Biological factors in dental caries: role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3)

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Dental caries is a complex disease process that afflicts a large proportion of the world, regardless of gender, age and ethnicity, although it does tend to affect more with a low socioeconomic status to a greater extent. Remineralization may be enhanced by providing low levels of calcium and phosphate, in conjunction with minimal amounts of fluoride. It is truly remarkable the difference that a very small amount of fluoride (<1ppm) has upon demineralization and remineralization. This is because fluoride acts as a catalyst and influences reaction rates with dissolution and transformation of various calcium phosphate mineral phases within tooth structure and resident within plaque adjacent to tooth surfaces. The incorporation of minimal amounts of fluoride into HAP yields FHAP that resists demineralization to similar level as FAP. New and emerging methods have been and are in the process of being developed. These hold great promise for preventing and reversing caries, especially in the one-fifth of the population that accounts for two-thirds of the caries experience. Still, the mainstay in caries prevention and remineralization is frequent exposure to low levels of fluoride. This may be accomplished with fluoridated toothpastes, supplemented with fluoride mouthrinses, CPP-ACP containing chewing gum and application of fluoride varnishes. The role of systemic fluorides appears to be limited and primarily has a topical effect.
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INTRODUCTION

Several decades ago, it was noted that clinically detectable white spot lesions could "involute" or remineralize over time (Table 1).[1,2] In fact, white spot lesions followed for 6 to 7 years either became arrested or reverted to sound enamel in 75% of cases. Only one-quarter progressed onto cavitation. This implied that remineralization may occur *in vivo* with clinically detectable white spot lesions. Intervening episodes of demineralization and remineralization are further suspected when it is realized that it requires up to 7 years for a white spot lesion to progress through the entire enamel thickness to the dentinoenamel junction. This length of time would indicate that there is ample opportunity for intervention to occur to facilitate remineralization. The incorporation of more acid-resistant mineral phases has also been noted with comparison of *in vitro* cariogenic challenges of white spot lesions and adjacent sound enamel.[3,4] Both mineral loss and increases in lesion depth are markedly less with white spot lesions when compared with adjacent sound enamel. When compared with artificial enamel lesions, natural white spot lesions have a greater ability to remineralize based upon decreases in lesion depth and gain in mineral content (Table 1).

REMINERALIZATION OF CARIES

The ongoing remineralization process, as well as post-eruption maturation, is exemplified by the increase in fluoride content in the superficial aspect of enamel caries lesions.[5-9] It has been noted that when compared with adjacent sound enamel, the surface zone and superficial body of the lesion contain a significantly higher concentration of fluoride. Based upon the prior information presented, it is evident that during periods of demineralization and subsequent remineralization, enamel lesions preferentially adsorb fluoride ions onto the partially

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Table 1. Natural History of White Spot Lesions

Clinically Detectable White Spot Carious Lesions after 6 to 7 Years Follow-up		
Cavity Formation	25%	
Arrested Lesion	43%	
Remineralized Lesion (Sound Surface)	32%	
Clinically Detectable Lesions: Mean Progression Time to Dentinoenamel Junction (DEJ)		
	50% of Enamel Thickness	**Lesion at DEJ**
US adolescents	16 months	43 months
Swedish adolescents	38 months	85 months
Resistance of Natural White Spot Lesions to *In Vivo* Cariogenic Challenge		
	Lesion Depth Increase	**Mineral Loss**
Natural White Spot Lesions	39um	1,482 vol%um
Adjacent Sound Enamel	110um	2,688 vol%um
Remineralization of Natural White Spot Lesions *In Vitro*		
	Lesion Depth Decrease	**Mineral Gain**
Natural White Spot Lesions		
2 Weeks Remineralization	19um	2,898 vol%um
4 Weeks Remineralization	21um	5,867 vol%um
Artificial Enamel Lesions		
2 Weeks Remineralization	1um	1,317 vol%um
4 Weeks Remineralization	7um	2,496 vol%um

Compiled from references: 1-4,72

demineralized HAP crystals or redeposit FHAP during periods of remineralization.[5,6,7,10-12] Redistribution of fluoride released from the advancing front with deposition of fluoride-containing mineral phases within the surface zone and superficial body of the lesion may also occur. These result in an increased resistance to further acid attacks along the enamel surface and a reduction in the critical pH required for mineral dissolution. In addition, enamel caries consistently has a higher uptake of fluoride from exogenous topical fluoride sources, when compared with sound enamel (Table 2).[5,6,9] From relatively low concentrations of topical fluoride agents, a 20 to 93% increase in fluoride uptake occurs with enamel caries when compared with sound enamel. Daily exposure to synthetic saliva and fluoride markedly reduces lesion formation and progression rates when compared with controls (Table 2, Figure 1).[13] The combination of fluoride and synthetic saliva rinsing provides an additional reduction in lesion depth.

Since the mid-1970's, the dental profession has become interested in the concept of remineralization.[3-9,13-33] The term remineralization refers to replacement of mineral lost during the demineralization process. The terms repair and healing of lesions has also been invoked. The process of remineralization is a natural component of the dynamic caries process. There are intermittent periods of demineralization via acid produced by cariogenic bacteria during carbohydrate fermentation. The periods during which there is a return to the resting plaque pH is when remineralization may occur. This involves transforming soluble calcium, phosphate and fluoride and less caries-resistant mineral phases into HAP and/or FHAP. The repair or healing of the lesion may occur by deposition of mineral on existing damaged crystals (crystal growth) or nucleation and *de novo* crystal formation. The crystals may have a similar size as that of the original crystal or be markedly larger. Loss of organic matrix with more closely apposed crystals may improve caries resistance by decreasing the permeability of enamel to acid influx.

Remineralization may be as simple as the immediate repair of recently acid-damaged enamel and occur on an "as-needed" basis with no clinical evidence of a lesion. Conversely, the repair process may require prolonged mineral deposition in order to reverse a clinically detectable white spot lesion. It is important to realize that remineralization is an ongoing process in each oral cavity, and that the degree of remineralization and whether a lesion will progress to be become detected clinically or even cavitate is dependent upon biological factors in the plaque and saliva, composition of enamel, oral hygiene, dietary habits and exposure to preventive agents.

Over the past several decades, remineralizing or calcifying fluids with variable formulations of calcium, phosphate and fluoride have been developed and tested in laboratory and *in situ* situations, using both artificial enamel caries and naturally occurring caries (Figure 1).[3-9,13,17-33] Along with the creation of remineralizing fluids, various methods have been instituted to assess remineralization of carious lesions. These include: 1) polarized light microscopy; 2) microhardness of the tooth surface and the lesion surface; 3) microradiography; 4) mineral analysis of calcium, phosphate and fluoride phases incorporated into the lesion; 5) confocal laser microscopy; 6) microprobe and electron diffraction analysis; and 7) transmission and scanning electron microscopy. The most commonly used methods for assessing remineralization of carious lesions are polarized light microscopy and microradiography.

Table 2. Fluoride Uptake and Remineralization *In Vitro* of Enamel Caries

Fluoride Uptake by Sound Enamel and Enamel Caries				
	Control	**0.2% NaF**	**0.4% SnF**	**0.123% APF**
Sound Enamel	946 ppm	1,323 ppm	1,494 ppm	2,728 ppm
Enamel Caries	1,405 ppm	2,416 ppm	2,940 ppm	3,293 ppm
Fluoride Rinse (0.2% NaF) and Enamel Caries Formation Rate				
	Lesion Initiation Period		**Lesion Progression Period**	
Control	31 um/day		41 um/day	
Synthetic Saliva	20 um/day		20 um/day	
Fluoride/Synthetic Saliva	15 um/day		17 um/day	
Oral Fluid (whole saliva, ten 60 minute treatments)				
	Lesion Area Decrease			
Oral Fluid	8%			
Oral Fluid with Fluoride (1ppm)	11%			
Calcifying Fluids (ten 6 minute treatments)				
	Lesions Area Decrease			
1mM Calcium	22%			
1mM Calcium with Fluoride (1ppm)	72%			
3mM Calcium	9%			
3mM Calcium with Fluoride (1ppm)	24%			
Enamel Crystal Diameters				
Sound Enamel	**35-40nm Surface Zone**	**Body of Lesion**	**Dark Zone**	**Translucent Zone**
Enamel Caries	40-80nm	10-30nm	50-100nm	25-30nm
1mM Calcifying Fluid	80-120nm	50-100nm	100-150nm	25-30nm
3mM Calcifying Fluid	50-75nm	50-75nm	50-100nm	25-30nm
Acid-Etching of Lesion Surface Prior to Calcifying Fluids (ten 1 minute treatments)				
	Lesion Depth Decrease			
1mM Calcium	(1ppm fluoride)		34%	
3mM Calcium	(1ppm fluoride)		24%	
Calcifying Fluids (ten 1 minute treatments)				
		Lesion Depth Decrease		
	Non-Acidulated (pH 7.0)		**Acidulated (pH 5.0)**	
1mM Calcium (1ppm fluoride)	18%		29%	
3mM Calcium (1ppm fluoride)	10%		22%	
Calcium Fluoride Formation (pmol/mm2) on Saliva-Coated Enamel Surfaces				
	Water	**0.2% NaF**	**2% NaF**	**APF**
With Saliva Film	<20	75	390	3,931
Without Saliva Film	<17	52	114	942
Demineralization/Remineralization Cycling and Effect of Bound Fluoride				
Treatment	**Bound Fluoride (outer 100um)**		**Mineral Loss**	**Lesion Depth**
Control	87 ppm		91 um	165 um
APF	180 ppm		41 um	102 um
DCPD-APF	423 ppm		21 um	76 um
Shark Enamel	30,000 ppm		4 um	24 um
Casein Phosphopeptide Amorphous Calcium Fluoride (CPP-ACP)				
Treatment	**Increase in Mineral Deposition**			
1.0% CPP-ACP Solution	64%			
0.1% CPP-ACP Solution	44%			
Chewing Gum with CPP-ACP	46%			
Dose Effect of CPP-ACP in Chewing Gum				
0.19 mg CPP-ACP	9%			
10.0 mg CPP-ACP	63%			
18.8 mg CPP-ACP	102%			
56.4 mg CPP-ACP	152%			

Compiled from references: 5,6,9,13,19-28,31,34,39-42,63

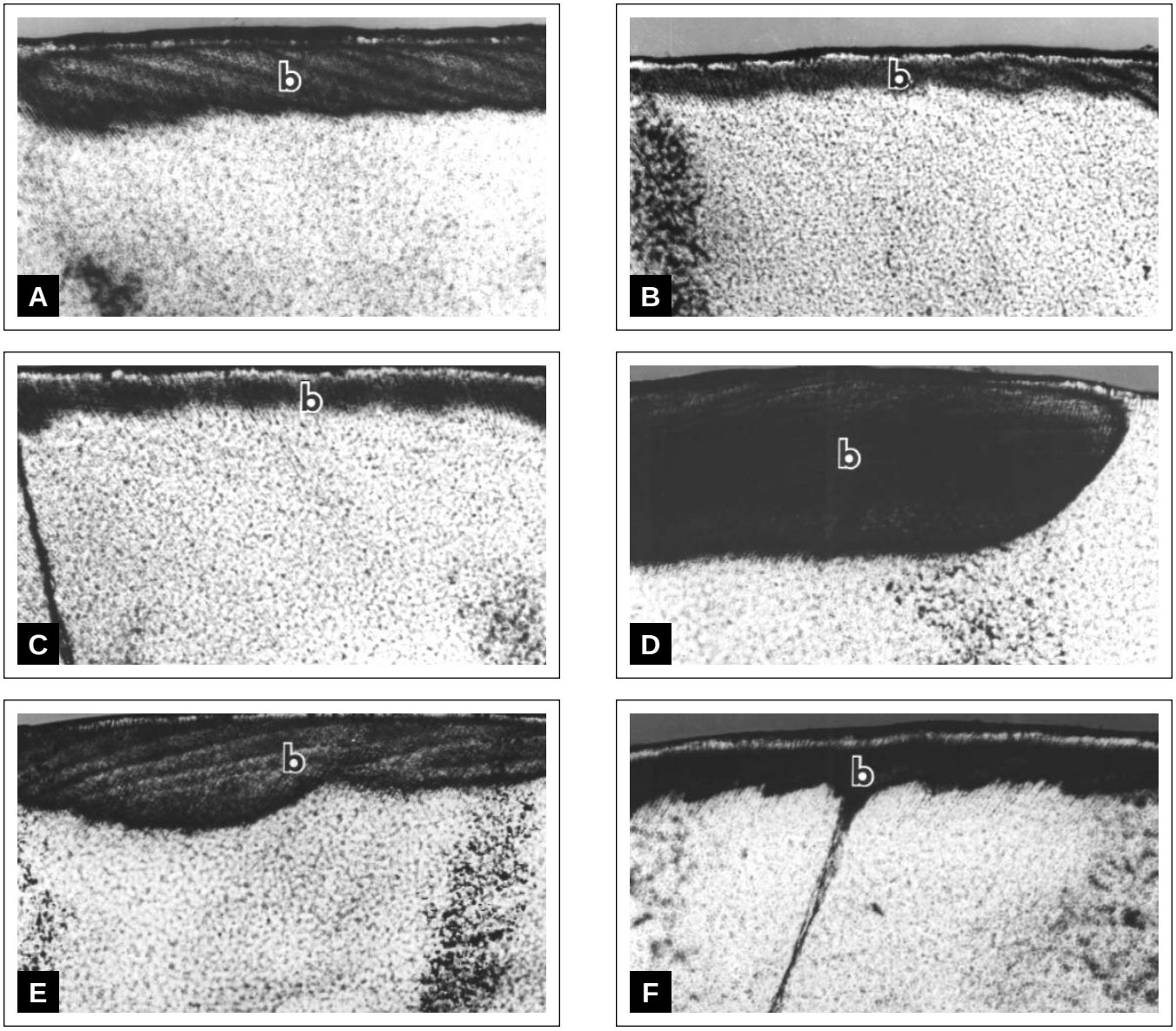

Figure 1. Daily exposure to low concentration sodium fluoride rinse and synthetic saliva affects caries-like lesion formation **(A-C)** and lesion progression **(D-F)** in enamel. (Lesion formation period: **A** = control, **B** = daily synthetic saliva rinse, **C** = sodium fluoride rinse and synthetic saliva rinse; Lesion progression: **D** = control, **E** = daily synthetic saliva rinse, **F** = sodium fluoride rinse and synthetic saliva rinse; b = body of lesion; polarized light microscopy, water imbibition; reprinted with permission: Hicks MJ, Flaitz CM, Silverstone LM: Initiation and progression of caries-like lesions of enamel: effect of periodic treatment with synthetic saliva and sodium fluoride. Caries Res 19: 481-9, 1985).

Qualitative polarized light microscopy allows for evaluation of the zones of demineralization (body of the lesion and translucent zone) and remineralization (surface zone and dark zone) with respect to area occupied, width and depth. With quantitative polarized light microscopy, pore volume changes associated with demineralization and remineralization within the various zones of caries can be determined. Microradiography allows for determination of lesion depth and area, and the volume of mineral loss, as well as a combination of these factors.

Remineralization may occur with oral fluids (whole saliva) and is facilitated by the addition of a small amount of fluoride (Table 2, Figures 1, 2); however the degree of remineralization is limited.[16,22-24] This may be due to the presence of organic substances that attach to the enamel surface and fill the underlying pores in the carious lesion, effectively blocking entry of calcium and phosphate ions. The time for treatment of these lesions to produce significant remineralization is considerable, but these laboratory studies indicate that whole human saliva has remineralizing abilities. More importantly with constant bathing of the carious lesion by saliva following periodic plaque removal, reversal of a lesion by endogenous saliva is possible.

The next important finding in remineralization was the effect of the concentration of calcium ions on the remineralization process (Table 2, Figure 2).[5,6,16,22,26,27]

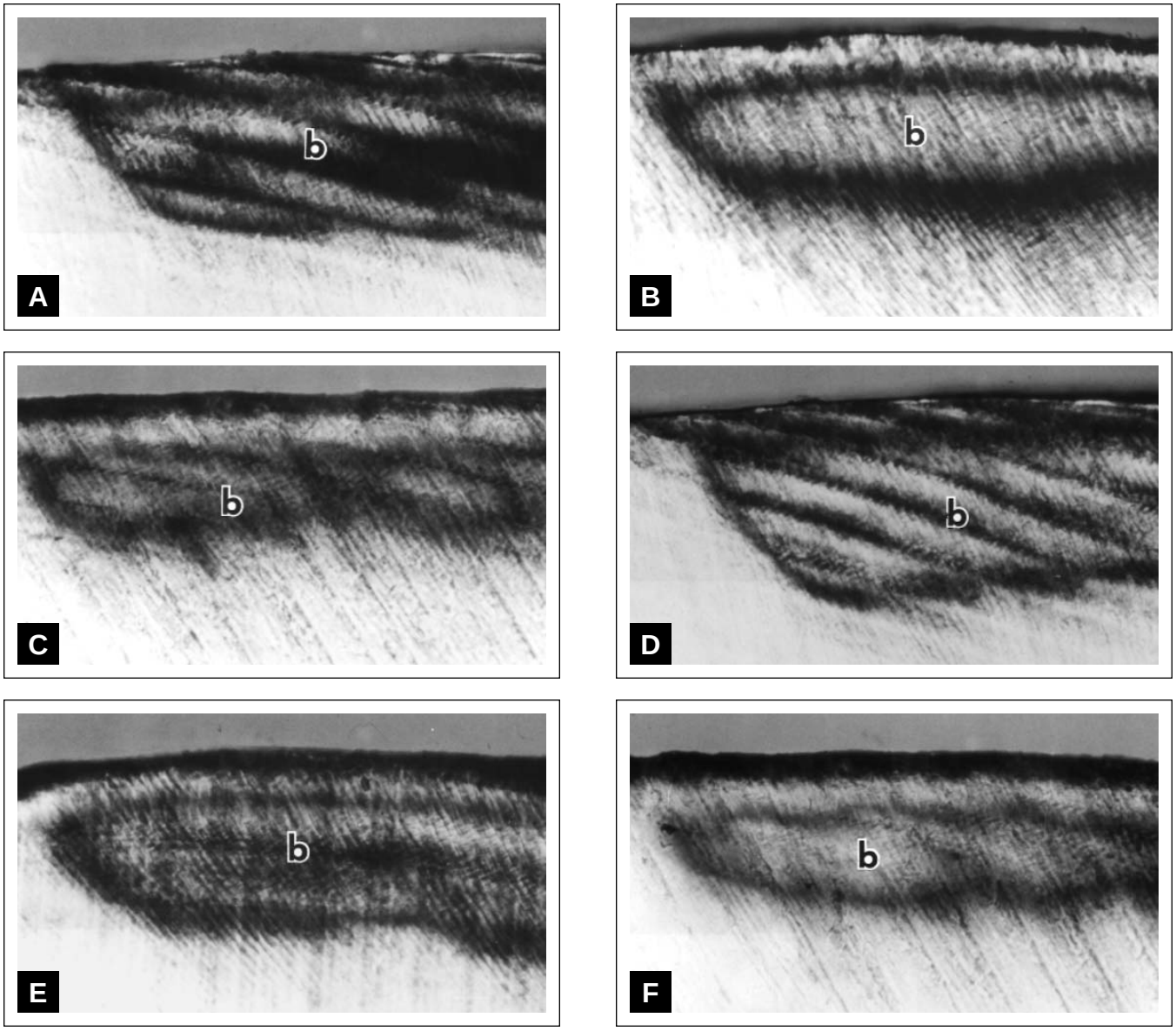

Figure 2. Remineralization of caries-like lesions of ename (b= body of lesion)l may be accomplished with relatively short repeated exposures to calcium, phosphate and fluoride containing calcifying fluids (60 second calcifying fluid rinses, 10 exposures, 30 minute synthetic saliva rinses). Acidulation of the calcifying fluids facilitates the remineralization process. (1mM calcium calcifying fluid remineralization: **A)** control lesion, **B)** 1mM calcium calcifying fluid at pH 7.0, **C)** 1mM calcium calcifying fluid at pH 5.0. 3mM calcium calcifying fluid remineralization: **D)** control lesion, **E)** 3mM calcium calcifying fluid at pH 7.0, **F)** 3mM calcium calcifying fluid at pH 5.0. Polarized light microscopy, water imbibition; Reprinted with permission: Flaitz CM, Hicks MJ: Remineralization of caries-like lesions of enamel with acidulated calcifying fluids: a polarized light microscopic study. Pediatr Dent 18: 205-9, 1996).

Relatively low concentrations of calcium have considerably different effects, although both remineralizing fluids have an identical calcium to phosphate ratio (1.67). It was noted that a 1mM calcium calcifying fluid resulted in a 2.3 fold increase in remineralization, as shown by reduction in lesion area, when compared with a 3mM calcium calcifying fluid. The reason for this is that the 3mM calcium remineralizing solution was supersaturated with all mineral phases (DCPD, OCP, HAP, TCMP) while the 1mM calcium fluid was not (supersaturated only with HAP). This resulted in relatively rapid precipitation of mineral phases within the superficial aspect of the lesion with the 3mM calcium calcifying fluid, while the 1mM calcium calcifying fluid penetrated the entire depth of the lesion. Qualitatively, the higher calcium remineralizing solution produced an increase in the width of the surface zone at the expense of the underlying body of the lesion, while decreasing lesion area modestly. In contrast, the 1mM calcium calcifying fluid penetrated the entire lesion and resulted in an average reduction of almost 25% in the lesion area. Dark zone and surface zone areas were increased, while there were reductions in the body of the lesion and the translucent zone areas. Determination of the supersaturation of these calcifying fluids predicted that the 1mM calcium fluid would favor crystal growth on already existing damaged

Table 3. Fluoride in Saliva and Plaque

Whole Saliva			
Non-Fluoridated Areas	0.007 ppm		
Fluoridated Areas	0.007 to 0.009 ppm		
Fluoridated Dentifrice Usage			
Immediately after brushing	60 to 250 ppm		
3 Minutes after brushing	3 to 11 ppm		
30 Minutes after brushing	0.03 to 0.1 ppm		
60 Minutes after brushing	0.007 to 0.009 ppm		
Plaque pH and Water Fluoridation			
	DMFS	**No Water Fluoridation**	**Water Fluoridation**
	<2	4.8	5.0
	2-8	4.6	4.8
	>8	4.6	4.6
Plaque Fluoride and Caries with Variable Fluoride Content in Dentifrice			
Fluoride Content	**DMFS**	**Plaque Fluoride**	**Salivary Fluoride**
1,000 ppm	6.54	1.91 ppm	1.48 ppm
1,500 ppm	5.95	2.21 ppm	1.88 ppm
2,500 ppm	5.11	2.54 ppm	3.03 ppm

Compiled from references: 29,58

crystals; whereas, 3mM calcium fluid would support crystal nucleation and new crystal formation.

In an elaborate scanning electron microscopic examination of the effects of the caries process and remineralization, the prediction regarding differences in HAP/FHAP crystal formation between 1 and 3 mM calcium calcifying fluids was proven (Table 2).[20,22,24,26,27] With sound enamel, the crystals had an average cross-sectional diameter of 35 to 40nm, quite similar to that previously described for HAP (25 to 40nm). During the caries process, enamel crystal diameters were decreased substantially within the zones of demineralization (translucent zone and body of the lesion); whereas, the diameters were increased substantially within the zones of remineralization (dark and surface zones), indicative of reprecipitation of mineral phases transported from the advancing front. With the 1mM calcium calcifying fluid, enamel crystal diameters were markedly increased in all zones, in agreement with the prior conjecture that remineralization occurs via crystal growth on existing crystals damaged during demineralization. In contrast, the crystals in lesions exposed to the 3mM calcium calcifying fluid possessed sizes quite similar to those within 3 of the 4 zones of untreated enamel caries (surface, dark and translucent zones). The major difference was the increase in crystal diameters within the body of the lesion following exposure to the 3mM calcium remineralizing solution. These findings tend to support the prediction, based upon supersaturation data, that the 3mM calcium fluid would results in crystal nucleation and *de novo* crystal formation.

The addition of fluoride to the calcifying fluids markedly affects the degree and extent of remineralization.[22-24,26,27] Using the same 1mM and 3mM calcium solutions, the addition of a minimal quantity of fluoride (1 ppm) resulted in an almost 3-fold reduction in lesion area with the 3mM calcium calcifying fluid (Table 2). An even more substantial reduction in lesion area was achieved with addition of fluoride to the 1mM calcium remineralizing solution (3.3-fold). Without fluoride, there was a 22% reduction in lesion area; while with fluoride in the 1mM calcium fluid, lesion area was reduced by 72%. A similar effect in lesion reduction was found when either 1ppm or 10ppm fluoride was added to the remineralizing fluids. Since that time it has been shown that as little as 0.1ppm fluoride in these solutions results in a significant improvement in remineralization. The low level of fluoride necessary to markedly increase mineral deposits in carious lesions attests to the role as a "catalyst" in the remineralization process and the fact that fluoride-containing HAP (FHAP) and other fluoride-containing mineral phases have a substantial resistance to a cariogenic challenge. Intraoral models have also found that low concentrations of fluoride in demineralizing and remineralizing solutions are more critical than fluoride within the enamel during demineralization and remineralization.[32] This is because fluoride in solution modulates the reaction rates in demineralization (limits) and remineralization (facilitates).

Although remineralization of enamel caries may be accomplished with low concentration calcium fluids containing minimal amounts of fluoride, the exposure time is considerable. The use of acid-etching of the lesion surface prior to exposure to calcifying fluids or acidulation of the calcifying fluids (Figure 2) has allowed for a reduction in the time necessary to induce remineralization (Table 2).[25,28] Acid-etching of the enamel surface with phosphoric acid only once prior to exposure to a series of 1 minute treatments with 1mM and 3mM calcium calcifying solutions containing 1ppm fluoride produced reductions of 34% and 24% in lesion depth, respectively.[28] Acidulation (pH 5.0) of these calcifying fluids (Figure 2) yielded similar decreases

Table 4. Current and Future Strategies for Caries Prevention and Remineralization

Systemic Fluoride
Optimal Water Fluoridation
Salt, Milk and Sugar Fluoridation
Fluoride Supplements
Topical Fluoride
Fluoride Rinses (prescription and over the counter)
Fluoridated Toothpastes
Topical Fluoride Gels and Solutions (professional application and prescription)
Fluoride Varnishes
Slow Release Fluoride Devices
Fluoride-Releasing Preventive and Restorative Materials and Cements
Antimicrobials
Chlorhexidine Rinses, Gels and Varnishes
Short-Term Antimicrobial Therapy with Oral Antibiotics
Triclosan-PVMA Containing Agents (rinses, dentifrices)
Iodine containing rinses
Anti-Plaque Agents
Surfactant/Detergent Rinses (sodium dodecyl sulphate)
Quaternary Ammonium Compound Rinses (cetylpyridinium chloride)
Essential oils (thymol or menthol) rinses
Plant extract rinses (sanguinarine)
Metal Salts (zinc or copper) rinses
Amorphous Calcium Phosphate-Casein Phosphopeptide Chewing Gum
Vaccination Against Cariogenic Bacteria
Active Immunization
Synthetic mutans streptococci peptides
Mutan streptococci antigens coupled to cholera toxin subunits
Mutan streptococci genes fused to avirulent salmonella
Liposome-coated delivery systems
Passive Immunization
Topically applied monoclonal antibodies
Immunoglobulin delivery via milk and whey
Egg yolk antibody
Transgenic plant antibody
Novel Plaque Inhibition
Inhibition of Glucosyltransferase
Competitive inhibitors
Anti-Glucosyltransferase antibodies
Plant and fungal products
Interference with Adhesion and Co-Aggregation
Soluble receptor analogues
Soluble adhesins
Improved Antimicrobials
Combination antibiotics
Slow-release devices

Compiled from references: 10,14,15,46,59-68

in lesion depth with a 29% and 22% reduction for 1mM calcium and 3mM calcium fluids, respectively.[25] The use of acid-etching or acidulation may create a more reactive surface that facilitates interaction with the mineral phases in the remineralizing solution and those within the underlying carious lesions.

As indicated previously with dissolution of dental hydroxyapatite in the presence of high fluoride concentrations, such as with a professionally applied topical fluoride gel or foam, calcium fluoride is formed.[31,34-36] With saliva-coated enamel (pellicle), the amount of calcium fluoride detected on an enamel surface after topical fluoride treatment is substantially increased (Table 2).[29] As expected, acidulated phosphate fluoride treatment deposits the greatest quantity of calcium fluoride on the saliva-coated enamel. Calcium fluoride during an acidic attack will be exposed to acid phosphate or phosphate ions. This may result in calcium fluoride

Table 5. High Risk Factors for Caries Development

	Low Risk	Intermediate Risk	High Risk
Salivary Factors			
Flow rate	>1mL/minute	0.7 to 1 mL/minute	<0.7mL/minute
Buffer Capacity	pH 5-7	pH 4-5	pH <4
Mutans Streptococci	<105 cfu/mL	105-106 cfu/mL	>106 cfu/mL
Lactobacilli	<104 cfu/mL	104-105 cfu/mL	>105 cfu/mL
High Acidity (low pH)			
Low Lysozyme, Lactoferrin or Lactoperoxidase Levels			
Low Arginine, Histidine-Rich Protein and Urea Levels			
High Viscosity			
Low Fluoride, Calcium and Phosphate Levels			
Low Immunoglobulin Levels			
Dental Plaque			
Acidogenic Bacteria (Mutans streptococci and Lactobacilli) with High Acid Production			
Increased Intracellular and Extracellular Polysaccharide Formation			
High Plaque and Gingival Indices			
Tooth Physical Characteristics			
Immature Enamel lacking Post-Eruption Maturation and Exposure to Fluoride			
Surface Enamel with Low Fluoride or High Carbonate, Magnesium and Organic Content			
Increased Acid Solubility			
Malocclusion with Plaque Retentive Areas			
Deep Pit and Fissure Morphology			
Malformed Teeth with Retentive Areas			
Enamel Defects (hypoplasia and/or hypocalcification)			
Dietary Habits			
Frequent High Sucrose Food Ingestion			
Adherent High Sucrose Food Ingestion			
Chronic Medication Intake with High Sucrose/Sweetener Content			
Trace Elements in Diet: High Selenium and Low Fluoride, Strontium, Molybdenum, Aluminum, Lithium and Boron			
Previous Caries Experience			
High Caries Experience in Primary Dentition and/or Permanent			
History of Early Childhood Caries			
Smooth Surface Caries, Especially with Anterior Teeth			
Family History of High Caries Rate			
Systemic Disease			
Xerostomia: Autoimmune Disease (Systemic Lupus Erythematosus, Sjogren's Syndrome, Diabetes Mellitus, Head and Neck Radiation, HIV Infection)			
High Carbohydrate Dietary Requirements (Cystic Fibrosis, Phenylketonuria)			
Chronic Illness Requiring Medications with High Sucrose Content			
Xerostomic-Inducing Medications			
Socioeconomic Factors			
Low Income with Limited Access to Dental Care Provider			
Young Parents with Low Education Level			
Behavior and Attitudes toward Health Care			

Compiled from references: 10,46,58-64,68,71-73,75-77

being hydrolyzed to FHAP.[10-12,31,34-38] In addition, calcium fluoride can act as a reservoir for both calcium and fluoride ions, and release these ions during acidic challenges. This will inhibit further HAP dissolution, and encourage FHAP formation. Although calcium fluoride is not bound to enamel, it tends to remain on the tooth surface for several weeks, especially in retentive niches, and continues to release calcium and fluoride during periods of demineralization.

Fluoride that is firmly bound to enamel represents an important factor in reducing demineralization and facilitating remineralization (Table 2).[31] Bound fluoride is higher with typical APF treatments and is significantly increased with a combined DCPD-APF exposure. Bound fluoride from APF reduces mineral loss (2.2-fold) and lesion depth (1.6-fold); however, the addition of DCPD to the APF treatment creates even greater reductions in mineral loss (4.5-fold) and lesion depth (2.2-fold).

An interesting development in the delivery of calcium and phosphate ions to the oral cavity has occurred over the past few years.[39-42] An amorphous form of calcium phosphate (ACP) stabilized by a phosphopeptide from the milk protein casein (CPP – casein phosphopeptide) has emerged and is available for delivery in chewing gum as a caries preventive and remineralizing agent (CPP-ACP). The casein phosphopeptides are multiphosphorylated peptides

derived from enzymatic digestion of casein from cow's milk. Via phosphoseryl residues, CPP stabilizes calcium and phosphate ions in solution as an amorphous calcium phosphate form (ACP). CPPs can bind up to 25 calcium ions, 15 phosphate ions and 5 fluoride ions per molecule. CPP-ACP has been shown to localize to tooth surfaces, and during acidogenic challenges may release calcium, phosphate and fluoride, and maintain the supersaturation of calcium and phosphate ions in the vicinity of the tooth surface. In addition, CPP-APP has a remineralizing effect on enamel caries. Using solutions containing small concentrations of CPP-ACP (Table 2), it is possible to increase mineral deposition by 44 (0.1% CPP-ACP) to 64% (1.0% CPP-ACP). In the concentration provided with commercially available chewing gum, mineral deposition increased by 46%. There appears to be a beneficial dose effect on remineralization with increased amounts of CPP-ACP (Table 2). Analysis by electron diffraction studies has show that the mineral deposits are HAP. The remineralization identified with CPP-ACP is quite encouraging because a simple pleasure, such as chewing sugar-free gum, may lead to a reduction in caries and remineralization of existing carious lesions. In addition to remineralizing effects, CPP-ACP has been shown to suppress adhesion of mutans streptococci to enamel and alter plaque microflora.

Finally, caries on tooth surfaces apposing teeth restored with fluoride-releasing materials may undergo remineralization.[43-45] Artificial enamel lesions adjacent to glass ionomer restorations[45] had a 2.4 fold increase in the area of the lesion remineralized, compared with lesions that had been exposed only to synthetic saliva. The degree of remineralization was similar to that for a fluoridated dentifrice (2.2-fold increase), but less than that for twice daily 0.05% sodium fluoride rinsing (3.7-fold increase)

ROLE OF FLUORIDE IN THE CARIOUS PROCESS

Although systemic fluoride in the form of water fluoridation has been touted in the past for the decline in dental caries, it has been realized that the primary reduction in dental caries is because of the topical effect of water fluoridation and the availability of fluoridated toothpastes.[10,14,15,46-58] In several European countries without water fluoridation, a similar level of caries reduction was found following introduction of fluoridated toothpastes. As the proportion of the population utilizing such fluoride-containing toothpastes increased, the caries experience incrementally declined to levels similar to that noted in the United States during the late 1980's and early 1990's. Water fluoridation is still of importance because it provides a readily available means to:[10,14,15,46-49,51-59]

1. Deliver the topical and systemic effects of fluoride on several occasions during the day;
2. Increase the level of fluoride in developing primary and permanent teeth;
3. Increase fluoride deposition during secondary and reparative dentin formation;
4. Increase the level of fluoride in root surfaces exposed due to periodontal disease in older age populations;
5. Increase fluoride concentration of plaque, saliva and tooth mineral (Table 3);

Topical fluoride mechanisms in reducing demineralization and enhancing remineralization include:[10,14,15,46,48,50-56,58]

1. Antimicrobial effect via inhibition of bacterial metabolic pathways following diffusion of hydrogen fluoride into bacteria;
2. Desorption of bacteria from HAP and reduction in bacterial adherence to HAP;
3. Elevation of plaque pH indirectly and limiting the action of mutans streptococci and lactobacilli;
4. Reduction in caries susceptibility of recently erupted teeth and root surfaces, via post-eruption maturation;
5. Inhibition of demineralization of the HAP crystals of enamel and root surfaces;
6. Enhance the remineralization process by acting as a "catalyst" and influencing the reaction rate in mineral phase formation and transformation;
7. Exchange fluorine ions for hydroxyl ions in HAP and allow for lower critical pH for tooth mineral containing FHAP;
8. Recharge fluoride-releasing preventive and restorative materials.

CURRENT AND FUTURE STRATEGIES FOR CARIES PREVENTION AND REMINERALIZATION

The mainstay of caries prevention and remineralization revolves around the delivery of fluoride, either in a systemic or topical form (Table 4).[10,14,15,45-58,60,61] As noted previously, systemic fluoride is considered to be important, but has less of an effect on demineralization and remineralization than topical forms of fluoride. In particular, fluoride agents that are available in low dosage, in order to avoid dental fluorosis, delivered over long periods of time and with frequent exposures (fluoridated dentifrices, over the counter fluoride rinses, fluoride varnishes) are most effective. High concentration fluorides with less frequent exposure (professionally applied topical fluorides, prescription high dose rinses, gels and toothpastes) are not be as beneficial in reducing caries experience and remineralization of white spot lesions. High dose fluoride agents are more effective in caries control with patients that have high caries activity and/or rampant caries. When restoration is necessary in patients with high caries activity, fluoride-releasing restorative materials should always be considered.

Antimicrobial agents that affect the composition of the plaque microflora may be of benefit in patients with active or rampant caries (Table 4).[50,59-70] Short-term use of prescription antimicrobials (chlorhexidine, iodine containing rinses, systemic antibiotics) may allow for plaque control and a reduction

in development of new lesions, while arresting and/or remineralizing existing lesions. Reassessment to determine the length of therapy and reinstitution of antimicrobial therapy following adequate control of caries activity is necessary. Several other nonprescription antimicrobials are available for use in reducing the plaque burden and cariogenic bacteria (Table 4). Several fluoridated toothpastes contain triclosan and a copolymer that are effective in reducing plaque and gingivitis, and may be beneficial for caries prevention and remineralization.

The future of caries prevention may be related to novel methods in eliminating cariogenic bacteria from the oral environs, neutralizing the effects and inhibiting plaque formation (Table 4).[68] Methods for instigating active and passive immunization against mutans streptococci exist, and there are several alternative methods to achieve immunity via vaccination. These investigational immunization protocols await further research in animals and humans, and approval by federal agencies. Various novel plaque inhibition methods are also in experimental stages. These involve inhibition of an enzyme system (glucosyltransferase) necessary for acidogenic bacteria to metabolize fermentable carbohydrates, interference with bacterial adhesion and co-aggregation necessary for colonization of plaque, and improved and targeted antimicrobial delivery systems.

HIGH RISK FACTORS FOR CARIES DEVELOPMENT

Many different factors are of importance in the dynamic process of demineralization and remineralization of tooth structures. A summary of factors that are important to consider in the patient at high risk for caries development is presented in Table 5.[10,46,59-64,68,70-76] Caries is not a simple process, but is multifactorial and includes assessment of saliva, dental plaque, tooth physical characteristics, dietary habits, previous caries experience, systemic disease, and socioeconomic factors. The culmination of many components from the oral cavity leads to whether a patient remains caries-free or is at high risk for caries development. Most important overall in prevention of dental caries and remineralizing existing dental caries is educating the patient in the necessity for a plaque-free environment, and providing the patient with the knowledge and means to perform adequate oral hygiene. Caries may be a multifactorial disease; however, dental plaque is the only cause of caries. The old concept that a "clean tooth never decays" still holds true in the 21st century.

SUMMARY

Dental caries is a complex disease process that afflicts a large proportion of the world, regardless of gender, age and ethnicity, although it does tend to affect more with a low socioeconomic status to a greater extent. The process of dental caries is dependent upon biological factors that are present within the saliva and dental plaque. There are many different agents within saliva and plaque that serve to protect the tooth surface against caries development. Salivary flow rate, buffering capacity, antimicrobial activity, microorganism aggregation and clearance from the oral cavity, immune surveillance, and calcium phosphate binding proteins all interact to inhibit or reverse demineralization of exposed tooth surfaces. Cariogenic bacteria levels within the saliva and plaque determine whether caries will occur or not, and the concentration in saliva and plaque are intimately related to the type of carbohydrate ingestion and the frequency of ingestion, as well as the oral hygiene practiced by the individual. The physicochemical properties of the mineral comprising the tooth surface and subsurface modulate the development, arrestment and remineralization of dental caries. Post-eruption maturation of enamel surfaces and exposed root surfaces is important in order for more susceptible mineral phases to be modified by incorporation of soluble fluoride from the plaque into dental hydroxyapatite. The chemical reactions that occur during acidic conditions when tooth mineral dissolves (critical pH) are determined by the supersaturation of calcium and phosphate within plaque and saliva, as well as if fluoride is present. Remineralization may be enhanced by providing low levels of calcium and phosphate, in conjunction with minimal amounts of fluoride. It is truly remarkable the difference that a very small amount of fluoride (<1ppm) has upon demineralization and remineralization. This is because fluoride acts as a catalyst and influences reaction rates with dissolution and transformation of various calcium phosphate mineral phases within tooth structure and resident within plaque adjacent to tooth surfaces. The incorporation of minimal amounts of fluoride into HAP yields FHAP that resists demineralization to similar level as FAP. New and emerging methods have been and are in the process of being developed. These hold great promise for preventing and reversing caries, especially in the one-fifth of the population that accounts for two-thirds of the caries experience. Still, the mainstay in caries prevention and remineralization is frequent exposure to low levels of fluoride. This may be accomplished with fluoridated toothpastes, supplemented with fluoride mouthrinses, CPP-ACP containing chewing gum and application of fluoride varnishes. The role of systemic fluorides appears to be limited and primarily has a topical effect. Modulation of the immune system by active and passive immunization and topical administration of biological plaque inhibitors is in the future. Although there is a considerable proportion of the population at high risk for caries, it is difficult to identify these individuals prospectively. This no doubt is due to the multifactorial and complex nature of dental caries. Additional epidemiologic and scientific evidence-based research is needed to identify these individuals in order to lessen their caries burden. As

stated previously, the old concept "a clean tooth never decays" still holds true in the 21st century and will continue to hold true for centuries to come.

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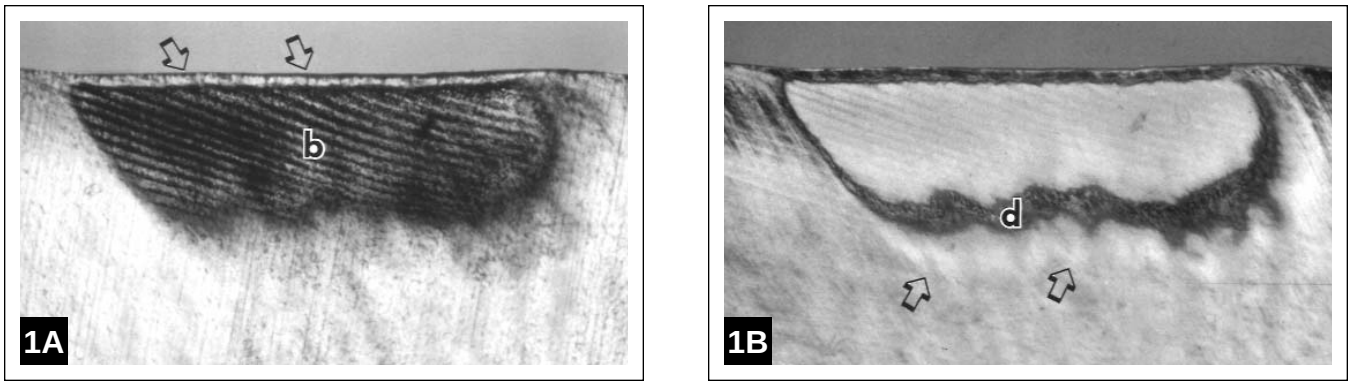
ERRATUM:

Only Figure 1A was published in the article entitled, "Biological factors in dental caries enamel structure and the caries process in the dynamic process of demineralization and remineralization (part 2)" by John Hicks / Franklin Garcia-Godoy / Catherine Flaitz, J Clin Pediatr Dent 28(2): 119, 2004, Figure 1B was not published. The entire legend was published, but it did not indicate the A) subheading or B) subheading.



Figures 1A and 1B. Histopathologic appearance of enamel caries lesion viewed with polarized light microscopy.

A) An intact negatively birefringent surface zone (arrow) is present overlying the positively birefringent body of the lesion (L). The negative birefringence of the intact surface zone (arrow) is due to a pore volume (mineral loss) of less than 5%; whereas, the positive birefringence of the body of the lesion (L) is due to a pore volume (mineral loss) of >5%. The adjacent sound enamel is negatively birefringent and typically has a pore volume of 0.1% as determined by quantitative polarized light microscopic means. (polarized light, water imbibition, refractive index of water = 1.33, refractive index of sound enamel = 1.62).
B) The translucent zone (arrow) represents the advancing front of the enamel caries lesion and is a zone of demineralized enamel that is negatively birefringent when imbibed with quinoline (refractive index of quinoline = 1.62, identical to refractive index of sound enamel = 1.62). The pore volume (mineral loss) of the translucent zone is approximately 1%, approximately 10 times that for the adjacent sound enamel (pore volume of 0.1%). The dark zone 9 (d) is a zone of remineralization that is adjacent to the translucent zone and may have a secondary dark zone adjacent to the overlying surface zone. This zone has a pore volume (mineral loss) of 2 to 4%, in contrast to a minimum pore volume of 5% for the body of the lesion. The positive birefringence of the dark zone, when imbibed with quinoline, is due to a microsieve network of porosities in this zone that preferentially excludes quinoline due to its molecular size and retains air within the smaller porosites (refractive index of air = 1.0; refractive index of quinoline). The differences in the refractive indices of pores filled with air compared with those filled with quinoline allows for detection of the dark zone by polarized light microscopy, when quinoline imbibition is used.

(Figure reprinted with permission: Hicks MJ, Flaitz CM, Silverstone LM: Fluoride uptake in vitro of sound enamel and caries-like lesions of enamel from fluoride solutions of relatively low concentrations. J Pedod 1986;11:47-61.)