978 AD	Maury Massler	Showed the clinical importance for the dentist to differentiate the outer infected active carious dentin from the deeper arrested carious dentin [59].
1980 AD	Theodore Koulourides	Lesion consolidation with remineralization and rehardening of enamel in calcifying solutions containing fluoride [60].
1981 AD	Martin Brännström	Bacterial microleakage into dentin and pulp causes recurrent decay, pulp inflammation and necrosis [61].
1986 AD	Walter J. Loesche	Developed the "specific plaque hypothesis" that stated caries was an acidogenic bacterial infection caused by mutans streptococci and lactobacilli species [62].

Table 1: Continued.

Date	Clinical/Scientist	Observations
1994 AD	Philip D. Marsh	Developed the "ecological plaque hypothesis" to describe the dynamic relationship within plaque biofilm consortiums where low pH selects for the growth of cariogenic microorganisms [63].
1998 AD	Eva. J. Mertz-Fairhurst et al.	Ten-year clinical outcome study of carious lesions with sealed dentin showed arrested lesion progression with no more clinical pulp failures when compared to the control group with conventional caries removal [64].
2004 AD	Edwina A. M. Kidd	Metabolic activity in the human plaque biofilm is the all-important driving force behind any loss of mineral from the tooth or cavity surface and resultant pulp inflammation [65].
2009 AD	Eric C. Reynolds	Concluded that calcium phosphate-based remineralization technologies showed promising adjunctive treatments to fluoride therapy in early caries management [66].

called affected dentin, often referred to as secondary caries, being composed of fewer bacteria and demineralized dentin [53].

Black's use of references is an indication of his erudite nature It was obvious his depth of reading, understanding, knowledge, and forward thinking about the cause of caries for that era surpassed many others [67]. He understood that caries disintegration always begins on the enamel surface of the tooth in some pit or irregularity and that acid was formed at the very spot where caries begins. His clinical experience showed him that certain foods were associated with higher levels of caries. He grasped the importance of bacteria feeding upon lodged food particles and fermenting them to organic acids. Black had made certain personal histological observations. Caries penetration of dentin occurs by following the tubules to the pulp; his extended observations showed that pulp exposures occurred with the least destruction of dentin; "exposure of the pulp will occur ... that is to say, the more perfect the development, the more complete the penetration is confined to the direction of the tubules." He demonstrated that carious softening tended to be in isolated tubules, whereas softening of a ground section of dentin in a mineral acid was seen at its whole entirety; their appearances are distinctly different. Black also observed that in the initial carious invasion, the internal diameter of the tubules became enlarged and using an aniline dye stain, he demonstrated the tubules were occupied with bacteria. Regarding enamel caries, Black's laboratory studies demonstrated that enamel rods fell apart at the periphery and not in the rod center. His 1884 article summarized many of previous observations, "Decay of the teeth is certainly a specific disease, running a specific course, and evidently arising from a specific cause, but this cause is not yet certainly known ... While there is no decay without the presence of an acid, there is not necessarily decay because of the presence of an acid [68]." It is important to realize that J. Leon Williams, a colleague of G. V. Black, also observed dental caries as an *in situ* phenomenon in teeth associated with an overlying "thick felt-like mass of acid-forming microorganisms" otherwise known as dental plaque [6–8].

9. A Complex Dimensional Disease: Several Layers of Carious Dentin

Using various microscopic techniques, Furrier illustrated six-zones of carious dentin: bacteria-rich, bacteria-few, pioneer-bacteria, turbid-layer, transparent and a vital reaction layer. However, from a clinical point of view, tactile discrimination of caries varied from clinician to clinician due to its softness [69]. The issue of caries discrimination was solved by Professor Fusayama and Terachima, using an *in vivo* stain. They demonstrated that softened carious dentin is composed of two layers [57]. Their research demonstrated an outer infected carious zone just below the enamel-dentin junction densely populated with facultative and anaerobic bacteria that secrete (1) organic acids capable of dissolving hydroxyapatite crystals, and (2) proteases that degrade collagen and other proteins causing detachment of apatite crystals leaving the once solid substrate to simply collapse on itself. This outer infected caries is completely dead, with no capacity to register any sensitivity to tactile or thermal stimuli and is not physiologically capable of remineralization. This fact makes its removal clinically painless as no anesthesia is necessary. The deeper affected carious dentin is generally 1,000 to 2,500 μm thick and generally contains only a few pioneer bacteria. It is somewhat softened due to organic acids dissolving the mineral rich crystals without proteases damaging the organic proteins [57]. This deeper carious zone is vital with a sensory capacity to respond to various stimuli. Once the clinician reaches this vital layer with minimally invasive instrumentation, they realize when to stop instrumentation as the underlying affected tubule complex is physiologically capable of remineralization with crystals that fill the lumen of dentinal tubules to become sclerotic [59, 65]. Importantly, the application of these principles has evolved into the therapeutic use of indirect pulp capping [70–72] and stepwise excavation [73–75] for the conservative preservation of the vital dental pulp during clinical caries removal as long as a "bacteriometic" seal can be maintained [61, 64, 76, 77].

"An Ounce Of Prevention Is Worth A Pound of Cure" [78]. This expression from Benjamin Franklin (1706–1790)

means it is better to avoid problems in the first place, rather than trying to fix them once they arise. In a 1886 lecture to students, G. V. Black stated "The day is surely coming, and perhaps within the lifetime of you young men before me, when we will be engaged in practicing preventive, rather than reparative, dentistry" [79]. We wonder what Black would think if he realized that most of today's dental schools throughout the world still teach a restorative focused curriculum; rather than a series of preventive courses? Since the 1970s, our profession has witnessed the introduction of caries detectors, acid etchants, glass ionomers and composites that seem more suited to minimal intervention than Black's extension for prevention concepts of amalgam placement.

The addition of fluoride to public water has proved effective to reduce caries in human dentitions [80]; postdevelopmental use of fluoride is known to cause a significant reduction in caries through topical interaction with surface enamel and dentin throughout life [60, 66, 81]. Other measures have shown that an alteration or reduction of dietary sugars also results in a major decrease of caries in experimental animal models [82, 83] and humans [55, 58].

It is interesting to pause and reflect on dental research since mid-1800. Once caries was known to begin on the external tooth surface and proceed inwards, the dental profession gained recognition amongst the worldwide populace. As the science of caries prevailed, the tooth worm faded into oblivion. New devices and technologies emerged in parallel fashion and became used in the laboratories of clinicians who were searching for answers to the biology of the tooth and caries.

North American notables such as Harris (1806–1860), Black (1836–1915), Webb (1844–1883), Williams (1852–1932), and Miller (1853–1907) all shared very common childhood experiences [5, 9, 10, 40]. They were not born of nobility or gentry, but grew up in humble rural surroundings and learned of life by spending long hours in the pursuit of Nature. American cultural history records that almost every home contained the popular textbook of the day of Comstock's Philosophy for family reading and group discussions after dinner time in the evening [84]. Each of these individuals had a similar introduction to dentistry and study, they used their own personal finances; no governmental agency dispensed research funds for their research. They pursued answers to questions that had evaded other colleagues and published their findings because they wanted to make sure new knowledge was available to colleagues worldwide. There was no academic pressure to publish or perish.

10. Remaining Challenges

Where should we go from here? It seems that much of the above information, although still available in the dental literature, remains somewhat lost in the academic teaching of caries for today's dental students. A fundamental knowledge of dental caries and the pulpal response to this bacterial insult remains illusive to many of today's clinicians and educators. Since the 1880s, we have learned that bacteria are the cause of caries [15] as a dynamic biofilm (dental plaque) [62, 63], and that bacteria are essential for pulpal disease [56]. Restorative procedures and devices have been developed to identify and remove caries. Has our current cosmetic-restorative era failed us? Are today's dental students integrating the appropriate clinical and scientific information for caries risk assessment, minimal intervention in caries removal, preservation of the vital pulp, and total prevention of dental decay within the human dentition? Thanks to the personal curiosity and initial research efforts of Harris, Webb, Black, Williams, Miller, and other colleagues of the late 1880s, our dental community now recognizes the cause of caries. The authors, again, remind the readers of Professor G. V. Black's challenge from 1886, "The day is surely coming, and perhaps within the lifetime of you young men before me, when we will be engaged in practicing preventive, rather than reparative, dentistry." [79]. The time is Now as we travel along this timeline from the past to the future. Our scientific community has made enormous advances in molecular biology to further our understanding of dental caries as a biological phenomenon [85–87]. We must integrate our current discoveries and past knowledge base into clinical practice. Let us not only prevent dental caries at all levels, but also preserve the vital dental pulp.

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Clinical Study

The Possibility of Digital Imaging in the Diagnosis of Occlusal Caries

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The aim of this study was to assess the possibility of digital image analysis of pit-and-fissure discoloration in order to diagnose caries. Digital images showing pit-and-fissure discoloration in 100 teeth of 19 patients were analyzed to obtain the fractal dimension (FD) and the proportion of the area of pit-and-fissure discoloration to the area of occlusal surface (PA). DIAGNOdent values were measured (DD), and dentists' diagnoses were also obtained. The sensitivity and specificity of FD, PA, DD, and the combination of FD and PA compared to the dentists' diagnoses were calculated. The sensitivities of FD, PA, DD, and the combination of FD and PA were 0.89, 0.47, 0.69, and 0.86, respectively, and the specificities were 0.84, 0.95, 0.91, and 0.86, respectively. Although further research is needed for the practical use, it is possible to use the analysis of digital images of pit-and-fissure molar discoloration as a diagnostic tool.

1. Introduction

In recent years, the concept of minimal intervention (MI) has prevailed in dentistry. MI can be defined as the maximum preservation of the healthy dental structure [1]. Therefore, the importance of diagnosing caries at an early stage has increased. In conventional procedures, the diagnosis of caries has mainly consisted of visual inspection and tactile assessment with probing. However, Lussi [2] reported that the sensitivity of detecting caries was 0.62 by visual inspection and 0.82 by probing. In addition, the pressure of probing can damage the demineralized fissure and increase the risk that caries progress [3, 4]. To promote MI, diagnosis without a probe has been recommended [3]. The laser fluorescence-based caries detection device DIAGNOdent (Kavo, Germany) has been introduced as an alternative. However, no single detection method for caries is sufficient; therefore, the combination of some detection methods has been recommended [5–7].

Recent improvements in the personal computer have made the process of digital imaging more efficient and convenient [8]. Nevertheless, few applications use the quantitative evaluation of digital images to diagnose caries. If the shape of caries can be quantified, and the relationship between the numerical value and the condition of the lesion can be demonstrated, this information would be helpful to diagnose dental caries. One of the indexes which evaluates shape quantitatively is fractal dimension. The authors of this paper have previously shown that the fractal dimension and proportion of the area of pit-and-fissure discoloration to the area of occlusal surface obtained by digital imaging were significantly correlated with the depth of the caries and the DIAGNOdent values in extracted teeth [9]. For assessment of the method as a diagnostic system, the ability

of the diagnosis, such as the sensitivity, the specificity, and the accuracy, should be researched in clinical situation. The aim of this study was to assess the possibility of the clinical application of the diagnosis of occlusal caries using digital imaging by examining the sensitivity, the specificity, and the accuracy in comparison with the DIAGNOdent values and the dentists' diagnoses.

2. Materials and Methods

One hundred teeth (36 premolars and 64 molars) with pit-and-fissure discoloration from 19 outpatients were examined at the Clinic of Oral Diagnosis and General Dentistry, Dental Hospital, Tokyo Medical and Dental University. The occlusal surface of each tooth was washed with the Robinson brush to remove dental plaque without any abrasive paste. Then, pit-and-fissure discoloration was dried by air and measured three times using DIAGNOdent. The mean scores were used as the DIAGNOdent values of the teeth (DD). Next, the occlusal surface of each tooth was photographed as large as possible with an intraoral digital camera (Penscope, Morita, Japan). Each image was stored in a personal computer using a video capture interface (PC-MDVD/U2, Buffalo, Japan). Without knowing the DD, a dentist preliminarily diagnosed each tooth using visual inspection and tactile examination to decide which treatment plan would be appropriate (preventive or operative). The clinical diagnosis of preventive treatment for teeth was classified as CO. On the other hand, carious lesions requiring operative treatment were removed in a conventional clinical way. If the resulting cavity preparation was limited in the enamel, then the clinical diagnosis was classified as C1. If the resulting cavity preparation reached the dentin, and sound tissue still remained between the cavity and the pulp chamber, then the clinical diagnosis was classified as C2. No lesion reached the pulp chamber in this study. Five dentists ranging from 3 to 15 years of professional experience examined the teeth after calibration of the criteria of the caries assessment conducted before the study; the calibration was done as follows; first, the five dentists examined 30 extracted teeth and decide which treatment plan would be appropriate (preventive or operative). At that time, the rate of accordance among the five dentists was 76.7%. Then, the teeth were sliced pallarel to the teeth axis and to the depth of lesion for each tooth was determined. At last, the dentists discussed to accord the treatment planning for each tooth referring the depth of the lesion.

The digital photographs obtained were processed and analyzed using image analysis software (Image J, NIH, USA). First, each image was converted to an 8-bit gray-scale image, in which the density of grayness of each pixel was linearly scaled from min 0 (black) to max 255 (white). Then, the occlusal surface in the image was isolated from the background using a density histogram of the image, and the area was measured. Pit-and-fissure discoloration was also isolated from the occlusal surface using the density histogram, and the area was measured. The proportion of the area of pit-and-fissure discoloration to the area of the occlusal surface was calculated (PA). Next, the image of the isolated pit-and-fissure discoloration was converted into a binary image, in which the density of pit-and-fissure discoloration was 0, and its background was 255, followed by calculating the fractal dimension of pit-and-fissure discoloration (FD).

Table 1: FD, PA, and DD of each clinical diagnosis.

Clinical diagnosis	n	FD (SD)	PA (SD)	DD (SD)
C0	64	1.09^{a} (0.16)	0.005^{b} (0.009)	16.9^{cd} (15.0)
C1	24	1.34^{a} (0.09)	0.012^{b} (0.008)	45.2^{c} (25.1)
C2	12	1.52^{a} (0.09)	0.051^{b} (0.022)	57.9^{d} (27.7)

a, b, c, d: Numbers with the same superscript letters are significantly different ($P < .01$).

Differences in FD, PA, and DD between each clinical diagnosis were analyzed using two-way ANOVA and Games-Howell test to reveal the clinical diagnosis and the effect of the examining dentists. The correlation between the clinical diagnosis and each FD, PA, and DD was analyzed using Spearman's correlation coefficient. Discriminant formulas were obtained using discriminant analysis with the treatment plan (preventive/operative) as the objective variable and FD, PA, and DD as explanatory variables. Sensitivity and specificity were calculated by applying FD, PA, and DD to each discriminant formula. The accuracy, ratio of the number of teeth showing accordance between the treatment plan decided by the dentists and the predictive treatment plan decided using the discriminant formula to the number of all the teeth, was also obtained. All the statistical analyses were performed using SPSS 16.0 (SPSS Inc., USA). The entire process was approved by the Ethics Committee of the Faculty of Dentistry, Tokyo Medical and Dental University (No. 317).

3. Results

FD, PA, and DD values corresponding to each clinical diagnosis are shown in Table 1. FD, PA, and DD increased with the depth of the caries. The two-way ANOVA revealed that the FD, PA, and DD were different among the clinical diagnosis ($P < .01$). On the other hand, the difference of the examining dentists did not affect the FD, PA, and DD. Spearman's correlation coefficients between the clinical diagnosis and each FD, PA, and DD were 0.743, 0.700, and 0.652, respectively ($P < .01$). There were also significant correlations among FD, PA, and DD ($P < .01$).

Table 2 shows the discriminant formula, sensitivity, specificity, and accuracy of each explanatory variable. Based on the discriminant formula of each explanatory variable, the thresholds of FD, PA, and DD between preventive and operative treatments were 1.20, 0.012, and 28.8, respectively. The sensitivity of FD was greater than that of PA, DD, and the combination of FD and PA. The specificity of PA was greater than that of FD, DD, and the combination of FD and PA.

Table 2: The results of discriminant analysis.

Explanatory variables	Discriminant formula	Sensitivity	Specificity	Accuracy
FD	$Y = 6.74\,\mathrm{FD} - 8.12$	0.89	0.84	0.86
PA	$Y = 63.3\,\mathrm{PA} - 0.77$	0.47	0.95	0.78
DD	$Y = 0.05\,\mathrm{DD} - 1.44$	0.69	0.91	0.83
FD, PA	$Y = 5.68\,\mathrm{FD} + 17.8\,\mathrm{PA} - 7.29$	0.86	0.88	0.87

The accuracy of the combination of FD and PA was greater than that of FD, PA, and DD.

4. Discussion

Previously, it was reported that the fractal dimension and the proportion of the area of pit-and-fissure discoloration to the area of occlusal surface were significantly correlated with the depth of the caries and the DIAGNOdent values in extracted teeth [9]. In this study, the same tendency was observed for patients' intraoral teeth. The fractal dimensions for C0, C1 and C2 in the former study were 0.97, 1.30, and 1.52, respectively [9]. These results indicate that image analysis of molar pit-and-fissure discoloration was clinically useful for the diagnosis of caries. An increase of the proportion of the area of discoloration corresponded to a change of the volume of caries lesion, while an increase of the fractal dimension corresponded to a change of the shape of the lesion caused by caries progression.

A fractal is a geometric shape, possessing characteristics of self-similarity or self-affinity, and widely observed in nature [10, 11]. Recently, fractals have been in the spotlight in the field of medicine, and research has been introduced regarding its use in the field of diagnosis [12–14]. Fractal dimension, is a quantifiable value that characterizes shape. The dimension increases in number with the complexity of the structure. For example, a point is described as the zero dimension; a straight line is described as the first dimension, and a plane is described as the second dimension. The fractal dimension is a decimal dimension between integers. Such decimal dimensions can be obtained by expanding the definition of the dimension as the rate at which the perimeter (or the surface area) of an object increases, and the measurement scale is reduced [10]. Several ways to measure the fractal dimension have been introduced. In this study, the authors used a simple way to determine the fractal dimension called box counting. In this method, a grid of squares is placed over the object, and the number of squares through which any part of the object passes is counted. This process is repeated with different grids having different sizes. The number of squares placed over the object versus the length of the side of the square are then plotted on log-log scale. When a regression line is obtained from the plots, the slope of the line is defined as the dimension. The degree of uneven complexity of a boundary or a coast can be quantified using this approach. In this research, the fractal dimension of discoloration increased from 0.8 to 1.6 as the depth of the caries increased, which corresponded to a change in the shape of the discolored area from a point or a line to an area based on the progression of the caries.

The sensitivity and the accuracy of FD were greater than that of DD. The sensitivity of PA was less than that of FD and DD. Generally, the addition of valuables into discriminant formula is one of the ways to improve the accuracy, however, in this study, the accuracy of the combination of FD and PA was similar to that of single FD. Therefore, further study to find other valuables is needed to improve the accuracy of this method by combination of valuables. Thus, the accuracy of the diagnosis of occlusal caries using digital images of discolored areas was comparable to that of DIAGNOdent; therefore, its clinical application as a diagnostic tool is possible.

Because this study was clinical, the final diagnosis of each examined tooth was not confirmed by a histological procedure but by a dentist's clinical examination. As mentioned above, the diagnoses of dentists were reported to vary [2]. In this study, the results of two-way ANOVA showed that the effect of the examining dentist on the DD, PA, and FD values was not significant. Therefore, we considered that difference of the diagnosis among the dentists would be small.

In the present study, to examine the possibility of digital imaging in the diagnosis of occlusal caries, DIAGNOdent was used as a comparative pre-existing dental caries detection tool. There are other caries detection tools, such as fiber optic transillumination (FOTI), digital imaging fiber optic transillumination (DIFOTI), quantitative laser or light fluorescence (QLF), and electrical conductive measurements (ECM). FOTI, DIFOTI, and QLF have been tested in vivo, however, the number of clinical studies has still been small [15, 16]. On the other hand, a comparatively long time has passed since DIAGNOdent was introduced to the market, and many findings have been reported. Sheehy et al. [17] reported that DIAGNOdent had greater sensitivity and specificity than ECM. The correlation coefficient between DIAGNOdent readings and the depth and the volume of caries lesions was reported to be 0.47 [18]. Several researches have pointed out that DIAGNOdent measurements are affected by other factors, such as hypomineralization, plaque, debris, staining and wetness [19–21], while a high correlation between inter and intraobserver agreements was also mentioned [22, 23]. We employed DIAGNOdent as a comparison because the diagnosis was provided as a number, the handling was easy, and, moreover, its clinical use has been discussed in other studies.

In the present study, diagnosis using digital images of pit-and-fissure discoloration depended on the statistical relationship between the shape of discoloration and the depth of caries. Namely, the method did not measure infected tissue of individual teeth directly. The shape of the discolored area in the occlusal surface is, however, possibly affected

by medication history, individual history, and lifestyle [6]. Consequently, diagnosis using digital images of pit-and-fissure discoloration is rather experimental. Additionally, the procedure cannot be applied to colorless lesions, such as acute caries. Therefore, diagnosis using digital images of pit-and-fissure discoloration should not be used for definitive diagnosis. Rather, initial diagnosis, screening such as mass examination would be suitable because of its good sensitivity of FD and, the convenience of the procedure. Actually, the core of the diagnostic system is digital imaging processing by computers. If computer programing for all procedures was achieved, then screening of hundreds of examinees would be automated after photograph taking, which would make mass examination less time-consuming with low cost. For such automated uses of the computer, the process of extracting colors from the image must be improved; in this study, the threshold for the colored area was decided one by one by an observer's visual inspection. How to calculate the fractal dimension is also open to discussion. We used the box-counting method attached in the image analysis software, IMAGE J. The box-counting method is only suitable for self-similar profiles, not for more general, self-affine cases [10], that is, the fractal dimension using the box-counting method might be the approximate value. A special computer program must be developed to measure the fractal dimension more accurately, for example, the Minkowski method or the Richardson method [8, 10]. As mentioned above, the shape of the discolored area on an occlusal surface is affected by many factors. Therefore, the thresholds or the discriminant formulas acquired from this research are not universal. Further research is needed to determine the discriminant formulas to diagnose caries using the image analysis of molar pit-and-fissure discoloration.

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Research Article

Clinical Implications of Power Toothbrushing on Fluoride Delivery: Effects on Biofilm Plaque Metabolism and Physiology

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Dental biofilms are implicated in the formation of caries and periodontal disease. A major constituent of the supragingival biofilm is *Streptococcus mutans*, which produces lactic acid from sucrose fermentation, enhancing enamel demineralization and eventual caries development. Caries prevention through F inhibits enamel demineralization and promotes remineralization. Fluoride also exerts effects on metabolic activities in the supragingival biofilm such as aerobic respiration, acid fermentation and dentrification. In experimental *S. mutans* biofilms, adding 1000 ppm F to an acidogenic biofilm resulting from 10% sucrose addition increased pH to pre-sucrose levels, suggesting inhibition of acid fermentation. F effects on metabolic activity and sucrose utilization in interproximal plaque biofilms were also recorded. Addition of 10% sucrose reduced pH from neutral to 4.2, but subsequent addition of 1000 ppm F increased pH by 1 unit, inhibiting acid fermentation. 10% Sucrose addition also stimulated denitrification, increasing production of nitrous oxide (N_2O). Addition of 1000 ppm F suppressed denitrification, indicating an additional mechanism by which F exerts effects in the active interproximal biofilm. Finally, fluid dynamic activity by power tooth brushing enhanced F delivery and retention in an experimental *S. mutans* biofilm, suggesting a potential novel benefit for this intervention beyond mechanical plaque removal.

1. Introduction

The accumulation of dental plaque biofilms plays an important role in the development of caries, gingivitis, and periodontitis. Bacteria in dental biofilms constitute a viable community of microorganisms with complex ecological relationships that influence the microenvironment in which they reside [1, 2]. As these bacteria proliferate, they utilize nutrients from their immediate environment. In the case of supragingival plaque bacteria, saliva or external dietary carbohydrates from ingested food are major nutritional sources, while for subgingival plaque bacteria, proteins from gingival crevicular fluid or tissue breakdown products are the main nutritional reservoirs [1]. The expanding biofilm forms an irregular heterogeneous sponge-like structure containing clusters of bacterial cells surrounded by channels through which liquids such as saliva, ingested fluids, or mouthwash can flow [3]. *Streptococcus mutans*, which produces lactic acid from the fermentation of sucrose and is instrumental in caries formation, is a major constituent of supragingival biofilms [4]. The cariogenic aspect of *S. mutans* biofilms is due in part to an increase in the dissolution rate of hydroxyapatite, a mineral which constitutes more than 95% of tooth enamel. As acidity increases such that the pH drops below 5, increased demineralization of the enamel surface in turn accelerates the development of cavities. In addition, cariogenic bacteria on the periphery of the cell clusters, many of which are aerobes and facultative anaerobes, actively consume dissolved oxygen, resulting in oxygen deprived niches that favor proliferation of anaerobic pathogens [5]. The progressive development of the biofilm ecosystem in terms of these aerobic or anaerobic niches is

heavily influenced by acid production from fermentation of dietary sucrose and other sugars. Thus, the link that occurs between an initially cariogenic biofilm to one where anaerobic periodontal bacteria eventually predominate underscores the need for early anticariogenic interventions as evidenced by the use of fluoride. Since the topical application of fluoride rather than systemic delivery is considered most effective in promoting caries reduction, understanding the mode in which fluoride is delivered to the plaque biofilm and underlying enamel is important [6, 7]. Much of the emphasis of this article will therefore focus on the various mechanisms of fluoride interference on localized areas of biofilm physiology. In addition, the role that power toothbrushes can play in enhancing fluoride delivery into biofilms provides an expanded dimension for preexisting oral care devices to new applications, underscoring the need to continue searching for new directions in anticaries interventions.

2. Clinical Relevance of Fluoride as an Anticaries Intervention

The use of fluoride as a preventive measure against dental caries is well established. Comprehensive summaries on the importance of fluoride in combating caries have been published by Wefel [8], ten Cate and Featherstone [9], and Stoodley et al. [5]. The clear therapeutic effect of fluoride on dental caries was first shown in 1945 in Grand Rapids, MI, with the addition of fluoride to drinking water [10–12]. 11 years after water fluoridation in this study, 30,000 schoolchildren were monitored and found to have reduced caries incidence in excess of 60%. In subsequent years, the potential for fluorosis to develop in individuals when fluoride was added to water with already elevated natural concentrations of the mineral led researchers to determine the optimal concentration of fluoride in treated water. The concentration that would still exert an anticaries effect while minimizing the probability of developing fluorosis was determined to be approximately 1 mg/L [13].

Three main mechanisms have been proposed to explain the anticaries effect of fluoride. Firstly, fluoride enhances the resistance of enamel to increased acid attack by reducing enamel calcium hydroxyapatite solubility in acid. This occurs through the replacement of ions in calcium hydroxyapatite to form fluorapatite, reducing the susceptibility to acid 10-fold by lowering the onset of acidic dissolution from pH 5.5 to approximately pH 4.5 [8, 14]. Secondly, fluoride has been linked to reduced acid production by mutans streptococci and lactobacilli [15]. This can occur through inhibition of the metabolic and physiological pathways in the cariogenic biofilm that result in lactic acid production [15, 16]. Thirdly, fluoride can inhibit demineralization (enamel dissolution) and enhance remineralization (enamel deposition) in tooth enamel, positively impacting the ongoing process of remineralization-demineralization in tooth enamel [17]. If exposure to acid is short, saliva will raise the pH naturally so that the enamel loss can be repaired through remineralization. However, continued exposure to acid (e.g., continuous sucking on sugar-containing candies or sipping sugary drinks) can create a situation whereby the remineralization rate may be insufficient to repair the loss from demineralization, increasing the likelihood of caries development [18]. Hence, the right balance in the rates of demineralization and remineralization influences the success of caries reduction following implementation of a caries protection strategy [19–21].

3. Fluoride Effects on Aerobic Respiration, Acid Fermentation and Nitrification

As mentioned, fluoride can affect metabolic conditions in the cariogenic biofilm. Microsensors such as microelectrode probes can be used to evaluate local physiological conditions in the cariogenic biofilm, particularly the *S. mutans* biofilm established through in vitro models. Microelectrodes have tip diameters of less than 10 μm, which is on a spatial scale relevant to plaque biofilms which are usually less than 1 mm thick and composed of cell clusters with diameters on the order of tens to hundreds of microns. Some of these localized conditions include oxygen (oxygen probe for measuring aerobic respiration and nonrespiratory oxygen consumption), pH level (pH probe for measuring acid fermentation), and oxides of nitrogen (nitrous oxide (N_2O) and nitrate (NO_3) probe for measuring denitrification, a form of anaerobic respiration), before and following sucrose consumption. In addition, the effect of fluoride in the context of these physiological processes in the biofilm can be assessed by evaluating fluoride ion transport and retention through the use of specialized diffusion chambers [18].

Fluoride effects on biofilm physiology include influencing the localized anaerobic and acidic microenvironments found near the surface of the biofilm, promoting acid-loving bacteria that play a role in cariogenic biofilms [18]. Previous work by Stoodley et al. [5] used oxygen electrodes to measure the effect of adding 1000 ppm fluoride on dissolved oxygen levels in an *S. mutans* biofilm grown on hydroxyapatite coated slides (to simulate tooth surface enamel) in a drip flow reactor and then placed in a flow cell system. Fluoride increased the level of dissolved oxygen to 35% of air saturation in the biofilm, thereby creating a less favorable environment for pathogenic anaerobes. (Figure 1(a)) The same study also used pH probes to measure the effect of adding fluoride on biofilm pH profiles. Addition of 10% sucrose dropped pH from 7.1 to 5.9, while addition of F increased pH back to 6.8, showing that fluoride inhibits acid fermentation by increasing pH following the low pH levels generated by sucrose-driven fermentation (Figure 1(b)). The temporal element of acid fermentation and effect of subsequent fluoride addition was also explored in another experiment as shown in Figure 2(a). Acid production following addition of 2% sucrose resulted in a continuous drop in pH within the first 4 minutes. Removal of sucrose and addition of fluoride raised the pH after 6-7 minutes of intervention, reflecting the progressive dispersion of fluoride within the biofilm. pH variability also occurs at different sites within the *S. mutans* biofilm grown on hydroxyapatite slides.As Figure 2(b) demonstrates,

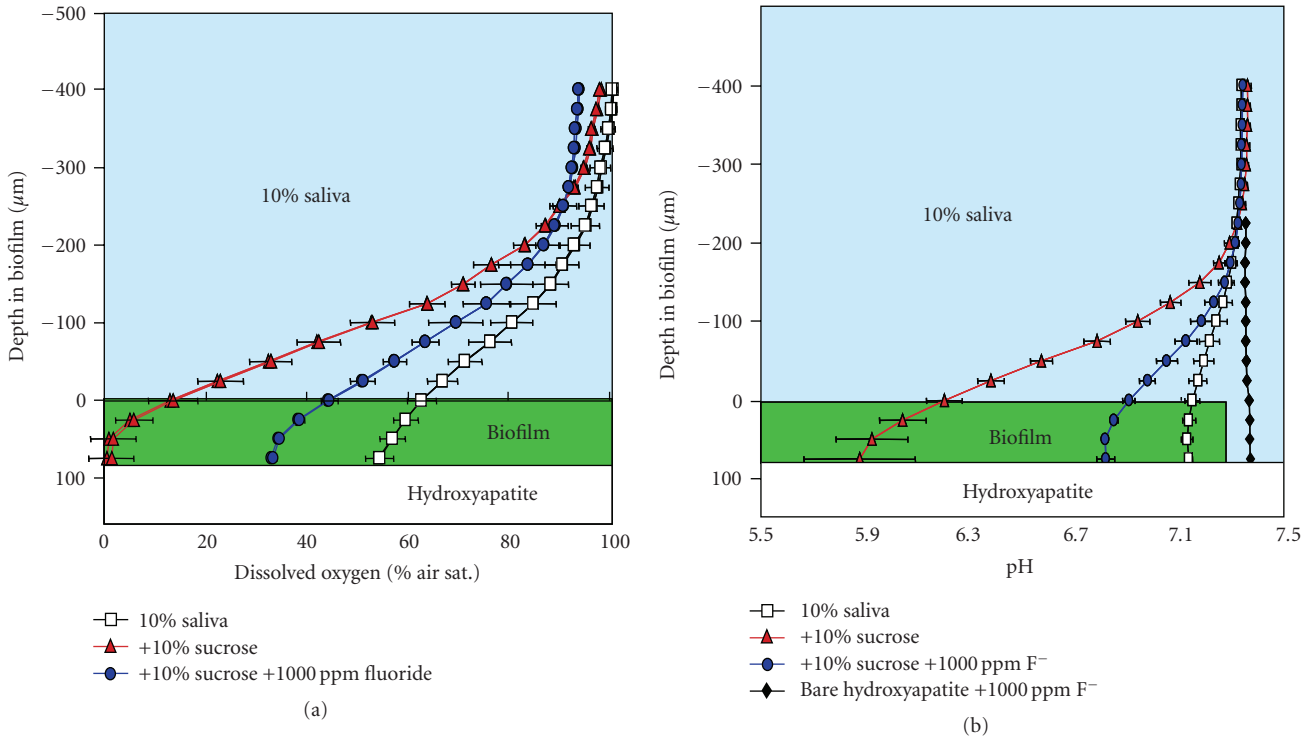

Figure 1: (a) Dissolved oxygen profile in 10% saliva after 10% sucrose addition increases anaerobicity to 50 μm. F addition reduced biofilm activity so that dissolved oxygen increased to approximately 35% of air saturation. (b) Adding 10% sucrose decreased pH from 7.1 to 5.9 due to sucrose fermentation. F addition increased pH back to 6.8, suggesting inhibition of fermentation.

placement of pH microelectrodes at three different sites within the biofilm after sucrose addition revealed substantial variability in pH response at different depths within these three microenvironments. Sucrose consumption and subsequent acid respiration resulted in a pH decline from approximately 7 to as low as 4.2, which is sufficient to cause tooth surface enamel erosion. Finally, a related experiment exploring the effects of fluoride used aerial flux to measure aerobic respiration and acid fermentation activity in a 75 μm thick *S. mutans* biofilm. Results of this experiment are shown in Figures 3(a) and 3(b). Effect of 10% sucrose addition on aerial O_2 flux increased the aerobic respiration rate by over 100% while subsequent fluoride addition (1000 ppm) reduced aerobic respiration rate by 17%. Relative proton (H^+) aerial consumption showed increased negative activity (i.e., H^+ production resulting in acidification) by a factor of 170 following sucrose addition, with subsequent addition of fluoride significantly inhibiting acid fermentation. In summary, what these various experiments validate is the role of fluoride in mitigating an acidic environment in the dental plaque biofilm by inhibiting acid fermentation of resident cariogenic bacteria such as *S. mutans*. Reduction of the acidic environment indirectly favors proliferation of the beneficial nonmutans streptococci that are harmed by the presence of high acid levels. The clinical significance of these health associated bacteria in the biofilm is that their presence is generally indicative of good oral health and hygiene.

These findings might be generalizable to other areas of the mouth. Experiments by Stoodley et al. [22] on tonsillolith biofilms have shown evidence of metabolic stratification similar to that found in dental biofilms. For instance, an overlapping denitrification zone was found between an upper layer characterized mainly by aerobic respiration and a lower (deeper) layer where acid fermentation predominated. Denitrification is the result of the decomposition of nitrite into nitrous oxide (N_2O) and sometimes nitric oxide (NO). Phagocytic cells, which form part of the innate host defense system, produce nitric oxide (NO) (which is a strong oxidizer) from arginine, specifically to attack bacteria as part of the phagocytic oxidative burst. However, bacteria can also produce nitric oxide (NO) from the reduction of nitrate ion (NO_3^-). Some bacterial species have evolved reductases that reduce nitric oxide (NO) further to nitrous oxide (N_2O) or nitrogen (N_2), rendering it harmless. It is not clear whether the ability of bacteria to degrade nitric oxide (NO) was an evolutionary adaptation to counter the bactericidal activity of phagocytic nitric oxide (NO) or to detoxify nitric oxide (NO) as a waste product of denitrification. Another possibility is that in an acid environment nitrite spontaneously oxidizes to nitric oxide (NO) so that the bacterial reduction of nitrate (NO_3^-) to nitrite (NO_2^-) in such an environment might be utilized by bacteria, not primarily for energy, but to attack competitive bacteria. Such a strategy would require that these bacteria have defenses against their own arsenal. A major contribution of this study was the use of microelectrodes to measure chemical gradients in biofilms grown on a mucosal surface. In addition, the use of tonsilloliths as a model for dental

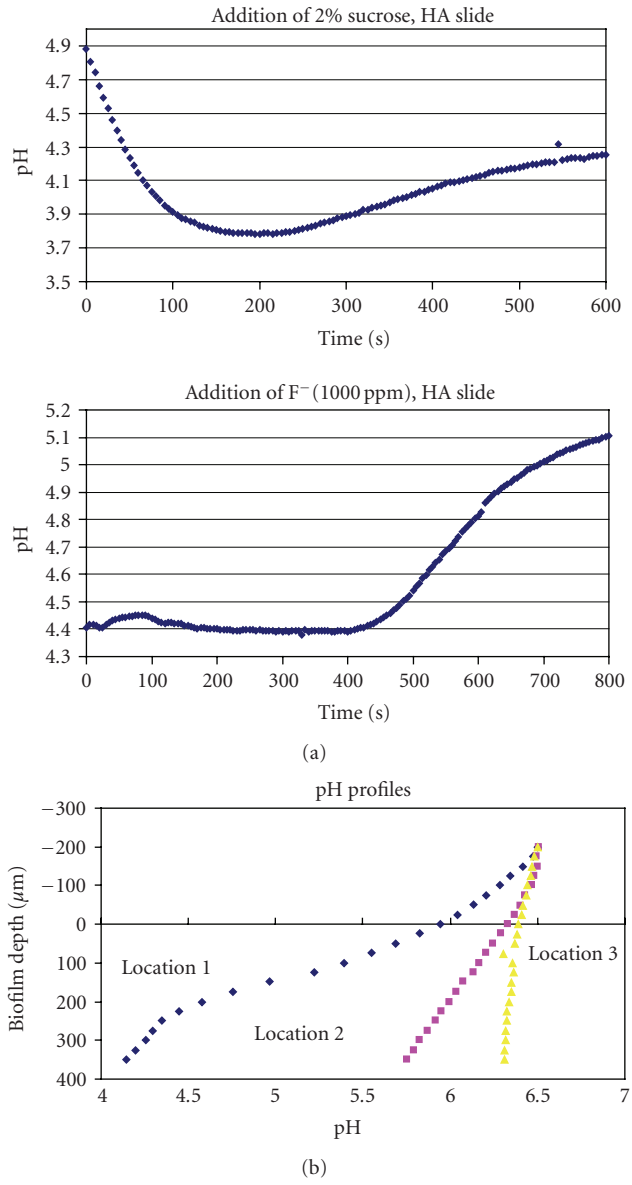

Figure 2: (a) *S. mutans* biofilm is grown on hydroxyapatite slides with microelectrode measurements revealing a drop in pH within the first 4 minutes after addition of 2 % sucrose. Removing sucrose and adding F raises pH after 6-7 minutes of intervention (graph redrawn from [5]). (b) Variability of microelectrode profiles within the biofilm after sucrose addition. In location 1 pH dropped to 4.2, while little change was seen in location 3.

biofilms allowed for cross functional insights into similarly stratified physiological activities present in dental biofilms, notably interproximal plaque.

To observe if select in vitro physiological activities observed earlier in *S. mutans* biofilms extended to "natural" multispecies dental biofilms, the activities were examined in interproximal plaque. Experiments were conducted on ex vivo interproximal plaque immersed in 10% saliva. Oxygen, pH, and nitrous oxide (N_2O) electrodes were used to measure aerobic respiration, acid fermentation, and denitrification, respectively, after sequential additions of sucrose and fluoride, or nitrous oxide (N_2O), sucrose, and fluoride for the denitrification studies (Figures 4(a), 4(b), and 4(c)). In the presence of 10% saliva alone, aerobic respiration predominates in ex vivo plaque where anaerobicity occurs below 200 μm. Supplementation with 10% sucrose induced acid fermentation, reducing pH from approximately 7 to 4.2. Subsequent addition of 1000 ppm fluoride increased pH to approximately 5.2, suggesting fluoride inhibition of acid fermentation. Nitrous oxide (N_2O) production after addition of sucrose provided evidence for denitrification in the interproximal biofilm. Following addition of fluoride, denitrification was suppressed, revealing yet another pathway of fluoride-induced inhibition in the active dental biofilm.

4. Dynamics of Fluoride Transport and Retention in *S. mutans* Flowcell Biofilm

As previously stated, the effectiveness of the topical application of fluoride in reducing caries underscores the need to understand how fluoride can be delivered to the plaque biofilm and ultimately to underlying enamel. Repeated exposure of plaque to fluoridated drinking water or dentifrice enables fluoride to bind to the sticky polysaccharide slime in the biofilm [23]. Even when the fluoride source is no longer present, bound fluoride in the plaque biofilm is slowly released over time, which can prolong anticaries activity. The biofilm acts as a storage reservoir for fluoride (and other ions such as calcium and phosphate) causing enhanced fluoride retention and exchange between these ions and tooth enamel, increasing the length of remineralization time to combat caries [18]. However, there is still insufficient knowledge on the exact mechanisms by which biofilms actively control fluoride passage through their complex layers, other than passive diffusion of fluoride through inert areas of the biofilm where there is virtually no fluid flow. Transport of small molecules or ions such as fluoride by diffusion is relatively fast across minute distances, but the time to attain a certain concentration at the base of the biofilm increases with the square of the thickness of the biofilm. Biofilm cell aggregates impede fluid flow (and hence fluoride mobility) through the cell clusters and to the tooth enamel surface itself, the ultimate target of fluoride activity.

5. Role of Toothbrushing in Fluoride Delivery

Even though fluoride was shown to be transported and retained in the *S. mutans* biofilm on account of diffusive flow alone, the role of fluid dynamic activity generated by power toothbrushes in enhancing fluoride delivery has not been sufficiently explored. Power brushing is designed to mechanically remove as much plaque as possible, particularly in inaccessible areas of the oral cavity. Such areas include fissures, interproximal and even subgingival areas, and possibly less exposed locations of the dentition such as posterior teeth. Increased penetration of fluoride into the biofilm through hydrodynamic forces could also enhance the period of fluoride retention and prolong its efficacy.

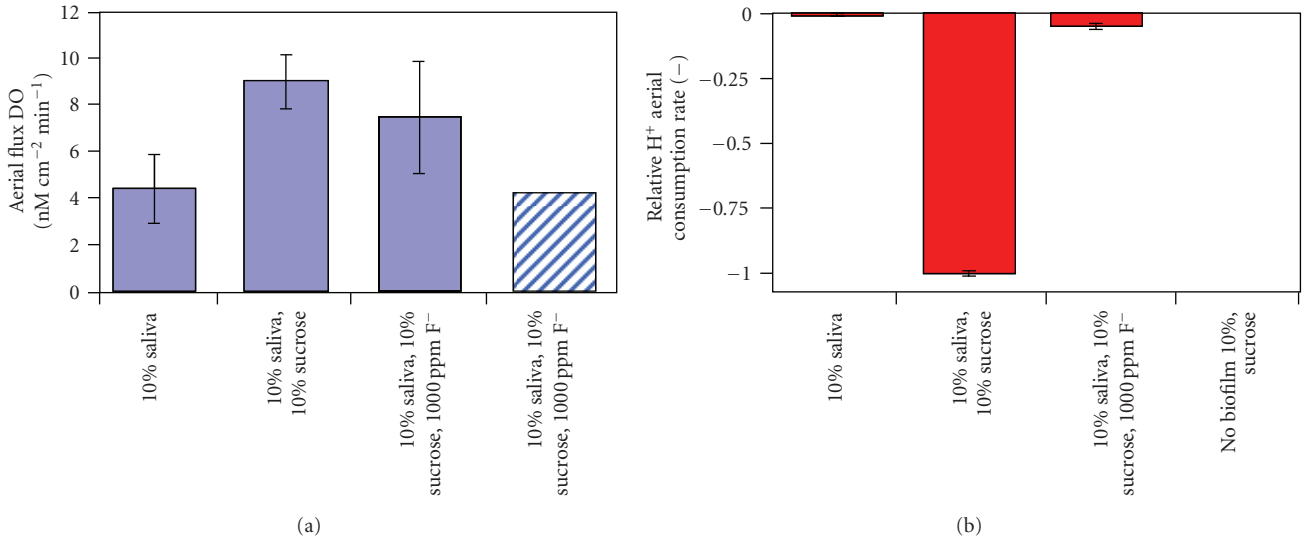

FIGURE 3: (a) In an *S. mutans* biofilm, addition of sucrose increased respiration rate by over 100% while fluoride addition reduced aerobic respiration rate by 17%. The aerobic respiration rate is shown in ex vivo interproximal plaque for comparison (hatched bar). (b) Negative H^+ consumption indicates acid production. The addition of sucrose increased activity by a factor of 170 while the addition of F significantly inhibited acid fermentation. Data for a sterile system are shown as a negative control.

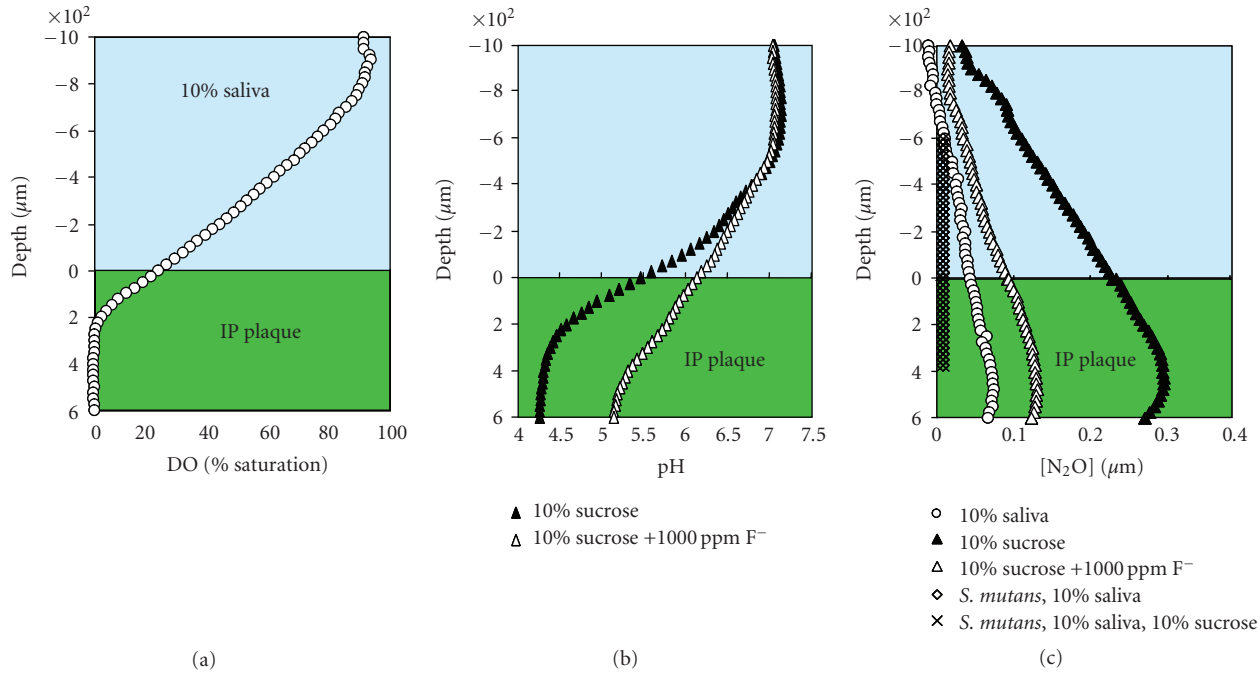

FIGURE 4: Metabolic activity in interproximal plaque. (a) In 10% saliva the plaque was anaerobic below a depth of 200 μm. (b) The addition of 10% sucrose reduced the pH from neutral to 4.2 within the dental plaque biofilm. NaF increased the pH by approximately 1 unit suggesting that acid fermentation had been inhibited. (c) N_2O production in the biofilm demonstrated that the interproximal plaque was denitrifying. Addition of sucrose stimulated denitrification while F suppressed it.

Since topical rather than systemic fluoride delivery results in caries protection, the efficacy of fluoride delivery to problematic sites is as important as concentration of salivary fluoride and frequency of fluoride exposure. Dental plaque biofilms formed in stagnant areas within the dentition can still result in caries if not physically removed or chemically managed. The motion from a sonic toothbrush has been demonstrated in vitro to drive fluid dynamic forces beyond the reach of the bristles into inaccessible interproximal spaces, resulting in biofilm removal in these areas [24]. As

a result, it is conceivable that fluid dynamics can also assist in the penetration of fluoride deeper into those areas of the interproximal biofilm that remains post brushing, allowing delivery of the extra few parts per million (ppm) of fluoride that is considered beneficial for added protection against caries.

A previously published experiment by Stoodley et al. [18] demonstrated fluoride delivery and retention into an in vitro *S. mutans* biofilm using a dual chamber system. Both chambers were separated by a permeable membrane colonized with *S. mutans* biofilm representing dental plaque to simulate in vivo interproximal plaque biofilm. The objective was to measure how quickly sodium fluoride passed through the colonized membrane from one chamber into the other during sonic brushing [18, 25–27]. A primary chamber served as the brushing chamber while a secondary measurement chamber served as the fluoride detection chamber to measure accumulating fluoride. A fluoride electrode in the measurement chamber measured how much fluoride was driven into it through the biofilm membrane following powered brushing in the primary chamber. The brushing chamber was filled with 1100 ppm fluoride solution and over a 4-minute monitoring period, the concentration in the brushing chamber never fell to less than 1050 ppm, suggesting that the concentration gradient driving the fluoride flux would remain more or less constant. Even with no brushing, fluoride concentration increased from 0.4 ppm to 0.5 ppm after 4 minutes due to the difference in fluoride concentration between the two chambers (passive diffusion). But with active brushing, the delivery of fluoride through the biofilm membrane increased considerably over a 4 minute brushing period for two power toothbrushes, with fluoride concentration measured in the measurement chamber at 0.65 ppm for one brush and at 0.8 ppm for the other, sonic brush. Fluoride delivery rate through the colonized membrane was measured as the mass transfer rate coefficient, which was significantly greater with power brushing than with passive diffusion alone.

6. Conclusions

The complexity of fluoride effects on microbial physiology can be summarized into three factors: saliva and overlying fluids, the plaque biofilm, and the underlying tooth enamel, into which fluoride exerts its main clinical benefits. In addition to the ionic interactions that occur among calcium, phosphate, and fluoride, other factors include transient pH that can range from pH 4 to 7, localized aerobic/anaerobic niches, temporal effects (seconds to minutes), and distance scales (micrometers to millimeters). The success of measurements to capture these physiological parameters depends on employing tools such as microelectrodes and compelling in vitro systems to model real life dental plaque biofilms.

The use of microelectrodes has proven to be a promising tool in studying localized fluctuations within in vitro plaque biofilms of physiologically relevant parameters such as pH, O_2, and nitrous oxide (N_2O) and has the possibility of being utilized extraneous beneficial agents such as fluoride. It is expected that further exploration into fluoride effects on biofilms in other niche sites such as the "subgingival" sites of the typodont would enhance previous observations that were recorded for the similarly less accessible interproximal sites.

Finally, increasing evidence suggesting the link between power toothbrushes and enhanced fluoride effects on the dental biofilm serves to steer new avenues of exploration for clinical benefits of power tooth brushes extending beyond mechanical bristle activity and plaque removal. The potential for enhanced fluoride delivery and retention into plaque biofilms through indirect but potent fluid dynamic action becomes even more useful where biofilms are located in hard to access areas, thus benefiting underlying enamel underneath these biofilms. Even the nature of brushing can impact efficacy of fluoride delivery. A four-day clinical trial revealed that sonic brushing increased the concentration of retained fluoride in plaque biofilm increased by greater than 40% compared to rotary brushing, manual brushing, and manual brushing and flossing [28]. Further research into the physical relationships among power brushing, fluid dynamic activity, and the role of localized oxygen gradients in oral biofilms should be explored in the context of increasing fluoride retention and delivery. Many of the more pathogenic, anaerobic bacteria reside deeper in the plaque biofilm where the availability of oxygen is low and where they are protected from chemotherapeutic agents. However, this environment also represents a target area for potentially successful interventions by increasing oxygen availability and by delivering antimicrobials directly to these anaerobes via power brushing. Meanwhile, the opportunity for delivering and retaining other broad-based anticariogenic or antimicrobial agents into dental plaque biofilms should be considered in developing novel innovative approaches to caries management, whether as an ancillary benefit of power brushing or the main benefit of an intervention where directed delivery of the anticaries agent is the primary objective.

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Research Article

Demineralization Depth Using QLF and a Novel Image Processing Software

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Quantitative Light-Induced fluorescence (QLF) has been widely used to detect tooth demineralization indicated by fluorescence loss with respect to surrounding sound enamel. The correlation between fluorescence loss and demineralization depth is not fully understood. The purpose of this project was to study this correlation to estimate demineralization depth. Extracted teeth were collected. Artificial caries-like lesions were created and imaged with QLF. Novel image processing software was developed to measure the largest percent of fluorescence loss in the region of interest. All teeth were then sectioned and imaged by polarized light microscopy. The largest depth of demineralization was measured by NIH ImageJ software. The statistical linear regression method was applied to analyze these data. The linear regression model was $Y = 0.32X + 0.17$, where X was the percent loss of fluorescence and Y was the depth of demineralization. The correlation coefficient was 0.9696. The two-tailed t-test for coefficient was 7.93, indicating the P-value = .0014. The F test for the entire model was 62.86, which shows the P-value = .0013. The results indicated statistically significant linear correlation between the percent loss of fluorescence and depth of the enamel demineralization.

1. Introduction

Dental caries is the most prevalent chronic disease in children. Although there is evidence that the prevalence of dental caries has significantly decreased over the past 20 years [1, 2], dental and oral diseases continue to plague children, especially young children. According to reports of the Centers for Disease Control and Prevention (CDC) comparing National Health and Nutrition Examination Surveys (NHANES), about 28% of preschool children experienced tooth decay between 1994 and 2004 [3, 4].

The development of dental caries is a dynamic disease process, especially for early lesions, which have repeated demineralization and remineralization cycles before being clinically detected. Demineralization occurs from acidic substrate or carbohydrate fermentation by acidogenic microorganisms, causing a subsurface enamel lesion to form. The continuation of demineralization leads to cavitation on the enamel surface. Restorative dentistry is often required at this stage. Untreated dental decay will cause pain and possible premature tooth loss which can be harmful to the permanent dentition and can cause tooth crowding problems, speech disorders, compromised chewing, delayed growth and development, and high treatment costs [5–7]. The natural repair response to demineralization is remineralization, which incorporates minerals from saliva into the demineralized lesion. Due to the ubiquitous use of fluoride, the progression of enamel caries becomes slower. It is likely that many incipient lesions could be arrested before they become clinically detectable [8, 9]. Fluoride improves saliva remineralization effects and forms an acid-resistant fluorapatite-rich surface of enamel. The fluoride in plaque also interferes with the bacterial metabolism with a subsequent decrease acid production [10].

The slow progression of enamel caries offers the opportunity for dental professionals to diagnosis and manage caries before there is irreversible destruction of the tooth. "With respect to dentinal caries, the diagnosis of the disease and the

detection of early lesions should be regarded as cornerstones of cost-effective dental health care delivery and quality of care." [11] However, there exist large variations in caries diagnosis and treatment decisions due to the lack of reliable methods to analyze the extent of the subsurface decay [12–18].

Traditional diagnostic methods, such as visual inspection, aided by radiography with or without tactile sensation, appear to have low sensitivity and high specificity for caries detection. The translucency, color, hardness, and radiographic interpretation are factors that lead to a dichotomous decision (either absence or presence of caries). Although they are simple, quick, and cost-effective, the methods have considerable limitations. The earliest lesions are detected at the white spot stage. In addition, the demineralization and remineralization processes are not quantifiable to be monitored with the current diagnostic techniques routinely utilized today. The use of an explorer to forcefully probe tooth surfaces may cause damage to newly erupted teeth or create cavitation at superficial lesion sites [19–23]. Radiographs are the most widely used diagnostic technique in conjunction with visible examination, but is limited to interproximal enamel caries detection.

A new visual method, the International Caries Detection and Assessment System (ICDAS), was developed to provide an international system for recording detected caries and comparing data collected in different locations at different points of time [24, 25]. The visual examination is carried out on clean, plaque-free teeth, aided by a ball-ended explorer to check surface contour, minor cavitation or sealant.

In recent years, many new diagnostic systems have been developed based on the measurement of different physical signals, such as visible light, laser light, electronic current, and ultrasound [26]. Fiber-optic transilumination (FOTI) is an advanced visual inspection technique based on light scattering properties in enamel. When the light is placed on the buccal or lingual side of tooth, the light is scattered in the enamel to result in a relatively darker region in demineralized areas, compared to sound tissue. This contrast is used to detect dental lesions, especially for inter-proximal carious lesions, and has shown low to good sensitivity and good specificity [27–30]. However, this technique still cannot be quantified or well documented in longitudinal studies. The digitized fiber optic transillumination (DI-FOTI) is an improved FOTI technique to collect transmitted images displayed on a computer monitor for evaluation, but the evaluation is still undertaken by the examiner's subjective visualization [31–35]. DIAGNOdent (KaVo, Biberach, Germany) is a different technology from FOTI to detect carious lesions, based on the difference in fluorescence between sound and demineralized enamel. The device uses a small laser system to produce an excitation wavelength of 655 nm, which is transmitted through optic fiber to a hand-held probe. The excitation light from the probe tip is absorbed by both organic and inorganic tooth substances. The emitted infrared fluorescence is collected by the probe tip and quantified to be displayed on an LCD panel. This technique has high sensitivity and specificity, especially for carious lesions on occlusal surfaces [36–39]. However, the device supplies the information as an arbitrary value, and has to be calibrated frequently for longitudinal comparisons. The probe must also be rotated in all directions to detect the highest reading, which is very technique sensitive.

Another dental diagnostic tool for detection of early carious lesions is quantitative light-induced fluorescence (QLF), which is based on auto-fluorescence of teeth. When the teeth are illuminated with high intensity blue light, the resultant auto-fluorescence of enamel is detected by an intraoral camera which produces a fluorescent image. The emitted fluorescence has a direct relationship with the mineral content of the enamel [40–44]. Thus, the intensity of the tooth image at a demineralized area is darker than the sound area. The software of QLF systems can process the image to provide user quantitative parameters such as lesion area, lesion depth, and lesion volume. These parameters can detect and differentiate the lesions at very early stages, and make the QLF system more sensitive to changes of caries over time. The image can be stored for longitudinal study and be used as patient motivators in a preventative practice [45–47].

QLF has been widely used as a quantification system for assessing early demineralization or remineralization of human enamel by thoroughly investigating the correlation between fluorescence loss and the status of mineralization under various treatments. In these studies, the changes of lesion area, depth, and volume are expressed as changes of fluorescence in the region of interest. In order to detect the stage of early demineralization, the absolute lesion depth needs to be quantified. Some studies have found a strong correlation between the changes in lesion and fluorescence with a current gold standard methodology-Transverse Micro-Radiography (TMR) [41, 48–50]. However, these validation tests prepared samples from teeth that were cut and ground to flat surface enamel, and the fluorescence loss is based on the difference of average fluorescence on acid treated and control areas.

In our study, we use teeth with natural surface curvature, without cutting or grinding the enamel surface flat, then validate the interpolation algorithm of QLF technology to estimate the changes of fluorescence. The purpose of this in vitro project was to simulate the clinical intraoral situation, and investigate the correlation between the fluorescence loss and demineralization depth, so that the absolute lesion depth could be estimated to evaluate the stage of early demineralization. In addition, according to the QLF image processing algorithm, there are random errors in reconstruction of sound values, and the measured maximum fluorescence loss from a single pixel is extremely sensitive to random noise [51]. We improved the QLF image processing algorithm and implemented it into a novel software, which produces more reliable results.

2. Methods and Materials

Six extracted permanent molars were obtained from different individuals with various ages and various exposures to fluoride histories. The teeth were stored in 0.1 percent thymol solution. The teeth were examined with a light

microscope at 10 times magnification to see that no white spot lesions or enamel imperfections were present. The teeth were coated with an acid-resistant varnish, leaving a 1×5 mm window of enamel exposed. The teeth were placed in an artificial caries solution (2.2 mM Ca^{+2} , 2.2 mM PO_4^{-3}, 50 um acetic acid) for four days to produce incipient demineralized enamel lesions [52].

The acid-resistant varnish was then removed carefully with acetone and the teeth were placed in deionized distilled water. Teeth were taken from the water, air dried, and a QLF image was obtained (Inspektor, Amsterdam, Netherlands).

The teeth were then cut longitudinally with a hard tissue microtome (Silverstone-Taylor; Scientific Fabrications, Lafayette, CO, USA), to obtain 100 um sections. These sections were photographed, using a polarized light microscope (Olympus; Model BX60FS, Olympus Optical Co., LTD., Tokyo, Japan) in an imbibition media of water, representing greater than one percent pore volume [53]. The lesion depths on these sections were measured with a computerized imaging system (NIH ImageJ software, http://rsbweb.nih.gov/ij/).

2.1. QLF Image Analysis. The principle of QLF software in analyzing the loss of fluorescence is "a two-step, two-dimensional, linear interpolation of the fluorescence radiance values at the sound edges of the lesion area" [51]. For example, to calculate the lesion at point M (intercross of line ef and line gh), the first step calculates the linear interpolation value $Lm(x)$ parallel to the X-axis (Figure 1) as

$$Lm(x) = Lg + (Lh\text{-}Lg) * \frac{(Xm\text{-}Xg)}{(Xh\text{-}Xg)}, \quad (1)$$

where Lg and Lh are intensity values at points g and h in the QLF image. The $(Xm\text{-}Xg)$ is length of line Mg, and $(Xh\text{-}Xg)$ is length of line gh.

The second step begins to calculate interpolation values $Le(i)$ and $Lf(i)$ at points e and f using intensity values at A, D, and B, C as step 1. The intensity differences between real intensity (Le, Lf) and interpolation intensity $(Le(i), Lf(i))$ at points e and f are calculated as

$$\Delta Le = Le - Le(i)\ , \qquad \Delta Lf = Lf - Lf(i). \quad (2)$$

Then, the linear interpolation parallel to y axis is calculated as

$$Lm(y) = \Delta Le\ +\ (\Delta Lf\text{-}\Delta Le) * \frac{(Ym\text{-}Ye)}{(Yf\text{-}Ye)}, \quad (3)$$

where $(Ym\text{-}Ye)$ is length of line Me and $(Yf\text{-}Ye)$ is length of line fe.

Finally, the desired interpolation value at point M is $Lm(i) = Lm(x) + Lm(y)$.

The loss of fluorescence at point M is $\Delta Lm = Lm(i) - Lm$, where Lm is the lesion intensity at M in the QLF image. By comparing ΔL at each lesion pixel, the maximum value of fluorescence loss is defined as ΔL_{max}.

However, there are significant errors associated with $\Delta L_{\max}$ measurement. $\Delta L_{\max}$ is taken from a single pixel value, which could be dramatically affected by random noise such as a hot or cold spot. Another potential error is a reconstruction error, which depends on the accuracy of each pixel value (fluorescence on sound tooth structure) on lines of AB, BC, CD, and DA. The random noise at these lines also can significantly affect interpolation values at lesion area. In addition, the rectangle may not fit appropriately for irregular lesion shapes. The edges may be far away from or interfere with lesion area.

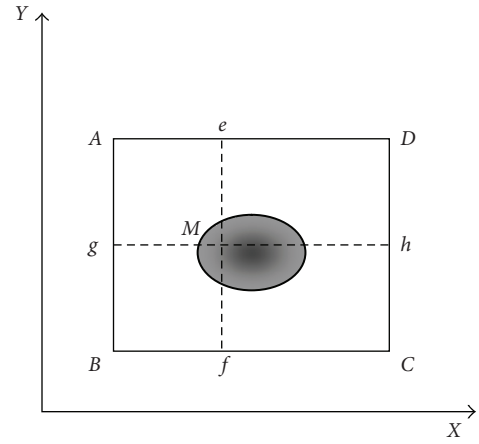


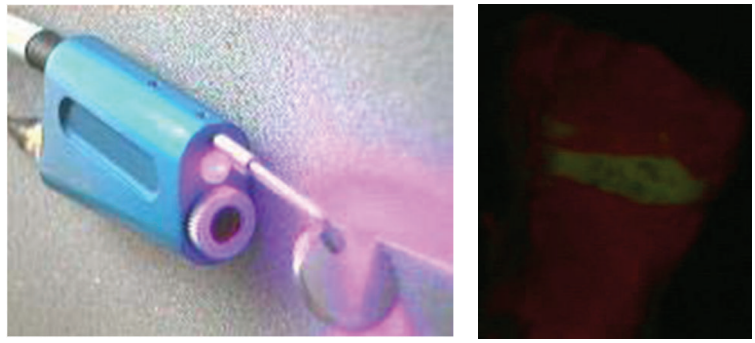
Figure 1: Interpolation description. The dark area represents lesion and others represent sound tooth structure. Thus, lines AB, BC, CD, and DA were seated on sound tooth structure and were used for interpolation.

To obtain precise measurements, we modified the interpolation algorithm and developed an image processing software. Instead of a rectangle, our software supports users to draw a polygon so that all edges can be on sound tooth structure and closely adapted to lesion shape. To reduce the errors for interpolation, the intensity values of each pixel on the polygon edges were recalculated as mean values of a 3×3 pixel area. In addition, to reduce the computational error of $\Delta L_{\max}$, the averages of 3×3 pixels of the ΔL value at the same site as that of $\Delta L_{\max}$ were compared. If there was significant difference between the mean value and $\Delta L_{\max}$, the large error was associated with $\Delta L_{\max}$. Then the second largest ΔL was selected for evaluation and the mean value of ΔL in a 3×3 pixle area represented the largest loss of fluorescence. This data was used to build a statistical model to estimate lesion depth.

2.2. Data Analysis. Microsoft Excel was used to analyze the data. The linear regression model was generated and an R-squared value was calculated.

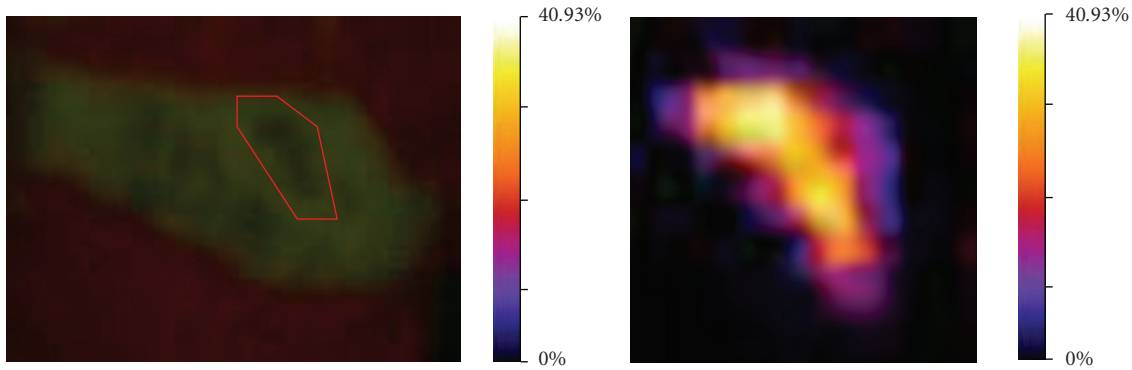
3. Results

The images collected by QLF (Figure 2) were processed. The region of interest (ROI) was selected, and then the interpolation methods were applied to calculate the percentage loss of fluorescence. The resulting image was displayed by Fire lookup table (LUT), and the associated color scale is shown



(a) (b)

Figure 2: Fluorescent tooth image (b) collected by Quantitative Light-induced fluorescence [QLF] (a).



(a) (b)

Figure 3: QLF images were analyzed by a novel image processing software. The region of interest [ROI] is shown as the red polygon (a). The resulting image of ROI [magnified ×5] after processing is colored according to the Fire Look-Up Table [LUT] (b).

Figure 4: The largest caries depth [d] is measured.

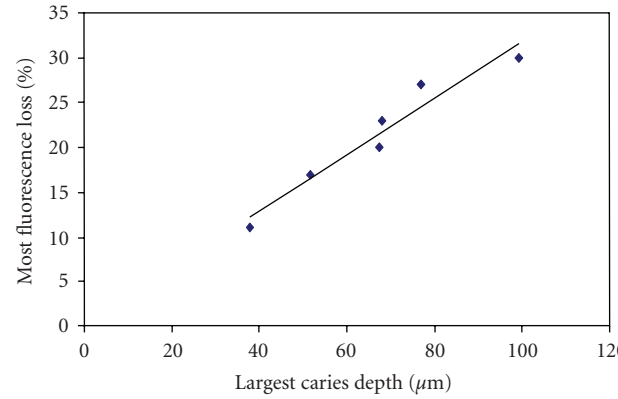


Figure 5: Linear regression analysis graph.

(Figure 3). The teeth were sectioned and imaged by polarized light microscopy (Figure 4). The deepest demineralization depth was measured as distance "d" by imageJ software. The largest depth data of demineralization and the most percentage loss of fluorescence are listed in Table 1.

The statistical linear regression was applied to these data (Figure 5). The correlation coefficient was 0.9696. The two-tailed t-test for coefficient was 7.93, indicating the P-value = .0014. The F test for the entire model was 62.86, which shows the P-value = .0013. Thus, results show significant linear correlation between the demineralization depth (X) and fluorescence loss (Y) as $Y = 0.32X + 0.17$.

Table 1: List of data for building statistical linear regression model.

Largest Caries Depth (μm)	37.8	51.6	67.6	68	77	99.2
Most Fluo. Loss (%)	11	17	20	23	27	30

4. Discussion

The results indicated statistically significant linear correlation between the percent loss of fluorescence and depth of the enamel demineralization. This would facilitate in-vivo measurement of demineralization using the noninvasive QLF technique, based on this linear model. This method provides dentists with critical information about the depth of demineralization when treatment planning dental care for patients.

Although extrapolating the findings for in vivo application may have potential, careful evaluation of how the oral environment, especially the moisture component and the angulation of teeth, may affect the QLF reading should be examined.

Compared to in vitro experimental conditions, in vivo application of QLF has certain difficulties, including access to lesions on occlusal and interproximal surfaces, measurement reliability, the effect of incorporation staining in lesions or sound surface, moisture in the oral environment, and angulations of light source.

The occlusal surface has complicated anatomic structures so that a complex light scattering pattern is generated, which may result in poor contrast between demineralization and sound tissue. A current approach to detect occlusal lesions is DIAGNOdent, where excited 655 nm wavelength light from the probe tip and emitted infrared fluorescence is collected and quantified as an arbitrary value to display on an LCD panel. However, the device needs to be calibrated for every usage, and is technically sensitive to angulation of the probe tip and occlusal staining.

Compared to the bulk of sound enamel structure on facial and lingual surfaces, the interproximal surface has its own complicated light-scattering properties, and the light scattering can also interfere with adjacent teeth. The QLF approach to detect interproximal lesions needs to be evaluated with other techniques such as FOTI or DI-FOTI using transmitted light.

Surface staining may cause technique difficulties to differentiate the lesion from sound tooth structure [54, 55]. The conventional professional prophylaxis is recommended before QLF application. Intraoral moisture may have high impact on QLF results. In vitro experiments apply air dry to teeth before QLF application. In dry teeth, the scattering of light is increased since the refractive index of dry enamel crystals is much greater than that of wet enamel crystals. Thus, in order to standardize the in vivo test, the drying time must be consistent [56]. In addition, the surface smoothness and curvature, and the angulation of the light source to tooth surfaces need to be evaluated by in vivo studies.

In this study, we modified the interpolation algorithms using the mean value of ROI (Region of Interest) border to interpolate the inside lesion area. The fluorescence loss at each pixel in the lesion is recalculated as the mean values of 3×3 pixel areas to avoid possible cold spots, which are created by system random error. In our study, we did find certain cold spots with significant fluorescence loss compared to its adjacent pixels in the QLF image (data not shown). These modifications minimize the system error so that the data is more reliable.

Early intervention with remineralizing agents could be evaluated for effectiveness, QLF examination at recall appointments allowing the clinician to see improvement or advancement in lesion depth.

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Review Article

Caries Detection Methods Based on Changes in Optical Properties between Healthy and Carious Tissue

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A conservative, noninvasive or minimally invasive approach to clinical management of dental caries requires diagnostic techniques capable of detecting and quantifying lesions at an early stage, when progression can be arrested or reversed. Objective evidence of initiation of the disease can be detected in the form of distinct changes in the optical properties of the affected tooth structure. Caries detection methods based on changes in a specific optical property are collectively referred to as optically based methods. This paper presents a simple overview of the feasibility of three such technologies for quantitative or semiquantitative assessment of caries lesions. Two of the techniques are well-established: quantitative light-induced fluorescence, which is used primarily in caries research, and laser-induced fluorescence, a commercially available method used in clinical dental practice. The third technique, based on near-infrared transillumination of dental enamel is in the developmental stages.

1. Introduction

Dental caries is one of the most prevalent chronic diseases of humans worldwide. When different stages of the disease are taken into account, from the initial to the clinically manifest lesion, very few individuals are truly unaffected. In most industrialised countries 60%–90% of school-aged children are affected. The prevalence among adults is even higher and in most countries the disease affects nearly 100% of the population [1].

During the last thirty years, however, major changes have occurred in the pattern of the disease. Progression of enamel caries is now slower [2], allowing time for preventive intervention before irreversible destruction of tooth substance occurs. During the early stages of the disease the process is reversible and can be arrested: noninvasive intervention can convert a lesion from an active to an inactive state [3, 4]. Appropriate diagnostic techniques are necessary to support such decisions about management of the individual lesion. The clinician needs to be able to monitor the outcome of noninvasive measures and in cases where there is evidence of lesion progression, make a timely decision to intervene, using minimally invasive techniques and restoring damaged tooth structure without weakening the tooth. Applying strategies to control, arrest, or reverse the disease process can reduce the economic burden, pain, and suffering of placing and replacing restorations [5].

This modern, conservative approach to clinical management of dental caries, which has been evolving during the past twenty years, has necessitated a critical appraisal of methods used today for clinical detection of carious lesions.

Complementing traditional diagnostic methods with advanced, more sensitive methods will improve caries diagnostic routines and hence the dental care and treatment of patients. The application of such complementary methods should offer objective information about the presence and severity of a lesion, to complement the clinician's subjective interpretation, providing evidence-based clinical caries diagnosis. In this context, there is also a place for more sensitive caries detection methods in clinical caries research. Clinical trials in which lesions are monitored in thousands of subjects over several years are no longer commercially viable. A quantitative method capable of measuring small changes would allow trials of much shorter duration and fewer subjects [6, 7].

Conventional examination for caries detection is based primarily on subjective interpretation of visual examination and tactile sensation, aided by radiographs. The clinician

makes a dichotomous decision (absence or presence of a lesion) based on subjective interpretation of colour, surface texture, and location, using rather crude instruments such as a dental explorer and bitewing radiographs [8]. Studies based on these methods often show low sensitivity and high specificity, that is, a large number of lesions may be missed [9–13]. Sensitivity and specificity are widely used measures to describe and quantify the diagnostic ability of a test [14]. In the context of caries research, sensitivity is a measure of the method's ability to correctly identify all surfaces damaged by caries, and specificity the measure of correctly identified all sound surfaces. Sensitivity and specificity are expressed as values between 0 and 1 (100%), values closer to 1 indicating a high quality result. For caries diagnostic methods, values should be at least 0.75 for sensitivity and over 0.85 for specificity [15].

Diagnostic techniques are also evaluated in terms of validity and reliability. To determine validity, the outcome as measured by the method is compared with a reference standard, a "true" situation. Reliability expresses the consistency of a set of measurements performed with the method. High validity is considered to confirm the absence of systematic errors and high reliability the absence of random errors of the method. The generalisability of a diagnostic technique is also described in terms of external and internal validity. The external validity reflects the extent to which the results of a study can be extrapolated to other subjects or settings, whereas internal validity reflects the degree to which conclusions about causes or relationships are likely to be true, in view of the measures used, the research setting, and the overall study design. Good experimental design will filter out the most confounding variables, which could compromise the internal validity of an experiment.

A wide variation in terms of sensitivity and specificity for conventional caries detection methods are found in the literature [9, 16, 17]. An overall low sensitivity of less than 0.50 is reported, which means that a guess would provide the same result when we correctly want to identify a caries lesion. A recently published comprehensive review [15] stated that the evaluations of diagnostic performance are based on limited numbers of studies of questionable internal and external validity attributable to incomplete descriptions of selection and diagnostic criteria and observer reliability. The quality of published studies is further compromised by the use of small numbers of observers, nonrepresentative teeth, samples with high lesion prevalence, a variety of reference standards of unknown reliability, and variations in statistical analysis of the reported results.

It is apparent that conventional methods for the detection of dental caries do not fulfill the criteria for an ideal caries detection method. These methods rely on subjective interpretation and are insensitive to early caries detection. It is widely recognised that the current methods cannot detect caries lesions until a relatively advanced stage, involving as much as one-third or more of the thickness of enamel [18].

The shortcomings of conventional caries detection methods and the need for supplementary methods have long been acknowledged. The series of published proceedings from the three "Indiana Conferences on Early Detection of Dental Caries" contains a wealth of detail of work in this area [19–21]. Over the past twenty years there has been intensive research into more sophisticated methods for early detection of dental caries [5, 7–9, 16, 17, 22–34]. There are a number of optical caries detection methods and some are summarized in Table 1. Several are in their infancy and there is significant work involved in developing these techniques. Therefore, validation studies are essential to determine their clinical utility before implementation in clinical practice.

Table 1: Summary of optical caries detection methods.

Optical Coherence Tomography	OCT
Polarized Raman Spectroscopy	PRS
Polarization Sensitive Optical Coherence Tomography	PS-OCT
Fibre Optic Transillumination	FOTI and DiFOTI
Quantitative Light-induced Fluorescence	QLF
Laser-induced Fluorescence	LF
Transillumination with Near-Infrared light	TI-NIR
Infrared fluorescence	IR fluorescence
Near-Infrared reflectance imaging	NIR reflectance imaging
Terahertz Pulse Imaging Multiphoton imaging	TPI
Time-Correlated Single-Photon Counting Fluorescence Lifetime Imaging	TCSPC FLIM

An initial effect of the caries disease process, increased porosity, results in a distinct change in the optical properties of the affected dental tissue, providing objective evidence of a caries-induced change. Caries detection methods based on changes in a specific optical property are referred to in the literature as optically based methods, optical methods, or dental tissue optics. The methods are based on the measurement of a physical signal, derived from the interaction of light with dental hard tissue. The following section presents a brief description of the principles underlying these methods.

2. Physical Principles Underlying Optical Caries Detection

Optical caries detection methods are based on observation of the interaction of energy which is applied to the tooth, or the observation of energy which is emitted from the tooth [26]. Such energy is in the form of a wave in the electromagnetic spectrum (Figure 1). The caries detection methods described in this paper use light in the visible and near-infrared range (NIR).

In its simplest form, caries can be described as a process resulting in structural changes to the dental hard tissue. The diffusion of calcium, phosphate, and carbonate out of the tooth, the demineralisation process, will result in loss of mineral content. The resultant area of demineralised tooth substance is filled mainly by bacteria and water. The porosity

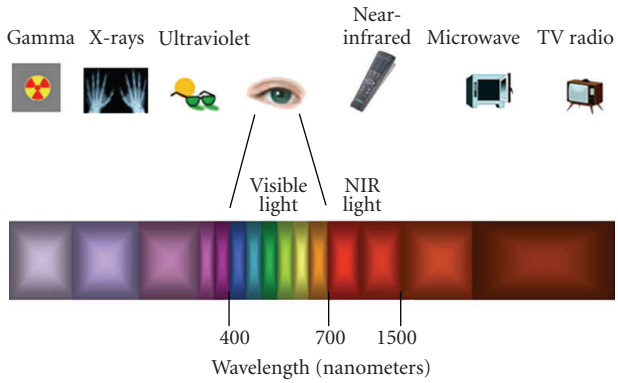


Figure 1: The electromagnetic spectrum.Wavelengths of interest in this paper are the visible light spectrum from 400 nm to 700 nm and the range of near-infrared light from 750 nm to 1500 nm.

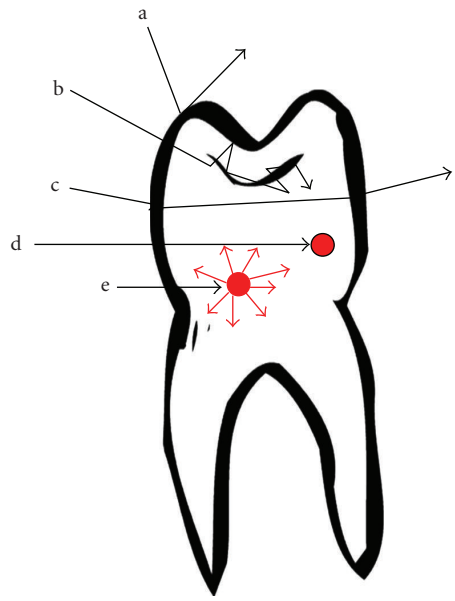


Figure 2: Light interactions with a tooth. How waves can interact with the dental hard tissue; (a) reflection, the wave rebounds; (b) scattering, the incident wave enters the tooth and changes direction. The photons then leave the tooth either as backscattering, where the photons leave through the surface by which they entered, or through another surface (scattering with diffuse transmission); (c) transmission, the wave is illuminated through the tooth and refracts on the surfaces; (d) absorption with heat production; (e) absorption with fluorescence. Most interactions of waves are a combination of these processes.

of this area is greater than that of the surrounding structure. Increased scattering of incident light due to this structural change appears to the human eye as a so-called white spot. Hence, the caries process leads to distinct optical changes that can be measured and quantified with advanced detection methods based on light that shines on and interacts with the tooth (Figure 2).

2.1. Scattering. Scattering is the process in which the direction of a photon is changed without loss of energy. The incident light is forced to deviate from a straight path when it interacts with small particles or objects in the medium through which the light passes. In physical terms scattering is regarded as a material property. A glass of milk is seen as white because incident light on the milk is scattered in all directions, leaving the milk without absorption [35]. Snow appears white because light incident in the snow is scattered in all directions by the small ice crystals. Light of all visible wavelengths exits snow without suffering absorption. Scattering is highly wavelength sensitive, shorter wavelengths scatter much more than longer ones [26]. Therefore, caries detection methods employing wavelengths in the visible range of the electromagnetic spectra (400 nm to 700 nm) are highly limited by scattering. An early enamel lesion looks whiter than the surrounding healthy enamel because of strong scattering of light within the lesion [23]. Methods measuring lesion severity are based on differences in scattering between sound and carious enamel.

2.2. Absorption with Fluorescence. Absorption is the process in which photons are stopped by an object and the wave energy is taken in by the object. The energy lost is mostly converted into heat or into another wave which has less energy and hence longer wavelengths. In physical terms absorption is also regarded as a material property. The previous analogy of the glass of milk appearing white can be extended to a cup of tea [35]; the tea is seen as transparent because it does not scatter light, but it looks brown because much of the light is absorbed by the tea. Likewise, mud and pollution in white snow can be seen as dark spots because certain wavelengths are absorbed by these polluted spots. Absorption of light in tissue is strongly dependent on the wavelength. Water is an example of a strong absorber in the infrared range. After absorption the energy can be released by emission of light at a longer wavelength, through the process of fluorescence. Fluorescence occurs as a result of the interaction of the wavelength illuminating the object and the molecule in this object. The energy is absorbed by the molecule with subsequent electronic transition to the next state, to a higher level state where the electrons remain for a short period of time. From here the electrons may fall back to the ground state and release the gained energy in terms of longer wavelength and colour, which is related to the energy given off and fluorescent light can be emitted. Autofluorescence, the natural fluorescence of dental hard tissue without the addition of other luminescent substances has been known for a long time [36]. Demineralisation will result in loss of autofluorescence [37] which can be quantified using caries detection methods based on the differences in fluorescence between sound and carious enamel.

3. Optical Caries Detection Methods

3.1. Quantitative Light-Induced Fluorescence. The quantitative light-induced fluorescence (QLF) is based on the principle that the autofluorescence of the tooth alters as the

mineral content of the dental hard tissue changes. Increased porosity due to a subsurface enamel lesion scatters the light either as it enters the tooth or as the fluorescence is emitted, resulting in a loss of its natural fluorescence. Bjelkhagen et al., [38], Sundström et al., [39] and subsequently de Josselin de Jong et al. [40] developed a technique based on this optical phenomenon. The underlying theory has been described extensively in several publications [41–43]. The changes in enamel fluorescence can be detected and measured when the tooth is illuminated by violet-blue light (wavelengths 290–450 nm, average 380 nm) from a camera hand piece, following image capturing using a camera fitted with a yellow 520 nm high pass filter (QLF; Inspektor Research Systems, Amsterdam, the Netherlands) (Figure 3(a)). The image is captured, saved, and processed: it is first converted to black-and-white so that thereafter the lesion site can be reconstructed by interpolating the grey level values in the sound enamel around the lesion. The difference between measured and reconstructed values gives three quantities: ΔF (average change in fluorescence, %), lesion area (mm^2), and ΔQ (area $\times \Delta F$), which gives a measure of the extent and severity of the lesion. Changes in fluorescence radiance and lesion area can be followed over time, to measure lesion development. Figure 3(b) shows the analytical stages of the method.

A high positive correlation is reported between QLF and absolute mineral loss, $r = 0.82$–0.92 [44–46]. At a consensus meeting in 2002, The International Consensus Workshop on Caries Clinical Trials (ICW-CCT) [47], it was agreed that QLF may offer one solution in the effort to reduce both the number of subjects and the duration of caries clinical trials. The method seems to have been rapidly adopted as a standard reference measure in clinical tests of the efficacy of preventive measures [42, 48–50]. Application for quantification of dental fluorosis [51], erosive lesions [52, 53], and staining, and bleaching of teeth [54–56] has been investigated. The QLF method can also measure and quantify the red fluorescence (RF) from microorganisms in plaque [57]. The RF observed in plaque can be of use when monitoring oral hygiene, denture plaque assessment, removing infected dentin, and detecting a leaking sealant or caries at the margin of a restoration. Two quantities are obtained, ΔR (average change in red fluorescence, %) and area (mm^2). So far there are a very limited number of studies performed with this feature.

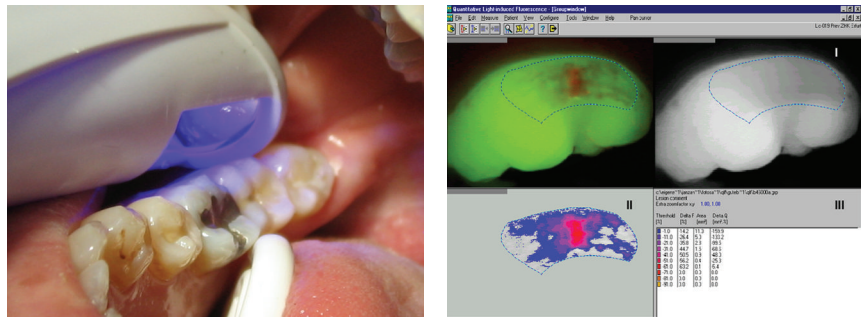
3.2. Laser-Induced Fluorescence. The DIAGNOdent (KaVo, Biberach, Germany) is a portable commercially available device (Figure 4(a)) for detection and quantification of caries [28, 58]. The method generates a simple numerical index of de- and remineralisation in enamel and dentin that can be recorded in the patient's file and monitored over time. The instrument is easy to handle and can also be purchased at a reasonable price. Red laser light ($\lambda = 655$ nm) is emitted by the device via an optical fibre and a probe to the caries lesion (Figure 4(b)). When the light interacts with certain organic molecules that have been absorbed into the porous structure the light is reemitted as invisible fluorescence in the NIR region. The NIR fluorescence is believed to originate from protoporphyrin IX and related metabolic products of oral bacteria [28, 59]: these products are chiefly responsible for the absorption of red light. The emitted light is channelled through the hand piece to the detector and digitally displayed on a screen (0–99). A higher number indicates greater fluorescence and by inference a more extensive subsurface lesion.

Two versions of the laser fluorescence (LF) device are currently available commercially. As well as the DIAGNOdent 2095 for application to smooth and occlusal surfaces, the latest version, the LF-pen (KaVo), has been designed for easier access to approximal surfaces. The original LF device has shown good performance and reproducibility for detection and quantification of occlusal and smooth surface caries lesions in in vitro studies, but the results of in vivo studies have been somewhat contradictory [60–70]. Among LF studies there is a wide variation in specific design features (the number of teeth included, the threshold for LF scores, validation methods, nonvalidated teeth, the outcomes expressed, etc.). A review by Bader and Shugars [24] disclosed that although several evaluations of diagnostic performance have appeared in the literature, the range of the LF device performances is extensive. For detection of dentinal caries, sensitivity values ranged widely (0.19 to 1.0) although most tended to be high. Specificity values exhibited a similar pattern, ranging from 0.52 to 1.0. In comparison with visual assessment methods, the LF exhibited a sensitivity value that was almost always higher and a specificity value that was almost always lower. The body of evidence was based primarily on in vitro studies. Extrapolation to the clinical setting is uncertain.

The LF pen has performed as well as the original device on occlusal surfaces in vitro [71]. To date, there is only one published study of the clinical performance of the LF pen on occlusal surfaces [72]. A moderately positive correlation (Spearman rho) was demonstrated, and greater variation of measurements was recorded with increasing clinically evaluated lesion depth. At a cut-off value of 25 for the threshold between enamel and dentinal caries, sensitivity was 0.67 and specificity was 0.79.

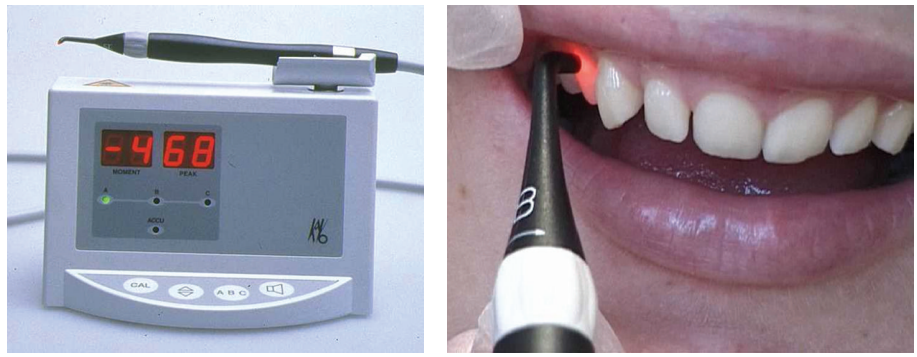
The LF method has also been investigated for longitudinal monitoring of the caries process and for assessing the outcome of preventive interventions [50, 63, 73–75]. The potential role of the LF device in detection of root caries lesions has not been extensively investigated and hitherto only three validity studies are available [76–78]. A low to moderate correlation was found when LF readings were correlated with histopathological lesion depth of root caries lesions [76, 77].

For the clinician to have confidence in using a caries detection method to support clinical treatment decisions, it is important that interpretation of readings is based on an understanding of the principles underlying the method and an awareness of potential shortcomings. With reference to LF, the question of what the method really measures has yet to be resolved. More research is needed to clarify the origin of the increased fluorescence caused by the excitation of 655 nm wavelength light. The NIR fluorescence is believed to originate from bacteria or their metabolites. Hence, there



(a) (b)

Figure 3: (a) Light of certain wavelengths is led by an optic fibre from the light source to a hand piece with a micro-Charge Couple Device video camera. (b) The image can be captured and saved for later analysis. Computer program: QLF 1.97e Inspector Research System BV, Amsterdam, The Netherlands.



(a) (b)

Figure 4: (a) The LF device operates with light from a diode laser transmitted through a descendent optic fibre to a hand held probe with a fibre optic eye. The emitted fluorescence is collected through the tip, passes into ascending fibres, and is finally processed and presented on the display as an integer between 0 and 99. (b) In the presence of carious tooth substance, fluorescence increases.

is a poor correlation between LF readings and the mineral content, but possibly better correlation with the presence of infected dentin.

In general, in vivo studies of LF for occlusal caries detection indicate moderate to high sensitivity and lower specificity [24, 60, 66, 68]. Lack of specificity, the increased likelihood of false-positive readings due to stain and plaque, and the absence of a single threshold are factors underlying the reluctance among authors to recommend the LF method unequivocally for caries detection. Therefore, the LF device should be regarded at most as a supplementary aid for detection of caries on coronal surfaces.

3.3. Transillumination with Near-Infrared Light. The caries lesion may also be examined by shining white light through the tooth. Wavelengths in the visible range (400–700 nm) are limited by strong light scattering, making it difficult to image through more than 1 mm or 2 mm of tooth structure [79]. Therefore, methods employing wavelengths in the visible range of the electromagnetic spectra (400–700 nm) such as QLF [40] ($\lambda > 520$ nm), LF [58] ($\lambda = 655$ nm), and Digital Imaging Fiber-Optic Transillumination (DIFOTI) [80]—which uses high intensity white light—are highly limited by scattering. Methods that use longer wavelengths, such as in the NIR spectra (780 to 1550 nm), can penetrate the tissue more deeply. This deeper penetration is crucial for the transillumination (TI) method. Research has shown that enamel is highly transparent in the NIR range (750 nm to 1500 nm) due to the weak scattering and absorption in dental hard tissue at these wavelengths [81–86]. Therefore, this region of the electromagnetic spectrum is ideally suited to the development of new optical diagnostic tools based on TI. Figure 5 illustrates the typical experimental set-up of a TI system with an NIR light source, an imaging camera such as a charge-coupled device (CCD), and software for computer-controlled acquisition. The image can be captured, saved, and stored in digital format.

This is a promising imaging technique for detecting the presence of caries and measuring its severity. The TI image is presented as a visually recognizable image, which is preferred by the average clinician. The method is nondestructive, nonionising, and reportedly more sensitive to detect early demineralisation than dental X-rays [83]. Identification of dental caries by TI is based on the fact that increased mineral loss in an enamel lesion leads to a twofold increase in scattering coefficient at a wavelength of

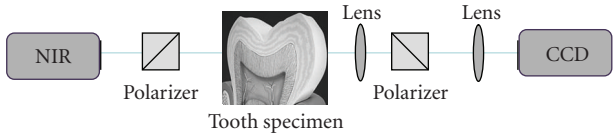


Figure 5: Transillumination (TI) with Near-Infrared (NIR) light. experimental set-up of the TI system. The tooth is illuminated with NIR light. Polarizers are used to experimentally block out the ambient light from saturating the detector, a Charge Couple Device (CCD).

1.3 μm [82, 83]. Caries thus appear as dark regions, since less light reaches the detector. Most research to date has used this wavelength, where low-cost light sources are available. When light illuminates the tooth the strong scattering effect in the enamel caries lesion results in less transparency. The decreased light transmission associated with the lesion can be detected when compared to that of the surrounding sound tissue.

The use of dental radiography should always be limited, even though it is the most often employed concept of routine examination. Dental radiographs also lack the sufficient ability for early caries detection [13, 16]. An initial caries lesion may be missed or underestimated in size in radiographs due to low attenuation of radiation in lesion, particular physical properties of the tooth structure, and imperfect technique such as overlapping. In contrast, the TI method offers the advantage of allowing for repeated projection to overcome some of these limitations.

The importance of the location of the caries lesion and how the resolution differs when the resultant image has to traverse a thick part versus a thinner part of the tooth to reach the detector is also of interest. Contrast calculation of the signal generated by a single lesion located near versus far from the CCD camera can be estimated. The ratio between the contrasts of images captured from both sides of the tooth can estimate the more precise location of the caries lesion on the approximal surface.

4. Summary

Both QLF and the TI methods enable imaging detection of enamel caries that can be digitally stored and viewed later. The QLF method also includes image analysis software which measures the difference in fluorescence between sound and demineralised enamel. Changes in fluorescent radiance and lesion area can be followed over time, to measure lesion development. The method seems to have been rapidly adopted as a standard reference measure in clinical tests of the efficacy of preventive measures.

Transillumination of enamel with NIR light is a promising technique for the detection and imaging of occlusal and approximal lesions. Application of repeatable, non-ionising radiation of the tooth allows the TI method to be used without restriction to monitor the caries process. The method overcomes some of the limitations of dental radiography such as overlapping. Moreover, the method can indicate the relative position of a lesion on approximal surfaces by calculating the ratio of contrast values obtained by illuminating tooth from the lingual or buccal surface, respectively. The method uses a range of wavelengths where low-cost light sources are available and the transmitted image can be detected by an ordinary CCD camera, similar to the one in mobile phones. The method can therefore be developed at reasonable cost as a fibre optic probe for intraoral use, connected to an ordinary computer screen.

The LF method generates a simple numerical index of de- and remineralisation in enamel and dentin that can be recorded in the patient's file and monitored over time. The instrument is easy to handle and can also be purchased at a reasonable price. New methods should be critically appraised according to strict criteria. Among LF studies there is a wide variation in specific design features (the number of teeth included, the threshold for LF scores, validation methods, the outcomes expressed, etc.). In vivo studies highlight the importance of rigorous clinical studies to confirm promising laboratory results. Results of the LF method in vivo have been somewhat contradictory. Therefore, The LF device should be regarded at most as a supplementary aid for detection of caries on coronal surfaces, pending the publication of further clinical studies.

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Research Article

The Evaluation of the Vector System in Removal of Carious Tissue

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The aim of this study was to evaluate the Vector system in comparison to the conventional technique in cavity preparation. Four extracted primary teeth with no restorations and similar fissure carious lesions and four permanent teeth extracted for orthodontic reasons were used. Class I preparations were made provided that the caries depth remained within the dentin limits. Two teeth were treated with an aerator, the other two had carious tissue removed with the Vector system. Prepared cavities were evaluated with scanning electron microscopy for the surface roughness of the dentine and enamel and for the carious tissue removal efficiency. This pilot study determined that it is possible to remove carious tissue and perform cavity preparation with the Vector system. According to this preliminary evaluation of surface quality, a cavity prepared with the Vector treatment system, allows for a slicker floor, and a more regular enamel-dentine line than that prepared with an aerator. However, the Vector system requires a longer treatment time which we believe may be a negative point, especially for young patients.

1. Introduction

Conventional cavity preparation and carious tissue removal are based on Black's principle of extension for prevention. This principle requires removing healthy tooth structure which is very destructive and leads to excessive tissue loss. In recent years, minimal invasive cavity preparation has gained popularity. Current practice keeps the size of cavities as small as possible. Conservative cavity preparation, which includes handpieces and burs, leads to the undesirable removal of healthy tooth structure. Due to this excessive loss of sound tissue, efforts have focused on new techniques [1, 2].

Over the last few years, new techniques and procedures for hard tissue removal were developed as alternatives to the conventional mechanical procedure [3]. Alternative carious dentin removal techniques have been proposed, including hand excavation, air-abrasion, air-polishing, ultrasonication, sonoabrasion, lasers, and chemomechanical methods [4, 5] (Table 1).

The Vector system is a new method combining both ultrasonic effects and microabrasive action of quartz crystal suspension. This method uses specially shaped metal tools for use with an abrasive slurry of silicon carbide (Vector Fluid Abrasive, grain size 40–50 mm) for micro-invasive preparation countouring and finishing of the tooth substance and nonmetal restorations. These instruments are available in different cylindrical and oval shapes for a precise preparation. The tissue removal is accurate, athermal, and of a gentle nature. Heat induction is almost eliminated so that only a small amount of liquid is needed. As a result the conditions of work during the preparation of carious lesions in teeth remain unchanged [6].

The manufacturer suggests that the Vector removes biofilm, plaque, calculus, and endotoxins. The resonating ring of the Vector System converts the ultrasonic dynamics of 25 kHz in a similar way to a hula-hoop. If it is pressed into the horizontal position it moves vertically with 90 degrees deflection. This allows a linear movement of the instruments parallel to the tooth surface and an adhering film of water or particle suspension. The ultrasound's energy is thereby indirectly coupled onto the tissues to be treated.

In contrast to diamond instruments, in the Vector system the energy is transmitted indirectly via the silicon carbide particles (average grain size approximately is 50 μm) carried in the water. Preparation with the Vector system is therefore carried out without exposure to high temperatures [7].

Table 1: Classification of techniques available for carious dentine excavation.

Category	Technique
Mechanical, rotatory	Handpieces and burs
Mechanical, non rotatory	Hand excavators, air-abrasion, air-polishing, ultrasonics, sono-abrasion
Chemo-mechanical	Caridex, Carisolv, enzymes
Photo-ablation	Lasers

Table 2: Cavity preparation time for Aerotor and Vector system.

	Average time
Primary teeth prepared with *Vector system*	5.7 minutes
Permanent teeth prepared with *Vector system*	7.7 minutes
Primary teeth prepared with *Aerotor*	1.5 minutes
Permanent teeth prepared with *Aerotor*	1.66 minutes

Therefore, the purpose of this study was to evaluate the Vector system in comparison to the conventional air-motor technique.

2. Materials-Methods

Four extracted primary teeth without restorations and with similar fissure carious lesions, and four permanent teeth extracted for orthodontic reasons were used. Class I cavities were prepared, provided that the cavity depth remained within the dentin limits. Two primary and two permanent teeth were treated with an aerotor, and in the other group teeth had their carious lesions removed and cavity preparation was done with the Vector system (Duerr Dental, Bietigheim-Bissingen, Germany). Cavity preparation was done according to manufacturer's instructions with a special diamond bur with 1.8 mm of diameter. During cavity preparation, a chronometer was used to time each procedure.

Crowns were separated from the roots with a separator. The prepared cavities were kept in ultrasounded solution for 30 minutes to eliminate dust and other tooth particules. According to electron microscopy instructions, specimens were coated with gold (150 seconds) with the Snonputter technique (Polaron Sputter Coater) for electron microscopy images.

Prepared cavities were evaluated under a scanning electron microscopy (Jeol JSM-5600, SEM) and dentine and enamel surface roughness and efficiency of carious tissue removal were studied.

3. Results

Cavity preparation times with the Vector system and the aerotor are shown in Table 2. The time required for cavity preparation using the Vector system was much longer than the aerotor treatment.

4. SEM Evaluation

4.1. Cavity Floor. There were bigger and deeper cracks and fractures on the floor of the cavities prepared with the aerotor in comparison to the vector system. In cavities which were prepared with the vector system, cracks were smaller and less deeper. Cavity bordes which were prepared with the Vector system were also as smoother (Figures 1(a)-1(b), Figures 2(a)-2(b)).

4.2. Dentine. In the dentine surface, the orifice of dentinal tubules was seen almost plugged. Typically, these surfaces have previously been described as scaly or flaky, or as an irregular surface.

Smear layer was less evident at the cavities prepared with the vector system. While in cavities prepared with the Vector system open tubule orifices could be observed, at cavities prepared with the aerotor it was observed that most of the tubule orifices were obstructed with a smear layer. Also, the dentin surface of the cavity floor was smoother in cavities prepared with the Vector system in comparison to the aerotor (Figures 3(a)-3(b), Figures 4(a)-4(b)).

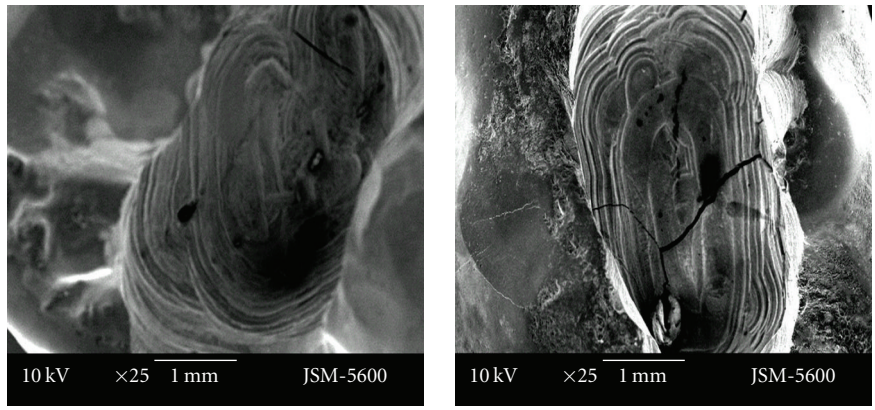
Although cavity surfaces seem relatively smooth at ×250 magnification, rough cavity surfaces with irregular particules were seen at the higher magnifications (×500, ×1000). More smear layer was seen in cavities prepared with the Vector system than those prepared with the aerator. At vector system cavities, indented structure of dentin tubuluses was observed much more clearly. Particules were seen as to be nailed to the smooth dentin surface in the cavities prepared with the Vector system. Tubule entries were observed much clearer with ×1000 enlargement. But tubules were observed as obstructed with debris, poor quality, and pointed surface view (Figures 5(a)-5(b), Figures 6(a)-6(b)).

4.3. Enamel. Enamel surface morphology was determined similarly in both cavity preparation systems. Enamel lines were streamlined in both the aerotor and vector system (Figures 7(a)-7(b), Figures 8(a)-8(b)).

5. Discussion

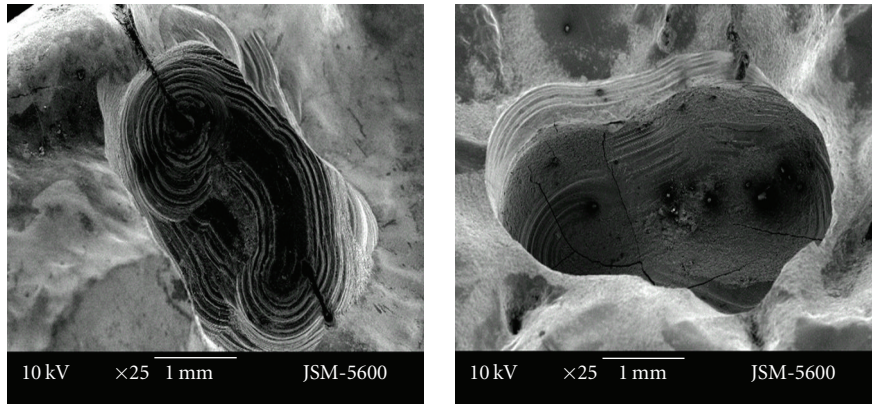
This pilot study determined that it is possible to remove carious tissue and perform cavity preparation with the Vector system. According to this preliminary evaluation of surface quality, a cavity prepared with the Vector treatment system is slicker, and the enamel-dentine line is more regular than that prepared with an aerator. However, the Vector system requires a longer treatment time.

The necessity of using the mechanical nonrotatory instruments has emerged to eliminate the negative effects of the conventional methods. One of these is the removal of carious tissue by chemomechanical methods. Chemomechanical caries removal involves the selective removal of soft carious dentin without the painful removal of sound dentin. The Carisolv system that is a popular chemomechanical system for caries removal consists of a gel with amino acids and sodium hypochlorite, and special hand instruments.



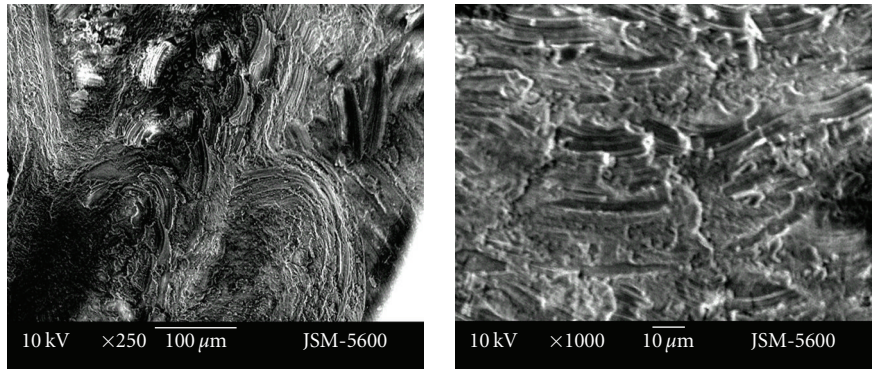
(a) (b)

Figure 1: Representative photographs of cross cut section of permanent (a)/primary (b) tooth cavity, prepared by aerotor (magnification ×25).



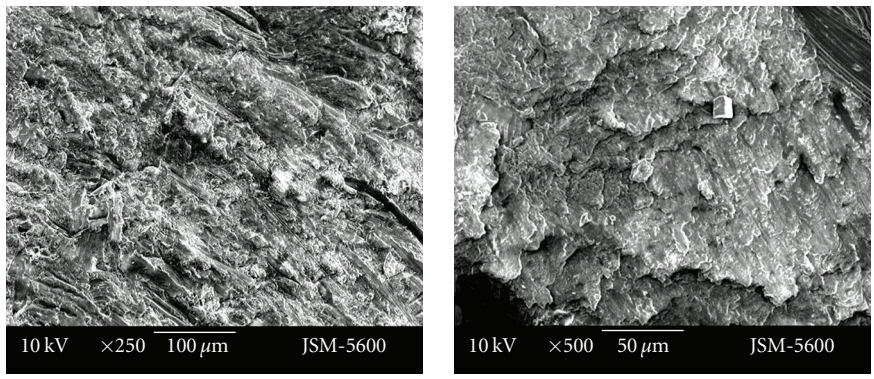
(a) (b)

Figure 2: Representative photographs of cross cut section of permanent (a)/primary (b) tooth cavity, prepared by vector system (magnification ×25).



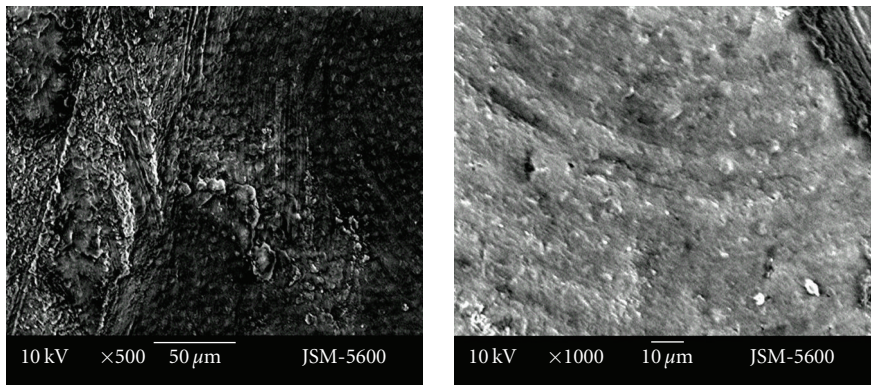
(a) (b)

Figure 3: Representative photographs of cross cut section of permanent tooth dentine surface, prepared by aerotor (magnification ×250 and ×1000).



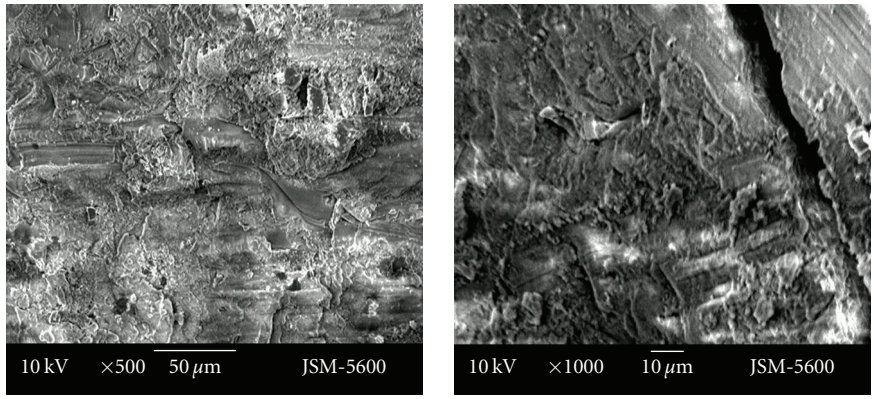
(a) (b)

Figure 4: Representative photographs of cross cut section of permanent tooth dentine surface, prepared by vector system (magnification ×250 and ×500).



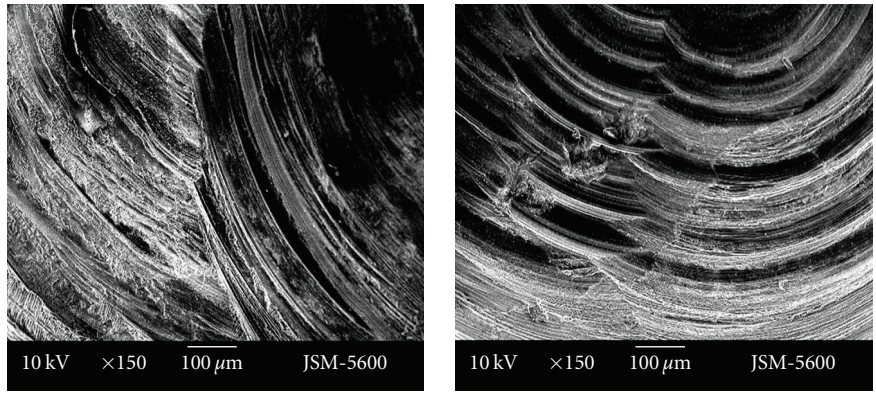
(a) (b)

Figure 5: Representative photographs of cross cut section of primary tooth dentine surface, prepared by aerotor (magnification ×500 and ×1000).



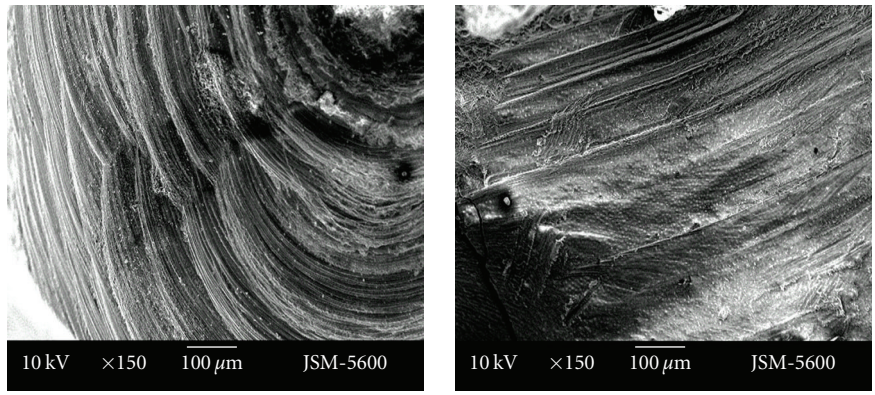
(a) (b)

Figure 6: Representative photographs of cross cut section of primary tooth dentine surface, prepared by vector system (magnification ×250 and ×500).



(a) (b)

Figure 7: Representative photographs of cross cut section of permanent (a)/primary (b) tooth enamel surface, prepared by aerotor (magnification ×150).



(a) (b)

Figure 8: Representative photographs of cross cut section of permanent (a)/primary (b) tooth enamel surface, prepared by vector system (magnification ×150).

One of the major advantages is the increased patient compliance to this technique of removing carious dentin compared to drills. In addition, unwanted removal of sound dentin is avoided and the need for local anesthesia is less. However, most of the studies reported that this method prolonged treatment time when compared with rotatory instruments [4, 8].

It should be noted that there is as of yet no evidence in the current literature of using the Vector system for minimal invasive cavity preparation (in spite of manufacturer recommendations), and hence the initiation of this pilot study. Therefore there has been no means of comparing these results with others. But after applying this method, it is evident that there could be advantages such as high level of patient acceptance because of reduced pain response, reduced risk of injury due to other tissue sound pulp protection (in deep lesions), high touch sensitivity and minimal use of water cooling (aerosols eliminated and infection minimized). There are some limitations of the method such as reduced visualization due to suspended particles (but there is a possibility of additional flushing of the preparation site), reduced removal rate compared with rotating burs, and the method is not suitable for the removal of extensive soft dentine caries. However, on the basis of the findings reported here, further research in a longer term prospective study comparing the Vector system with a conventional approach using high speed burs, local analgesia, and a rubber dam is now needed [6, 8]. The time required for preparation with the Vector system was significantly longer (9.5 minutes for CS and 16.8 minutes for CM) than when using conventional method (CS 3.9 minutes, CM 5.5 minutes t-3.91; $P < .0002$) [6]. On the other hand, all results consistently showed a definite advantage of the rotatory instrument approach with respect to time.

Pain perception by Hochmans scale showed that 54.8% of individuals treated by the use of the Vector system did not experience any pain as opposed to 29.1% using the conventional method. Children felt the conventional method to be more painful than the Vector system [6]. Rotatory instruments have some disadvantages, such as the nonselective removal of hard tissue, unpleasentness to the patients, necessity of local anesthesia, and potential adverse

effects to the pulp due to heat and pressure [3, 9]. It is suggested that the Vector systems have higher patient acceptance, and reduced pain response (sensitive children, anesthetic is often not needed) [8, 10].

The cavities prepared by a diamond bur in a conventional high-speed drill showed a box shaped configuration, with sharp cavo-surface edges and well-defined geometric internal angles, flat floor and wall cavity, with smear layer covering enamel and dentin surface in a typical morphological pattern as observed in Freitas et al.'s studies [3]. In contrast to diamond instruments, the energy is transmitted indirectly via the silicon carbide particles carried in the water with the Vector system. Preparation with the Vector system is therefore carried out without exposure to high temperatures and is extremely gentle on the pulp. Loosening of the enamel prisms is avoided, which results in optimum marginal qualities for subsequent restorations, and with a lesser smear layer than cavity preparation with diamond bur.

6. Conclusion

The present study determined that it is possible to remove carious tissue and perform cavity preparation with the Vector system. According to both primary and permanent findings, evaluating surface quality, a cavity prepared with the Vector treatment system has a cavity floor that is slicker and a more regular enamel-dentin line than a cavity prepared with an aerator. While being soundless, nonvibrating, and not using local anesthesia are advantages for clinical practice, a longer treatment time is a negative point, especially for young patients.

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Research Article

Determining the Effect of Calculus, Hypocalcification, and Stain on Using Optical Coherence Tomography and Polarized Raman Spectroscopy for Detecting White Spot Lesions

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Optical coherence tomography (OCT) and polarized Raman spectroscopy (PRS) have been shown as useful methods for distinguishing sound enamel from carious lesions *ex vivo*. However, factors in the oral environment such as calculus, hypocalcification, and stain could lead to false-positive results. OCT and PRS were used to investigate extracted human teeth clinically examined for sound enamel, white spot lesion (WSL), calculus, hypocalcification, and stain to determine whether these factors would confound WSL detection with these optical methods. Results indicate that OCT allowed differentiating caries from sound enamel, hypocalcification, and stain, with calculus deposits recognizable on OCT images. ANOVA and post-hoc unequal N HSD analyses to compare the mean Raman depolarization ratios from the various groups showed that the mean values were statistically significant at $P < .05$, except for several comparison pairs. With the current PRS analysis method, the mean depolarization ratios of stained enamel and caries are not significantly different due to the sloping background in the stained enamel spectra. Overall, calculus and hypocalcification are not confounding factors affecting WSL detection using OCT and PRS. Stain does not influence WSL detection with OCT. Improved PRS analysis methods are needed to differentiate carious from stained enamel.

1. Introduction

White spot lesions are areas of demineralized enamel that represent an early stage of caries, since they can progress to cavitated lesions if untreated [1]. These lesions appear chalky white when dried and are not visible radiographically. The demineralization process can be arrested or reversed by noninvasive means, including oral hygiene counselling and/or topical fluoride application [2, 3]. Therefore, early detection of white spot lesions (WSLs) is desirable since early preventive treatment can avert the need for future restorative treatment.

New technologies such as the DIAGNOdent and quantitative light fluorescence (QLF) devices have been developed for the detection of early carious lesions. These techniques are intended to be adjuncts to clinical decision making and aid in planning preventive treatment [4]. Despite the potential of these methods, the results from these tools are affected by confounding factors in the oral environment, thereby compromising the sensitivity and/or specificity of these techniques. For example with the DIAGNOdent, stain, calculus, plaque [5], as well as developmental hypomineralization can produce a fluorescence output that results in false-positive readings [6]. A review of the literature evaluating the DIAGNOdent device found that, compared to visual assessment methods, the sensitivity was consistently higher but the specificity was lower, concluding that the increased risk of false-positives limits its clinical usefulness

[7]. For QLF, stain, plaque, fluorosis, or any developmental hypocalcification results in false-positives [8–11]. Composite resins pit and fissure sealants as well as prophy pastes could also lead to false-positive results. Clearly, a new technology is needed that will not be affected by factors in the oral environment. A new optical approach to detect white spot lesions clinically is jointly being developed at the National Research Council of Canada-Institute for Biodiagnostics, the University of Manitoba and Dalhousie University, using a combination of optical coherence tomography (OCT) and polarized Raman spectroscopy (PRS). OCT is a nondestructive technique for high-resolution (10–20 μm) depth imaging that gives information about the morphology and depth of white spot lesions up to 3 mm into enamel [12]. This method is similar to ultrasound technology, but instead of sound waves, light waves are utilized and the imaging is limited to near-surface tissues. Changes in the refractive indices of structures cause light backscattering, creating an image that is different for sound enamel and demineralized enamel. Previous studies have demonstrated the potential of OCT for caries assessment [13–16]. PRS is a noninvasive spectroscopic method that provides details on the biochemistry and molecular structure of white spot lesions. The energy difference between the incoming excitation light and scattered photons is proportional to the vibrational energy of molecules within the sample being studied, known as the Raman effect. The caries process results in biochemical and structural changes that can be followed by PRS, which uses scattered light to determine differences between the mineral matrix of sound enamel and demineralized enamel [16]. Our previous studies have shown that OCT and PRS can be used to distinguish sound enamel from white spot lesions (WSLs) *ex vivo* [14, 15, 17]. However, the effects of calculus, hypocalcification, and stain on OCT and PRS have yet to be established. By combining OCT and PRS, false-positive results from one technology can potentially be eliminated, thereby increasing the sensitivity and specificity of the new method.

Calculus consists of an organic matrix with the inorganic components of dicalcium phosphate dehydrate (DCPD), hydroxyapatite (HA), octacalcium phosphate (OCP), and β-tricalcium phosphate (TCP). Raman spectra of human enamel are characterized by a main peak at $\sim$959 cm^{-1} arising predominantly from the symmetric phosphate groups in carbonated hydroxyapatite, the major mineral component of dental enamel [18, 19]. Clinically, hypocalcification appears visually similar (chalky white) to a WSL and must be differentiated from a WSL since hypocalcification is a developmental defect that does not need to be treated. In hypocalcified enamel, the crystals are arranged normally but there are pores due to larger spaces between enamel rods [20]. Stain is often classified as intrinsic, extrinsic, or internalized [21]. Intrinsic stain occurs during tooth development due to alterations in the structure or thickness of enamel or dentin, extrinsic stain accumulates on the acquired pellicle, and internalized stain occurs when extrinsic stain is incorporated into areas of enamel defects after tooth development.

The objective of this study was to determine whether calculus, hypocalcification, and stain are confounding factors affecting WSL detection with optical coherence tomography and polarized Raman spectroscopy.

2. Methods and Materials

Teeth were obtained from consenting patient volunteers undergoing extractions for other reasons. Ethics approvals were obtained from the human ethics committees of the authors' institutions. Postextraction, the teeth were rinsed with water and clinically examined by two clinicians independently. Samples were separated into 5 groups: sound enamel, WSLs, calculus, hypocalcification, and stained enamel. Figure 1 is a diagrammatic representation of sample sizes and allocation criteria for the above groups. Since patient histories were not available, it cannot be determined if hypocalcified enamel was caused by excess ingestion of fluoride; therefore, this category is referred to as "hypocalcification".

Teeth were stored in deionized water prior to measurement with OCT and PRS. Figure 2 is a diagrammatic summary of data collection and analyses with OCT and PRS.

A Humphrey's system 2000 optical coherence tomography scanner (Zeiss Humphrey Systems, Dublin, CA, USA) operating at 850 nm was used for OCT measurements. For all data, the laser was focused to the thinnest line on the tooth surface and the scan length was 2.0 mm. Scans were acquired vertically along the incisal/occlusal to cervical direction. Three scans were collected across the area of interest with the proximal surface of interest oriented perpendicular to the laser beam. Three vertical scans were also taken of sound enamel on the same tooth surface for comparison. OCT images had display resolutions of 500×100 pixels, and transverse resolutions of 10–20 μm. Figure 3(a) displays a photo of a tooth being scanned with the OCT system (laser scan line circled) with the corresponding 2-dimensional depth image of sound enamel (Figure 3(b)). MATLAB software (The MathWorks, Natick, MA, USA) was used to plot OCT images.

A LabRamHR Raman microspectrometer (HORIBA Jobin Yvon, Edison, NJ, USA) was used to acquire spectral data. The laser excitation was 830 nm and a 10x microscope objective was used (power at the sample was 125 mW) with an acquisition time of 5 seconds and 6 accumulations. On each surface, point measurements were taken with parallel- (p1) and cross- (p2) polarizations at a minimum of three different points. The optical configurations for parallel- and cross-polarization measurements have been described previously [17]. Background optics spectra were recorded and subtracted from each sample spectrum. Raman data were analyzed using MATLAB software to calculate the depolarization ratios ($I_\perp/I_\parallel$), where $I_\perp$ is the area under the Raman band from 925–1000 cm^{-1} for cross-polarization (p2), and $I_\parallel$ is the similar area for parallel-polarization (p1) [17]. This wavenumber range was used since it centres at 959 cm^{-1} (the main peak due to phosphate groups such as those found in apatite).

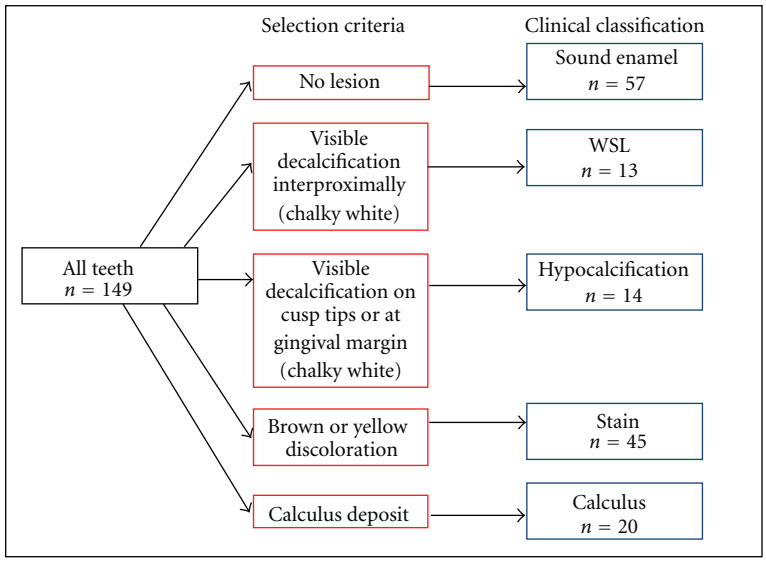

Figure 1: Schematic diagram outlining the sample sizes and allocation criteria for each of the 5 sample groups.

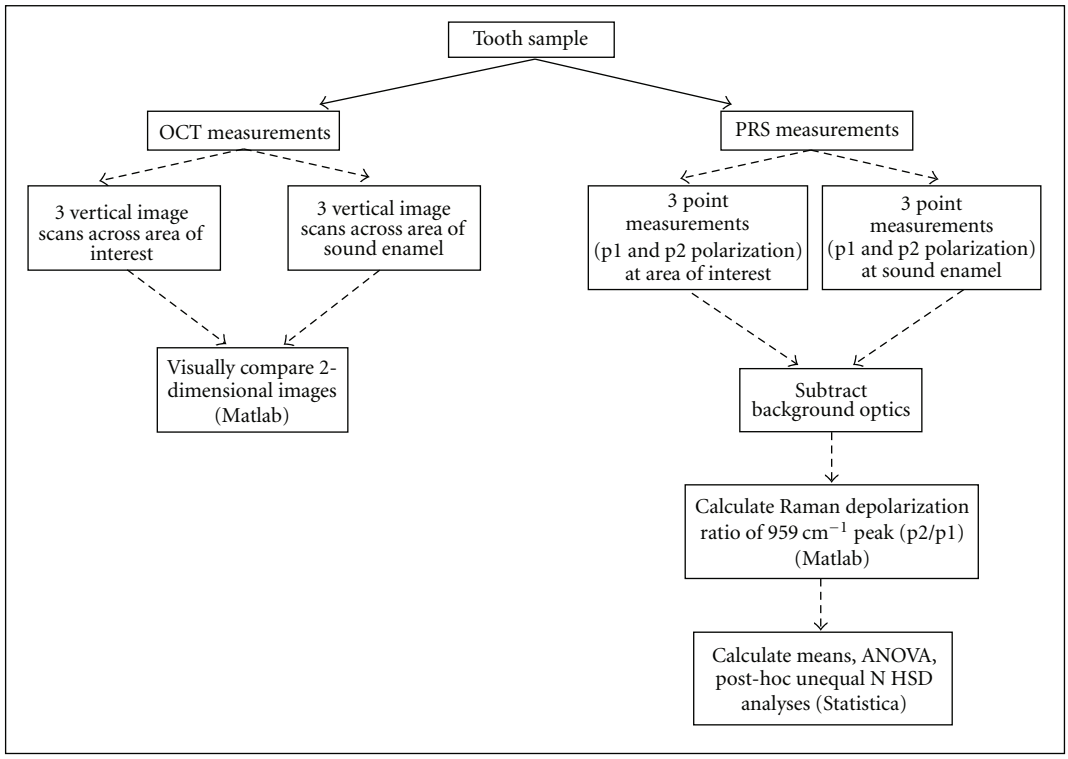

Figure 2: Flowchart of the OCT and PRS data collection and analysis methods.

3. Results

Two-dimensional OCT images are shown of sound enamel (Figure 3(b)) and a WSL (Figure 4(a)). In the sound enamel image, there is intense light backscattering at the tooth-air interface, and no significant signal with depth into the enamel. In contrast, the OCT image of a WSL shows significant light backscattering beneath the surface with a triangular shape characteristic of a subsurface lesion as observed on histological sections. The two-dimensional OCT image displayed as Figure 4(b) shows a deposit of material on the tooth surface that is attributed to calculus. The OCT image of hypocalcification in Figure 4(c) shows diffuse light back-scattering and a more irregular subsurface pattern of scattering across the entire region scanned compared to sound enamel. The light back-scattering of hypocalcified

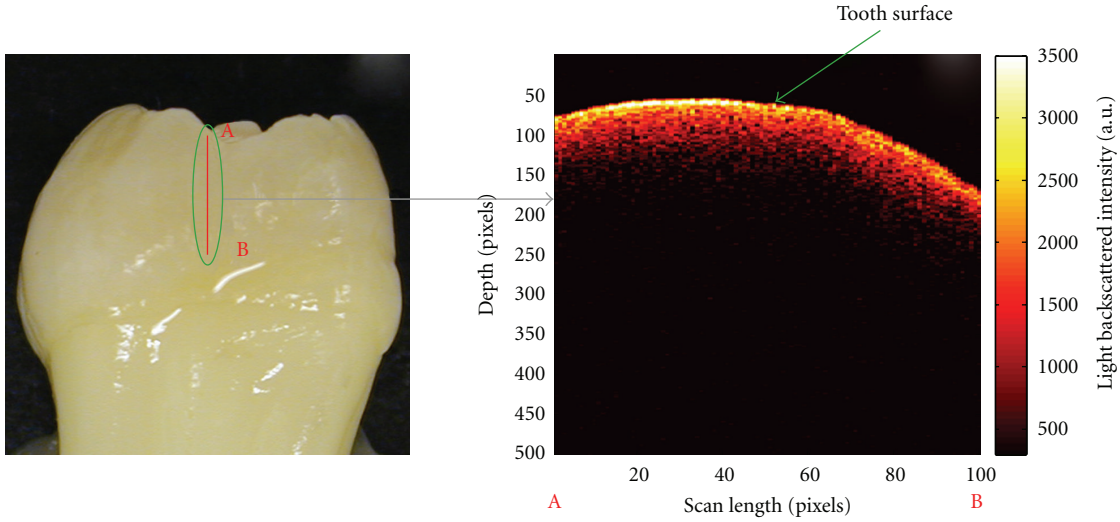

Figure 3: (a) Photo of an extracted human tooth with a line indicating the position of the OCT scan and (b) the corresponding OCT 2-dimensional depth image of sound enamel acquired from the laser line. Labels (A) and (B) indicate the two endpoints of the scan (a.u. = arbitrary units).

regions is also more irregular than WSLs, which have a characteristic triangular shape. The OCT image of stained enamel in Figure 4(d) demonstrates increased light backscattering when compared to sound enamel, but again there is no triangular-shaped subsurface light back-scattering as observed with WSLs.

Average Raman depolarization ratios (ρ) from the various groups are depicted in a box-and-whisker plot (Figure 5). In order to determine whether the mean depolarization ratio values of the various groups were statistically significant from one another, a one-way analysis of variance (ANOVA) followed by unequal N HSD post-hoc comparisons (Statistica) was performed (Table 1). It was determined that the mean values were statistically significant in all cases at $P < .05$ except for three cases. Sound enamel was not statistically significant from hypocalcified enamel, carious enamel was not statistically significant from stained enamel, and lastly stained enamel was not statistically significant from hypocalcified enamel. In Raman spectra acquired from areas of stain (Figure 6), there is a large background sloping fluorescence that is not observed in spectra from areas of sound enamel or WSLs without stain where these spectra have a flat background.

4. Discussion

The development of new technologies for WSL assessment requires that these devices undergo clinical validation prior to becoming an accepted clinical method. In addition to demonstrating the performance of these methods for providing high sensitivity for WSL detection, high specificity is also desirable. Optical coherence tomography and polarized Raman spectroscopy are methods that potentially can address the need for a technology with high sensitivity and high specificity. To date there are no studies outside our research group that investigate a combination of OCT and PRS to detect WSLs, and in particular the effects of the calculus, stain, or hypocalcification on the utility of these two technologies.

OCT imaging allows differentiation of sound from demineralized enamel on the basis of the characteristic triangular shape of the back-scattered signal beneath the tooth surface in images of WSLs. This subsurface scattering pattern is believed to be due to WSLs having porous enamel matrices, allowing incident light to travel further into the enamel and causing more scattering to occur, which is detected by the OCT system.

When calculus is present, it appears clearly as a deposit in the two-dimensional OCT image. Similar to conventional caries assessment methods, scaling of calculus is recommended before an assessment is made using OCT and PRS. OCT can possibly be used to alert the clinician when calculus is still present in the region of interest and that further scaling is necessary. OCT images of hypocalcification can be differentiated from WSLs, since the light back-scattering found with hypocalcification is more irregular than WSLs and lacks the characteristic triangular shape. To some extent, hypocalcification can be differentiated from sound enamel with OCT since hypocalcification has a more irregular pattern of scattering at the surface and subsurface compared to sound enamel. Although areas of stain are not easily distinguishable from hypocalcified enamel or sound enamel with OCT, they can be readily differentiated from WSLs because areas of stain lack the subsurface triangular-shaped back-scattering pattern characteristic of

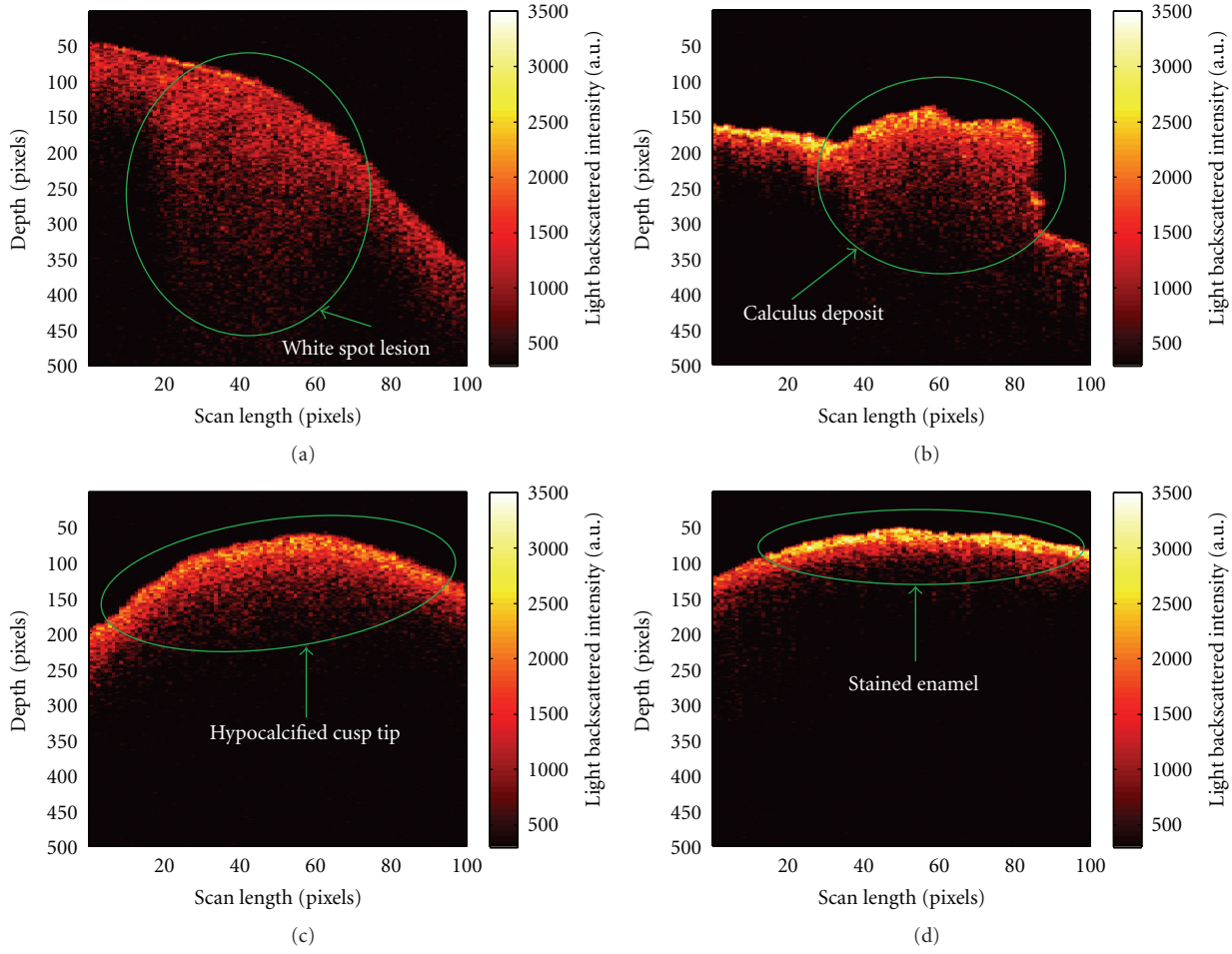

Figure 4: Representative OCT depth images of a (a) white spot lesion, (b) calculus deposit on a tooth surface, (c) hypocalcified cusp tip, and (d) region of stained enamel (a.u. = arbitrary units). All areas of interest are highlighted by the green markings.

Table 1: Results of ANOVA followed by unequal N HSD post-hoc comparison analyses with the P values shown. Mean Raman depolarization ratios (ρ; ± standard deviation) for each group are also displayed.

Group	Caries	Sound Enamel	Enamel with Calculus	Stained Enamel	Hypocalcified Enamel
Caries $\rho = 0.14 \pm 0.07$	—	$P < .001$	$P < .001$	$P = .97$	$P < .05$
Sound Enamel $\rho = 0.06 \pm 0.04$	$P < .001$	—	$P < .001$	$P < .001$	$P = .84$
Enamel with Calculus $\rho = 0.22 \pm 0.14$	$P < .001$	$P < .001$	—	$P < .001$	$P < .001$
Stained Enamel $\rho = 0.13 \pm 0.11$	$P = .97$	$P < .001$	$P < .001$	—	$P = .09$
Hypocalcified Enamel $\rho = 0.08 \pm 0.06$	$P < .05$	$P = .84$	$P < .001$	$P = .09$	—

WSLs. Further image analysis is required to nonsubjectively distinguish sound enamel from stained and hypocalcified enamel.

Statistical analysis revealed that mean Raman depolarization ratios were statistically significant in all cases at $P < .05$ except for three cases: (a) sound enamel compared to hypocalcified enamel, (b) carious enamel compared to stained enamel, and (c) stained enamel from hypocalcified enamel. Since hypocalcified enamel can be mistaken for WSLs upon visual clinical examination, it is reassuring that the Raman depolarization values from hypocalcified enamel are distinct from caries, thereby increasing the specificity of the method. Based on the analysis focusing on the depolarization ratio of the 959 cm^{-1} peak, hypocalcified enamel could not be distinguished from sound enamel. This observation indicates that, fundamentally, hypocalcified

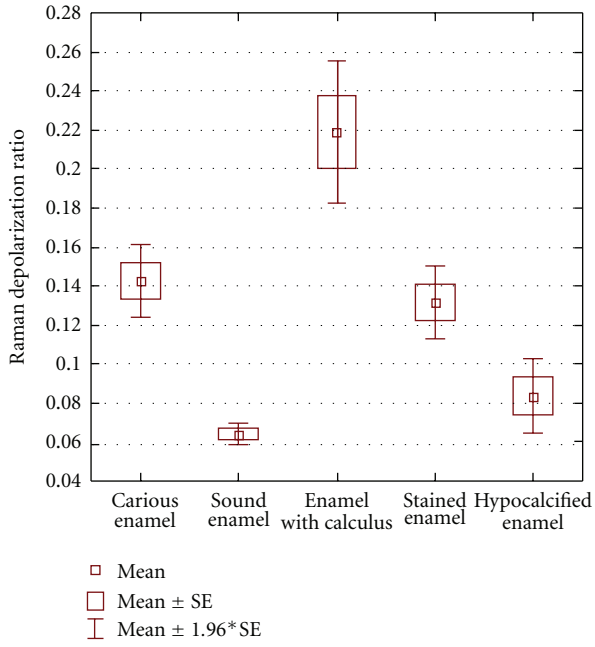

Figure 5: Box- and whisker- plot of the Raman depolarization ratios (mean, mean with standard error [SE], and mean with 1.96*SE) calculated from Raman spectra of regions of caries, sound enamel, enamel with calculus, stained enamel, and hypocalcified enamel.

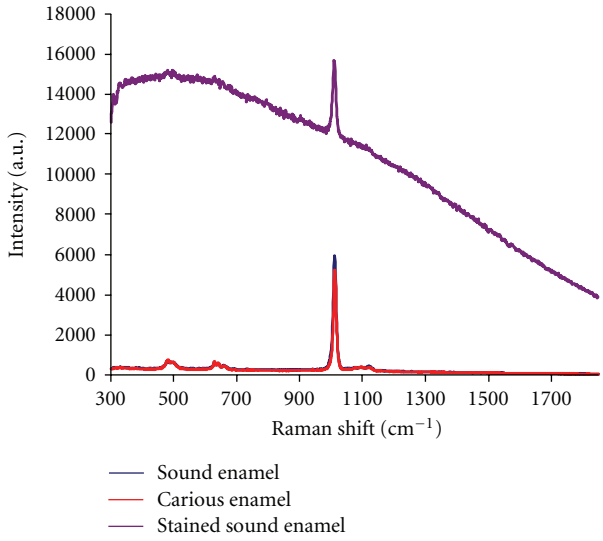

Figure 6: Representative parallel-polarized Raman spectra of unstained sound enamel, carious enamel, and stained sound enamel (a.u. = arbitrary units).

enamel is like healthy sound enamel and not in need of treatment. In order to make this separation, further studies are needed which include examining the peak positions and peak width of the phosphate hydroxyapatite peak, which have been shown to be affected by the mineral crystallinity [22]. Furthermore, other peaks could be surveyed to look for peaks characteristic of specialized forms of hypocalcification such as fluorosis.

The analyses indicate that stain complicates the use of PRS for discriminating stained sound enamel from carious enamel. In reviewing the Raman spectra acquired from areas of stain, it is observed that stained sound enamel spectra show a large background fluorescence that is not found in spectra from areas of sound enamel or WSLs without stain. In these preliminary analyses, a straight-line background in the region of the peak was simply subtracted for calculating the areas under the peak. Clearly, this initial approach is not enough as the curved background confounds this calculation. Further studies are consequently required using various algorithms to robustly fit the fluorescence background for elimination [23]. In addition, there are various instrument-based methods proposed to suppress the background fluorescence in Raman spectra [24]. With fluorescence-based devices (DIAGNOdent, QLF), stain chromophores from any source can lead to false-positive readings. With PRS, the fundamental basis of the method is the phosphate moieties specific to the dominant hydroxyapatite component from the mineral matrix. This peak itself is not due to staining and provides information on mineralization states as required for a method to detect demineralization in caries development. Therefore, with improved methods for fluorescence background subtraction/suppression, it is anticipated that staining will no longer confound PRS analyses.

The statistical analyses also indicated that, based on the Raman depolarization ratio, stained sound enamel cannot be distinguished from hypocalcified enamel. This is also not surprising since both groups are overall noncarious intact enamel with one group containing extrinsic staining. The underlying biochemistry of the enamel matrix is largely similar in both cases as revealed by the Raman spectra. Subsequent analyses such as those described above for examining spectra of hypocalcified enamel could provide insights for discriminating these two groups.

The presence of calculus leads to high Raman depolarization ratios. This result suggests that, like regions of demineralization, the apatite in areas of calculus has a disordered crystal structure and orientation as shown by higher depolarization ratio values. Calculus is easily observed on the OCT image, and therefore OCT will be the first technology used with the fibre optic probe to screen for WSLs and to determine whether PRS analysis of the lesion is necessary. If calculus is detected on the OCT image, the area will be scaled before the PRS method is applied to determine the Raman depolarization ratio. By combining OCT and PRS technologies, it is possible to rule out false-positive readings that might occur from using Raman depolarization ratios alone. Furthermore, according to the box- and whisker- plot (Figure 4), setting a depolarization ratio threshold of ~0.18 can help discriminate carious enamel from enamel with a calculus deposit.

It is important to note that this study was limited to extracted human teeth. In the oral environment, there will be a combination of many possible confounding factors present, and an additive effect may result in the oral cavity that was

not observed when testing the possible confounding factors separately. These issues will be addressed in our subsequent studies as we transition to using fibre-optic-based devices for in vivo measurements with patient volunteers.

In conclusion, calculus and hypocalcification are not major confounding factors affecting WSL detection using OCT and PRS. Stain does not influence WSL detection with OCT. With improved analysis methods, the current limitations with PRS analysis in the presence of stain will be overcome thus allowing better discrimination between carious enamel and stained enamel. The combination of OCT and PRS technologies can decrease the risk of false-positive reading and increase the potential for the detection of WSLs with high sensitivity and specificity. This initial study has pointed out limitations that should be taken into consideration when using these methods and highlighted further analyses that need to be undertaken to better understand the effects of calculus, hypocalcification, and stain on OCT and PRS technologies.

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