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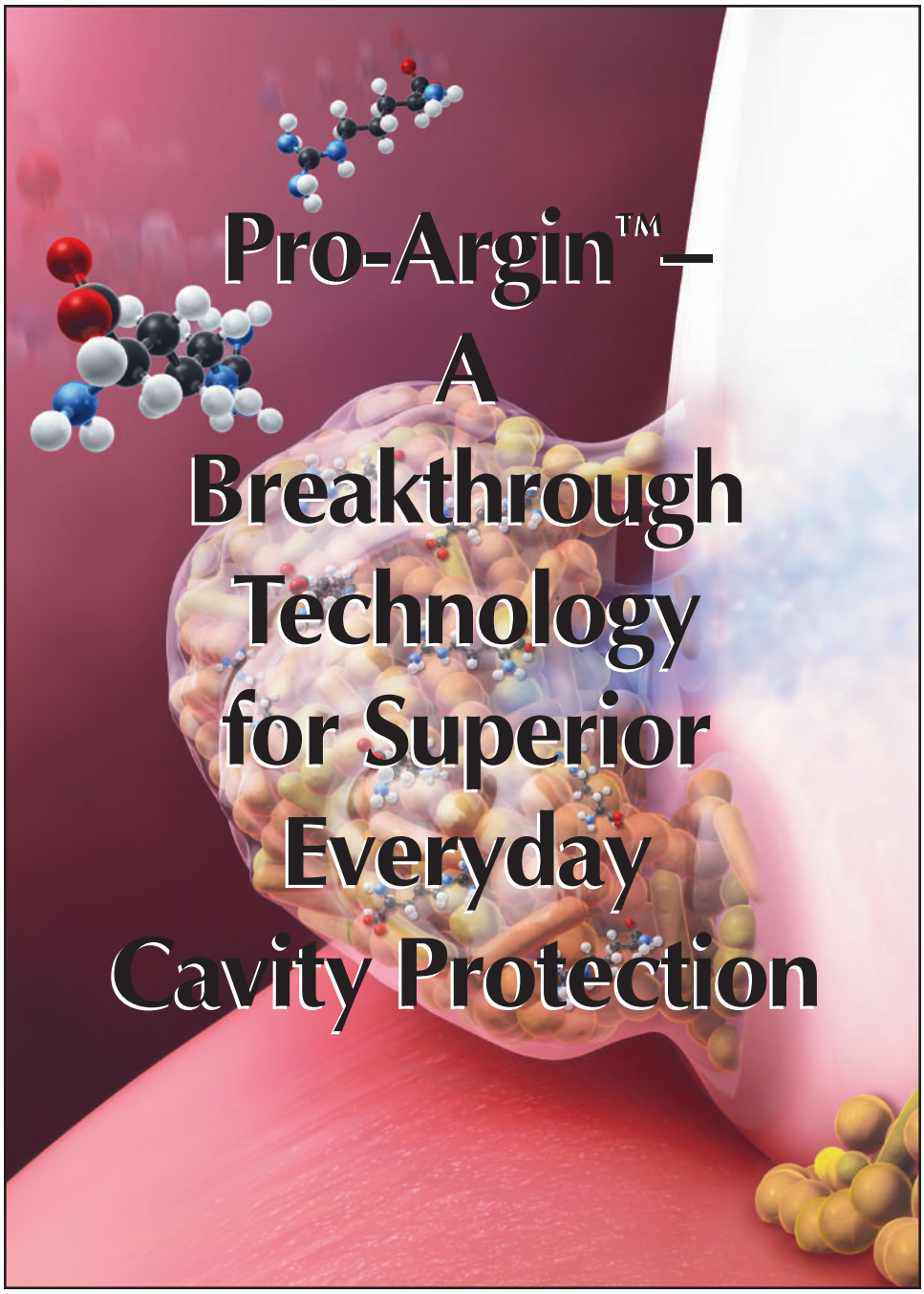

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Dental Caries: A Disease Which Remains a Public Health Concern in the 21st Century– The Exploration of a Breakthrough Technology for Caries Prevention

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Abstract

This paper provides an overview of modern concepts of dental caries, including its etiology, prevalence, and risk factors. The multifactorial nature of the disease is reviewed, and the concept of reducing caries initiation and progression by reducing pathological factors and restoring caries balance is discussed. In addition, the role and efficacy of fluoride in reducing and preventing caries is highlighted, demonstrating its successes and limitations.

A novel technology, based upon arginine and an insoluble calcium compound, has been identified which targets dental plaque to prevent initiation and progression of the caries process by reducing pathological factors. As the mechanisms of action of arginine and fluoride are highly complementary, a next-generation dentifrice has been developed, which combines arginine, an insoluble calcium compound, and fluoride, and has been clinically proven to provide superior caries prevention.

(J Clin Dent 2013;24[Spec Iss A]:1–14)

Introduction

Dental caries is a globally prevalent and ubiquitous oral health and public health concern.[1-3] It can affect children at a very early age,[4-6] and will certainly afflict most individuals in adolescence and throughout adulthood.[7-9] Many decades of scientific research have greatly increased our understanding of dental caries, and the application of this knowledge has led to the successful implementation of fluoride-based therapies to help arrest caries development and progression.[10] Nonetheless, dental caries remains a prevalent disease in society today.

Dental caries is a multifactorial oral condition with a complex etiology.[1,11] The interplay between dental plaque, constituents of the diet, and the host tissue, as well as genetic and environmental factors, have each increasingly been recognized for their importance in the pathogenesis of dental caries.[10,12,13] Some of the most important scientific concepts which have resulted from contemporary understanding include: 1) dental plaque is a complex oral biofilm which displays unique behavior that is quite different from the behavior of its constituent planktonic species;[1,14-16] 2) dental caries results from an ecological shift in dental plaque, from a healthy to a pathogenic flora;[12,17] 3) dental caries is a process, not an endpoint, and up to the point of cavitation this process may be arrested or reversed;[18,19] and 4) the caries process is a dynamic balance between pathological and protective factors which can progress if the pathological factors are dominant, and be reversed if the protective factors prevail.[20, 21]

These core scientific concepts, which have stimulated paradigm shifts in thinking,[12,21] are driving new avenues of caries research today, and are expected to underpin future developments in the prevention of dental caries.[10-12,21] More specifically, they are leading to: 1) enhanced understanding of the complex phenomena associated with the etiology and pathogenesis of dental caries;[11-17] 2) increased appreciation of the importance of caries risk factors in determining an individual's predisposition to caries;[7,22-24] 3) improved methods of caries detection and measurement of caries progression;[2,25] and, importantly, 4) new insights and better tools to aid the development and evaluation of new caries preventive measures.[10,17,21]

This paper provides an overview of: 1) the multifactorial nature of dental caries and the concept of caries balance; 2) global caries prevalence, the factors affecting the changing patterns of caries in developed and developing countries, and the development of caries within life stages; 3) caries risk factors and the concept of reducing caries progression by reducing pathological factors and restoring caries balance; 4) fluoride's efficacy and its role in reducing and preventing caries; and 5) new technologies to deliver a step-change improvement in everyday caries management and prevention.

The papers which follow in this Special Issue will describe the science underlying the development and clinical validation of a new and innovative dentifrice technology. This technology, which is based on arginine in combination with an insoluble calcium compound and fluoride, works in two complementary ways to reduce caries risk and restore caries balance, thereby providing superior anticaries efficacy.

The Multifactorial Nature of Dental Caries and the Concept of Caries Balance

Recognition that dental caries is a chronic infection caused by the indigenous oral flora has had important consequences for its prevention and treatment.[12] Dental caries is induced by protracted contact between dental plaque, tooth surfaces, and constituents of the diet, especially sugar.[1,11] The relationship between these three factors was first schematized by Keyes[26] in the now famous Venn diagram shown in Figure 1. The dimension of time was not explicitly illustrated, though time is a critical factor in determining caries severity.[7] Some authors have overtly incorporated the time dimension, while others have included additional factors now known to be associated with caries, such as saliva function, behavior, education, and socioeconomic status, as illustrated in Figure 2.[12,13]

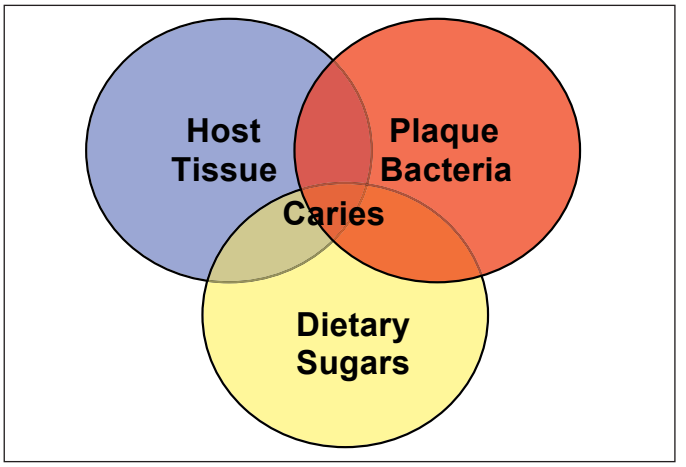

Figure 1. *Venn diagram of the three critical factors in dental caries. (Adapted from Keyes[26])*

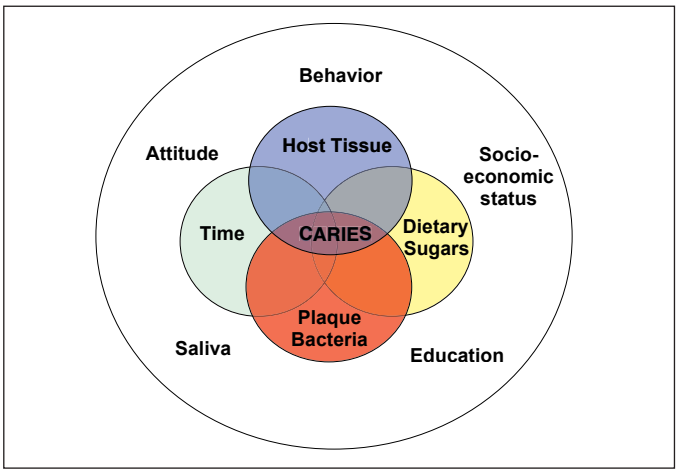

Figure 2. *Biological, environmental, and behavioral factors driving the developmen of dental caries. (Adapted from Brambilla, et al.[13])*

Dental plaque is a complex, highly diverse oral biofilm which develops over time on tooth surfaces, especially on hard-to-brush areas and areas adjacent to the gingival margin, and on soft tissue surfaces, especially the tongue.[1,11] While many individuals may have fewer than 100 bacterial species in their mouths, dental plaque may contain as many as 1000 species depending on its maturity and location in the oral cavity.[11,16,17] Less than half of these have been identified using classic microbial methods of plating and counting. Use of fluorescently labeled monoclonal antibodies (Mabs), deoxyribonucleic acid (DNA) probes, and real-time polymerase chain reactions (PCR) has enabled identification of the oral species that are non-cultivable.[11,27] The identification of specific bacteria associated with dental caries has been an area of extensive research and some controversy. While there are few researchers who currently believe that a single species is the unique culprit, it is believed that a limited number of acid-producing oral bacteria are highly associated with caries development and progression, which include the mutans streptococci.[7,18]

Dental plaque displays properties that are typical of biofilms. It is a highly structured, spatially organized, and metabolically integrated community of bacteria which interact and communicate by gene transfer and by secretion of signaling molecules. This organization renders specific species within the community co-dependent, and confers increased metabolic efficiency, greater resistance to stress, and enhanced virulence to the community as a whole.[14,27] The distribution of species within dental plaque varies from site to site (*e.g.*, on the teeth versus on the tongue) and within sites on a specific substrate (*e.g.*, deep in pits and fissures versus at gingival margin), demonstrating the subtle influence of the habitat. The composition of a community within a particular site can remain relatively stable over time in microbial homeostasis, reflecting a dynamic balance among the component species. However, this stability can be perturbed by significant changes in the environment, which can lead to overgrowth of previously minor species within the community.[28] Such changes trigger a change from a "healthy," to a more "pathogenic" plaque, thereby predisposing the site to disease.

Dental plaque bacteria create their own sticky, highly hydrated exopolysaccharide matrix, largely comprised of glucan, which acts as a "glue" and provides binding sites to the teeth and to other bacteria, thereby encouraging plaque formation.[1,11,27] Sugar, especially sucrose, promotes this matrix formation. A group of enzymes, known as the glucosyltransferases (GTFs), are produced on the tooth surface by specific species of oral bacteria, especially the cariogenic mutans streptococci, where they synthesize glucan from sucrose in the diet.[1,11] These GTFs account for the special relationship between sucrose and caries by allowing cariogenic bacteria to accumulate and form a critical mass that triggers the caries process.[18] By this means, cariogenic bacteria and sugar play a critical role in plaque pathogenicity and virulence in dental caries.[1,11]

It took many years of research to establish that the presence of fermentable dietary sugar is a critical factor in dental caries.[29] During the caries process, the acid-producing bacteria within dental plaque rapidly metabolize this sugar, producing acid at the tooth surface. By this means, the cariogenic bacteria and sugar play a second important role in plaque pathogenicity and virulence. When acid is formed in sufficient amounts and for sufficient time periods to create conditions that favor dissolution of calcium and phosphate from the tooth enamel, then demineralization occurs and tooth mineral is lost.[1,11,29] pH values as low as 4.0 have been observed at the tooth surface 10–15 minutes following exposure to sugar. Lactic, acetic, and formic are the most commonly detected acids.[1,11] The effect of this acid production on plaque pH behavior is illustrated by the Stephan

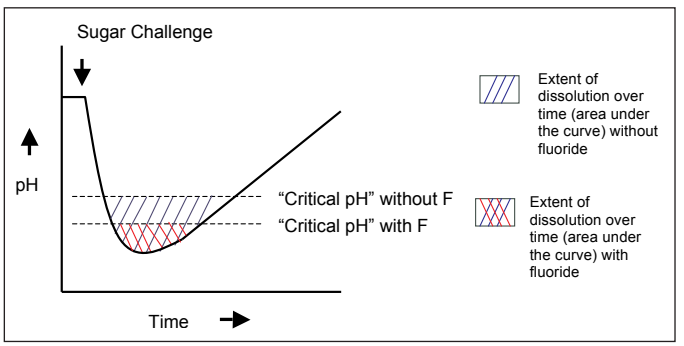

Figure 3. *Stephan curve showing the effects of a sugar challenge on plaque pH. Fluoride lowers the "Critical pH" below which dissolution of calcium and phosphate from the tooth occurs. Typical time to return to resting pH is 45–60 minutes. (Adapted from Kleinberg[29])*

curve shown in Figure 3.[29] Note the "critical pH" below which dissolution of calcium and phosphate ions from the tooth occurs. The extent of dissolution over time is determined by the extent and duration of the pH drop, *i.e.*, by the area under the curve, shown as the shaded region in the figure. When fluoride is present, the critical pH for dissolution is lowered, with the result that a lower plaque pH can be tolerated before dissolution of calcium and phosphate ions is initiated.[29]

Saliva plays an important role in modulating the Stephan curve. Saliva flow helps to disperse and dilute plaque acids, while saliva's buffering effects help neutralize plaque acids, together reducing the impact of a sugar challenge. Figure 4 compares the responses of saliva-deficient and normal individuals, clearly demonstrating the important role that saliva plays in modulating plaque pH behavior.[29-31] During a typical day, the teeth are subjected to multiple repeated exposures to sugar and, hence, to multiple repeated exposures to plaque acid, as illustrated in Figure 5.[13]

Adaptive mechanisms are important to the persistence of specific bacterial species, and to the plaque biofilm community as a whole. In the absence of adaptive mechanisms, microorganisms would be unable to survive the acid conditions created at the dental plaque-tooth interface. The species of plaque bacteria which are directly implicated in the pathogenesis of caries, for example the mutans streptococci, have evolved into sophisticated mechanisms which enable them to be acid-tolerant and, thus, to survive, to thrive, and sometimes even to dominate the ecology of dental plaque. In contrast, many of the non-pathogenic organisms do not possess these mechanisms of acid tolerance, with the result that they have difficulty surviving and may even disappear under extended cariogenic conditions.[1,11]

Adaptive mechanisms are also present in dental plaque to help counteract the mechanism of acid tolerance by which the acid-producing cariogenic bacteria persist.[1,11] Specifically, several oral organisms, including *S. sanguis*, have developed a pathway, known as the arginine deiminase pathway, which enables these organisms to break down the arginine present in saliva to ammonia and carbon dioxide. This base production serves to directly neutralize the acid produced within dental plaque.[1,11] It is noteworthy that a lack of alkali production by dental plaque is a critical factor in the pathogenesis of dental caries.[1] Thus, this mechanism can contribute to the stability of the plaque biofilm, and can help to prevent a change from a "healthy" to a "pathogenic" plaque.

As stated previously, one of the most important concepts underpinning caries prevention is the fact that caries is a process that is both dynamic and reversible.[20,21] This process comprises demineralization and remineralization steps. The demineralization step occurs when plaque acid on the tooth surface dissolves calcium and phosphate ions from the hydroxyapatite within the enamel structure, resulting in net loss of tooth mineral.[20,29] The remineralization step occurs when the acid challenge is removed, and free calcium and phosphate ions present in saliva are driven back into the demineralized zone in the enamel, resulting in a net gain of mineral by the tooth structure.[20,29] This process is shown in a highly simplified form in Figure 6. Fluoride accelerates the remineralization step, as we will discuss briefly later. The caries process initially leads to a sub-surface demineralized zone which is located below the intact enamel surface. Figure 7 illustrates the profile of such a lesion, by plotting mineral density as a function of depth below the tooth surface. This sub-surface zone is often referred to as an "early caries" lesion.[32] An early lesion in the active stage of demineralization may be arrested and reversed by remineral-

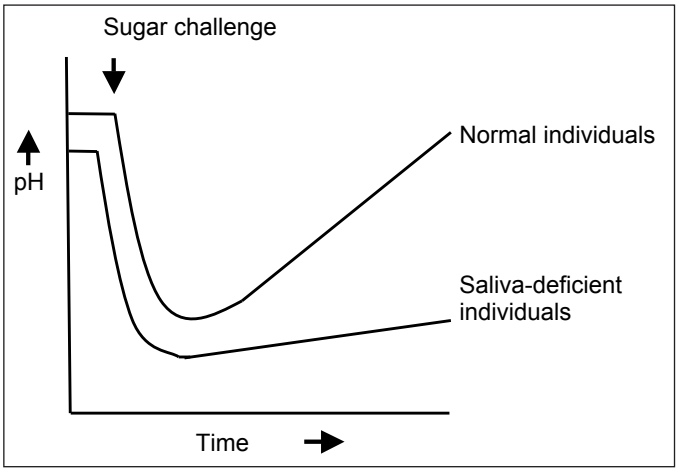

Figure 4. *Stephan curve showing the effects of a sugar challenge on plaque pH in saliva-deficient and normal individuals. (Adapted from Kleinberg[29])*

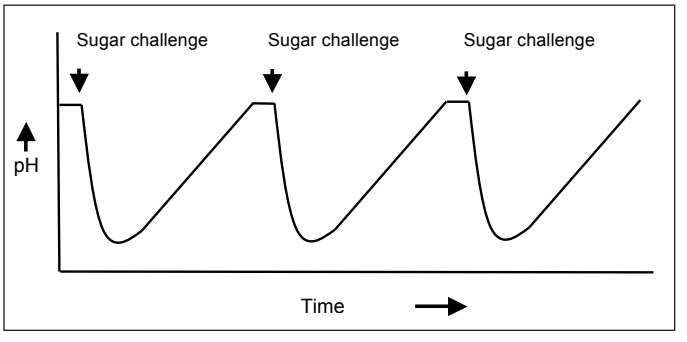

Figure 5. *Plaque pH resulting from repeated exposures to sugar during a typical day. (Adapted from Brambilla, et al.[13])*

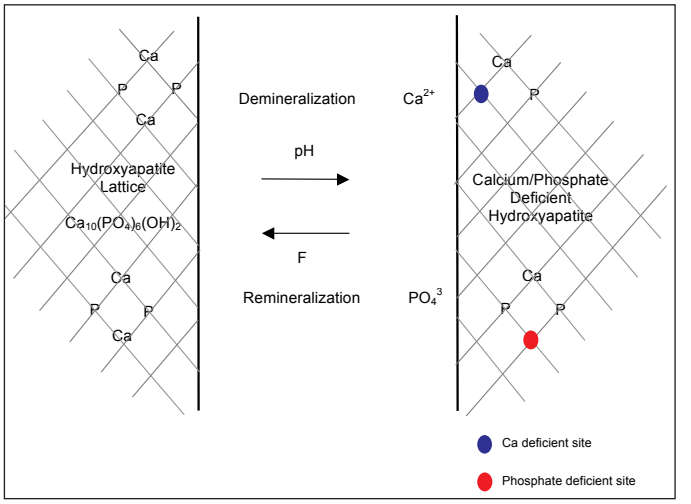

Figure 6. *Simplified illustration of the caries process, which comprises demineralization when plaque acid solubilizes calcium and phosphate ions from the tooth structure, and remineralization when free calcium and phosphate ions are driven back into the tooth structure.*

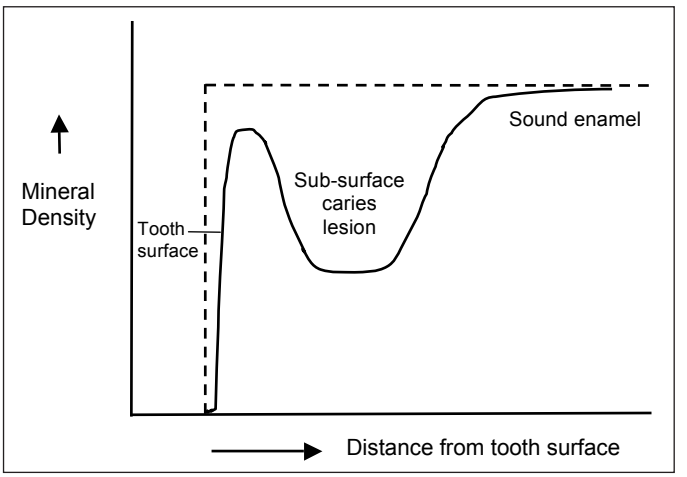

Figure 7. *Schematic illustration of the profile of mineral density from the tooth surface through a sub-surface caries lesion to the body of the enamel. (Adapted from Arends and Christoffersen[32]).*

ization. Only when a caries lesion continues to demineralize, and progresses beyond the point where it can be effectively remineralized, does it reach the clinical end point of cavitation.

The concept of caries balance was first introduced to simplify our understanding of the key factors involved in the caries process, and to make them readily applicable to clinical practice.[33] Subsequently, this concept has been embraced by a consensus group in California,[20] and at an American Academy of Pediatric Dentistry (AAPD) meeting.[21] In essence, the caries process is visualized as a balance between pathological factors and protective factors. Figures 8a and b illustrate the caries balance for caries-free and caries-prone individuals, respectively. If the pathological factors outweigh the protective factors, then the caries process leads to conditions of net demineralization and the formation or progression of a caries lesion. If, on the other hand, the protective factors dominate, then the caries process results in net remineralization, and existing caries lesions are arrested and reversed. In its most simplistic form, the caries balance is presented with three key factors on each side.[21] Additional factors could be added to represent the more detailed understanding that we now have about this multifactorial process. However, this simple approach has been favored because it makes the concept applicable to the clinical environment. Dental professionals can use this model to assess the pathological and protective factors in each individual patient, and to recommend specific preventive and treatment steps to reduce pathological factors and increase protective factors to help develop and maintain caries balance.[21]

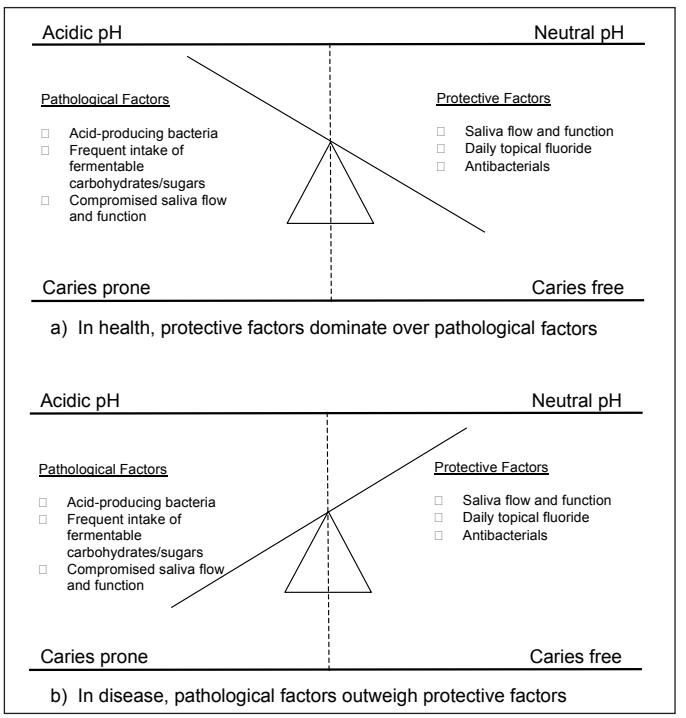

Figure 8. *Schematic illustration of the caries balance in a) health, and b) disease. (Adapted from Featherstone[21])*

The corollary to the concepts of the caries process and caries balance is that caries is a continuum of disease states, ranging from sub-clinical early lesions to advanced, clinically detectable lesions in enamel and dentin. Clinical researchers have classified the various stages of development and advancement of primary coronal caries lesions, and have defined diagnostic thresholds for use in caries clinical trials and in clinical practice. Accordingly, caries lesions detected by traditional visual/tactile methods have been designated D1 (intact), and D2 through D4 (cavitated), and have been depicted as the "above water" portion of an iceberg. In contrast, early caries lesions, which require more advanced and discerning methods of detection, have been depicted as the "below water" portion of an iceberg, as illustrated in Figure 9.[34] This is a critical issue with traditional caries clinical trials, and has important consequences for the development and validation of new preventive measures which are targeted to arrest and reverse early caries lesions.

Caries lesions can also occur adjacent to restorations in both the primary and permanent teeth. Such lesions may be referred to as "secondary caries" or "recurrent caries." As these lesions have similar characteristics to primary caries lesions, they are classified using the same principles. They also have the same basic etiology as primary caries, so an early secondary lesion can also be arrested and remineralized.[35]

The preceding sections have implicitly focused upon coronal (enamel) caries, but the discussion is also highly relevant to root

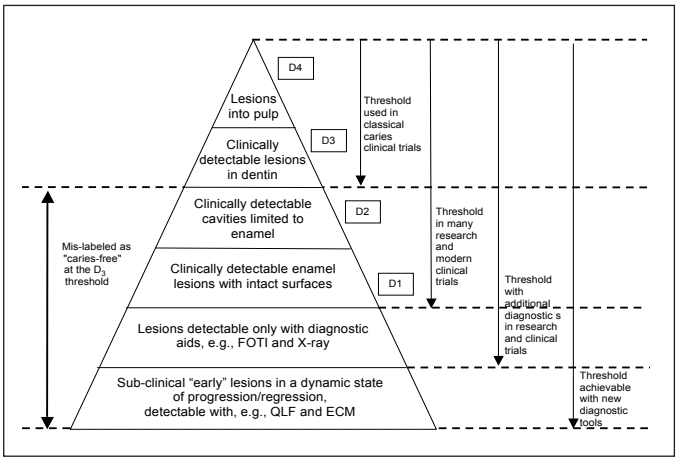

Figure 9. *Stages of development and advancement of coronal caries lesions. (Adapted from Pitts[34])*

(dentin) caries. The key factors—dental plaque bacteria, host tissue, and sugar—are also critical to the development of root caries. An additional factor is the presence of gingival recession and the host tissue is the exposed tooth root.[5] Root caries generally presents on the root surface close to the cemento-enamel junction. The base of a lesion is classified as "soft," "leathery," or "hard" to probing.[36] The critical pH for dentin demineralization is higher than the critical pH for enamel, which has suggested that more efficacious measures may be required to reduce demineralization and enhance remineralization of dentin as compared to enamel.[37] On the other hand, root caries sites are often easily cleaned of retentive plaque which may help reduce progression of the caries process and enhance remineralization. In practice, soft and leathery root caries lesions can be arrested and reversed and are, thus, amenable to preventive measures that restore caries balance.

Global Caries Prevalence, Factors Affecting the Changing Patterns in Developed and Developing Countries, and the Formation of Caries with Life Stages

An understanding of global dental caries patterns has grown over the past several decades, facilitated by the World Health Organization (WHO) and the Federation Dentaire Internationale (FDI) through procedures for the collection of standardized oral health data,[38] and by the development of a global goal for oral health of DMFT < 3 by the year 2000.[39]

A review of the data from a wide range of sources, including large national health surveys such as the National Health and Nutrition Examination Survey (NHANES) and National Institute of Dental and Craniofacial Research (NIDCR) survey conducted in the US,[40,41] and small dental health surveys such as the survey of caries status of 5- to 6-year-old children in the Caribbean[42] yields two important messages: 1) caries experience varies widely among populations, both within individual highly developed countries, such as the United States (US),[43] and from country to country in developing and emerging economies;[44] and 2) divergent trends in caries experience are correlated, at least in part, with two key variables, sugar consumption and oral hygiene behaviors.[45,46] The following section will delve more deeply into the underlying data from which these messages are drawn.

A review of the changing global patterns of caries experience among 12-year-olds over the period 1970–2001 has pointed to several important conclusions.[38] First, there has been a dramatic decline in overall caries experience in many established market economies, including the US, Canada, Western Europe, Australia, and Japan.[40,41,47-50] Current caries levels are reported to be low in these established market economies (mean DMFT ~1 in 12-year-olds).[38,39] Second, caries levels in several developing and emerging economies, including Latin America and Central and Eastern Europe, are moderately high (mean DMFT = 3–4 in 12-year-olds).[38,42,48] Current caries status in these countries does not yet meet the WHO/FDI global goal.[39] While some countries, such as Latvia and Poland, appear to have experienced some decline in caries over the past decade, others, such as Hungary, have been relatively static, and yet others, such as Croatia, have experienced an increase.[38] These levels of caries experience have been attributed, at least in part, to the introduction and availability of dietary sugars coupled with infrequent or inadequate oral hygiene.[39] Third, caries experience appears to be "low" to "moderate (mean DMFT = 1–2) and relatively constant over time in populations in Sub-Saharan Africa and the Middle East.[51-54]

A second review paper has drawn similar broad conclusions.[55] The author described three patterns of caries prevalence: 1) "Rural populations," giving examples of China, Africa, and remote areas of South America having low levels of caries; 2) "Newly industrialized populations," giving examples of Taiwan, Chile, India, Uganda, and Thailand having high levels of caries associated with increasing exposure to sugar; and 3) "Industrialized populations," giving examples of North America, Europe, and Australia where oral health status is broadly characterized by a decline in caries in children and adolescents from previously high levels, and by an increase in tooth retention in older adults.[55] Interestingly, this author noted that restorative dentistry has concomitantly changed focus from primary cavities in children and adolescents in the 1970s to "recurrent" caries in adults in 2000, reflecting the pattern of caries across life stages.[55]

Clearly, the data demonstrate that overall caries experience varies widely among populations at different stages of economic development. Interestingly, in 2012, a critical review contrasting caries trends in children in Vietnamese and Australian populations confirmed the wide variability in caries experience driven by socioeconomic inequality.[56] Likewise, a systematic review of dental caries in adults, published in the same year, revealed that social factors, such as education level, income, and occupation, are associated with caries.[57] At face value, the data from the industrialized countries suggest that caries is effectively managed and is no longer a major health problem there. However, an in-depth analysis of the data reveals that this is not the reality.

Surveys have reported a dramatic decline in dental caries in the US population.[40,41] Since the 1970s, there has been a decrease of 57.2% in DMFT and 58.8% in DMFS in the permanent teeth of 6- to 18-year-olds.[58] Despite this remarkable progress, dental caries remains a significant problem in the US today.[59,60] The NHANES III study showed that 16.3% aged 2–4 years, 28.5% aged 6–8 years, 17.9% aged 12–15 years, and 25.3% aged 35–44 years had untreated caries.[41] The magnitude of the caries problem in the US is more pronounced seen through the caries severity data. The mean DMFS among adolescents aged 12–15 years was 4.5, and among adults aged 35–44 years was 42.5.[41] Clearly, caries is not simply a childhood disease, it is also a prevalent adult problem. Importantly, the data show that caries experience in the US is not homogeneous. To the contrary, 80% of caries experience was found in 25% of US children.[43] The caries problem is largely found in a "high risk… subset of the US population, particularly in certain racial and ethnic groups and in groups of low social economic status.[41] Thus, it is readily apparent that dental caries in the US is not close to eradication, and is not likely to be so any time soon.

Surveys conducted in Europe have reported similar changing patterns of caries experience. A recent review has shown a dramatic decline in overall caries experience across many countries in Western Europe.[48] However, widespread disparities between

different subgroups have also been reported in Great Britain and in cities such as Zurich and The Hague.[48,61] Divergent trends were seen between high and low social economic status groups, especially among recent immigrants.[48] This pattern clearly mirrors the trends seen in the US. A recent paper on caries status of 12-year-olds in Italy concluded that overall caries experience has declined dramatically over the past twenty years, from DMFT > 5 to its present level of DMFT ~ 1. But it also noted that differences remain between children with different socioeconomic backgrounds, confirming that the conclusions regarding disparities and high risk groups, made from data collected in other established market economies a decade or more ago, remain highly relevant today.[62]

A recent survey conducted in Australia confirmed the pattern of declining caries experience seen in other industrialized countries, and demonstrated disparities between indigenous and non-indigenous Australians[63] which were attributed to poor oral hygiene, diets rich in refined sugars, and a number of factors stemming from low economic status.[64]

Two systematic reviews have been conducted to assess caries experience in three developing regions: Latin America and the Caribbean, Middle East and North Africa, and Sub-Saharan Africa.[42,51] Mean caries prevalence and caries severity were lowest in Sub-Saharan Africa and highest in Latin America and the Caribbean, reflecting the population's access to sugar in these regions. Caries rates were reported to have decreased from 1970 to 2004 in Latin America and the Caribbean, and have remained static in the other two regions.[42,51]

A large national survey of oral health status in China was published in 2002.[65] At age 5, caries experience was high; 76.6% were affected with a mean DMFT of 4.5. Caries experience was significantly lower in adolescents and young adults. 45.8% of 12-year-olds were affected, with a mean DMFT of 1.0; 55.3% of 18-year-olds were affected with a mean DMFT of 1.6; and 63% of 35- to 44-year-olds were affected with a mean DMFT of 2.1. In contrast, caries experience was very high in the oldest population; 64.8% of 65- to 74-year-olds were affected with a mean DMFT of 12.4. Caries levels were higher in urban areas among adolescents and young adults reflecting diet, whereas they were higher in rural areas in older adults reflecting a lack of oral hygiene. At the national level, changes in caries experience in adolescents were small, but some provinces with extensive preventive programs experienced reductions in caries, whereas others with limited preventive programs had increasing caries levels.[65]

As we have seen from the prevalence data, dental caries is a disease which may affect individuals during each life stage, from birth through adolescence and adulthood to old age.[39] Teeth are susceptible to dental caries as soon as they erupt. Caries incidence peaks between 2–5 years in the deciduous dentition, and in early adolescence in the permanent dentition.[39] While it has been reported that 50% of 12-year-olds in the US are now caries-free,[18] it is apparent that being caries-free as an adolescent does not mean being caries-free for life. The "fact… that 50% of US school children have never had a cavity has also been disputed.[59] Several studies in established market economies, have shown increasing caries prevalence with age.[39] Based upon the data from developing market economies, reviewed herein, it appears that increasing caries experience with age is a broad global phenomenon. In addition, gingival recession increasingly occurs in older adults, exposing the tooth root and posing root caries as a potential additional problem.[39]

The sustained impact of dental caries in adults is also evident in the cost of dental services. In an analysis of the distribution of dental services across life stages in the US, diagnostic and preventive dentistry costs were evenly spread across the entire age range from early childhood (up to 5 years) to late adulthood (90 + years), as were the costs of basic restorative dentistry. In contrast, major restorative dentistry costs increased substantially from adolescence to 50–59 years old, and remained at a plateau until age 80–84 years. This suggests that, even in countries where dental caries has declined dramatically over the past several decades, it remains an ongoing oral health and public health problem throughout life.[7]

Early childhood caries (ECC) typically affects the buccal surfaces of the maxillary incisors and the occlusal surfaces of the first molars.[5] ECC first presents as a band of white decalcification along the gumline or on the occlusal surfaces coincident with the presence of plaque. As the surfaces of the primary teeth are highly susceptible to acid dissolution, ECC progression may quickly result in severe cavitation and even tooth loss.[6]

Today, caries in children and adolescents is largely manifest in interproximal and occlusal surfaces coincident with plaque, which is more difficult to remove from these sites.[66] This pattern of caries experience continues through to adulthood. However, caries may also be observed on buccal surfaces in high risk individuals,[67] including patients undergoing orthodontic treatment.[68]

Secondary caries can occur in the primary dentition of young children, but is generally observed in the permanent dentition of adolescents and adults. Secondary caries is associated with restorations and is the major cause of restoration failure in children, adolescents, and adults alike. Secondary caries results in repeated tissue breakdown and repair which may, ultimately, result in tooth loss.[35]

Although root caries can be present in young individuals, it is generally a problem of the dentate older adult.[37] In the US, it has been reported that increases in root caries with increasing age are substantially higher than the corresponding increases in coronal caries.[69] Thus, it is anticipated that root caries will become an increasingly important oral health concern in the future, as people live longer and increasingly remain dentate.

Taken together, the data lead this author to the following overall conclusions:

- Dental caries is a prevalent condition throughout today's world. It invariably begins in childhood and increases throughout adolescence and adulthood. Even in countries which have experienced a dramatic decline in the prevalence and incidence of caries, the majority of adolescents and almost all adults have experienced caries to a greater or lesser extent.
- On a global basis, caries experience has been driven by two key behavioral factors; increasing levels of caries have been driven by increasing consumption of refined sugars, while decreasing levels of caries have been driven by improved oral hygiene and the effective use of fluoride. A model of caries development which explains the diverse and changing pat-

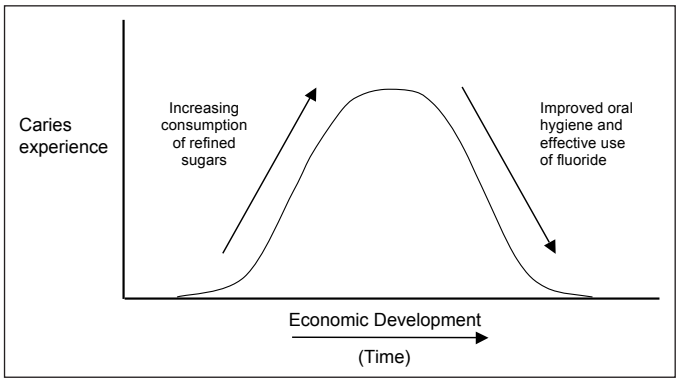

Figure 10. *Model to explain changing global caries patterns based on two key factors that drive caries experience: consumption of refined sugars and oral hygieneluse of fluoride.*

terns observed today is shown in Figure 10. In essence, caries levels reflect the relative importance of these two factors at both the individual and population level.

- The divergent trends in caries experience, driven by deeper-routed social and environmental factors, are clearly apparent in established market economies; such trends may become more apparent in developing and emerging markets in the future.

The fact that dental caries is a globally prevalent disease does not, alone, merit it as a major issue. However, the significant cost to and impact on individuals and society does elevate dental caries to an oral health and public health concern. The most tangible costs are, of course, for dental restorations. In the US alone, the cost of dental services rose to more than $60 billion per year in 2000, of which the greater proportion was for the treatment of dental caries.[60] More recent data estimated the annual cost of dental services in the US in 2008 to be $80–82 billion, with services targeted at dental caries at 55 ± 10% or $45 billion.[70] Despite this high expenditure, more than 30% of US adults did not receive necessary treatment because of the prohibitive cost of dental services.[60] For these individuals, and for many populations around the world who cannot afford or do not have access to dental care, the costs are equally important, if less tangible. The pain and suffering associated with caries can diminish the quality of life and may lead to malnutrition and other health problems, while the cosmetic consequences of dental caries can impact self-esteem and self-confidence.

Caries Risk Factors and the Concept of Reducing Caries Progression by Reducing Pathological Factors and Restoring Caries Balance

Risk is the probability that an event, generally an adverse event, will occur within a meaningful time frame. Interest in risk is low when the risk is zero or 100% because the outcome is inevitable. In contrast, intermediate levels of risk are interesting because risk factors, which may be biological, environmental, or behavioral in origin, can be explored and risk management strategies can be applied to reduce risk and to improve the anticipated outcome.[71]

The concept of assessing and managing risk in dental caries is a relatively recent concept which has grown out of our understanding of the factors driving the changing patterns of caries experience at the population level. These changing patterns have stimulated questions regarding why some individuals experience caries, whereas others do not.[71] Because of the complexity of dental caries, there are many factors involved to a greater or lesser extent in the development of caries.[71] There is general acceptance that the following are true risk factors in the development of dental caries: the presence of susceptible tooth surfaces; acid-producing bacteria, especially *S. mutans*; frequent sugar intake; impaired salivary function, especially low flow and poor buffering capacity; poor oral hygiene, especially infrequent or inadequate tooth brushing; past caries experience; inadequate fluoride exposure; limited access to dental care; and low socioeconomic status.[7,21-24,71-74]

Consideration of the specific risk factors for caries leads to the idea of reducing and managing caries development and progression through targeted prevention at both the population and the individual level. At its simplest, caries risk can be reduced by reducing caries risk factors.

Clearly, risk factors that are environmental or behavioral in origin, such as low socioeconomic status and limited access to (medical and) dental care, are critically important to society in general and to the future of dental caries. They certainly need to be addressed to trigger a homogeneous decline in caries on a global basis, and are beginning to be addressed through, for example, national public health initiatives. Other risk factors with similar origins, such as poor oral hygiene and frequent sugar intake, can be addressed, at least in part, through more widespread oral health education. However, as each of these factors is beyond the scope of this paper, they will not be discussed further.

The risk factors that are amenable to modification and can be addressed by the development of new and improved oral care products are the two key biological factors: susceptible host tissue and cariogenic bacteria in the plaque biofilm, with its consequence, bacterial acid production.

Historically, practical application of caries research to prevention and treatment measures has been focused on the host tissue. Reduction of the tooth's susceptibility to acid attack can be achieved by changing the chemistry of the tooth's surface, rendering it less vulnerable to demineralization. Likewise, remineralization can be increased by enhancing the uptake of calcium and phosphate ions into demineralized enamel (and dentin). Each of these measures has the potential to increase protective factors and to restore the caries balance.

With respect to the cariogenic bacteria themselves, potential measures to reduce the risk or impact of a cariogenic challenge include: 1) reduction of total plaque burden; 2) inhibition of plaque metabolism, especially acid production; and 3) promotion of microbial homeostasis in sites with dental plaque, preserving the dynamic balance in favor of non-pathogenic organisms, and preventing environmental perturbations that lead to an overgrowth of acid-producing bacteria, especially *S. mutans*. Each of these measures has the potential to reduce pathological factors and to restore the caries balance.

Fluoride's Role and Efficacy in Reducing and Preventing Caries

The primary route to prevent and control plaque-related oral health problems, including dental caries, is through thorough mechanical removal of dental plaque from all tooth surfaces on a regular and sustained basis.[75] In reality, however, this is nei-

ther practical nor attainable for many individuals, as they lack the knowledge, skills, or motivation to do so. For this reason, it is a widely accepted practice to use therapeutic agents to supplement normal mechanical oral hygiene procedures.[76,77] Many years of research have provided a sound understanding of why fluoride and other proven therapeutic agents are clinically effective. In summary, they are delivered and effectively released into the oral cavity during application, and are retained in the mouth for a sufficient time period to exert sustained biological activity.[78]

Fluoride is, without question, a highly successful caries preventive agent; the dramatic decline in caries prevalence and severity observed over the last several decades has been attributed to fluoride's widespread use. Indeed, the widespread use of fluoride toothpaste has been widely acknowledged by academic experts, the dental profession, and professional health organizations to be the single most important factor contributing to the decline observed in caries over the past several decades.[79] Perhaps surprisingly, fluoride is the only clinically proven anticaries agent that is accepted for routine use in oral hygiene products on a global basis. Importantly, the benefits of fluoride are clinically proven in all segments of the population, from young children to older adults.[80]

Initially it was thought that fluoride affected tooth development and mineralization, and that systemic fluoride administration was necessary for optimum benefit. A paradigm shift in understanding the mechanism of action of fluoride drove development and validation of topical treatments for the prevention of dental caries.[12] Today, consumer products encompass toothpastes and mouthrinses for self-care, whereas prescription products encompass varnishes and gels for professional in-office treatment, and high fluoride toothpastes and mouthrinses for prescribed home care.

Fluoride is clinically effective in preventing the caries process and reducing the formation of cavities, because it acts directly on the tooth mineral to prevent mineral loss. During use of a fluoride toothpaste or mouthrinse, low levels of fluoride are delivered to the oral cavity where they are retained in reservoirs on the tooth surface and the soft tissues for sustained time periods after application.[33,81-83] These low, sustained levels of fluoride are able to modify the critical pH value below which calcium and phosphate ions are solubilized from the tooth structure and, thus, are able to reduce demineralization.[83-85] These low, sustained levels of fluoride are likewise able to enhance remineralization of demineralized tooth enamel and dentin.[83,86] This is believed to occur via formation of calcium fluoride on the tooth surface which acts as a reservoir during periods of homeostasis, and is triggered by significant pH drops to drive calcium ions into calcium-deficient hydroxyapatite sites in the caries lesion.[87]

The scientific literature abounds with clinical studies which demonstrate the benefits of topical fluoride products on dental caries. There is a significant and clinically meaningful benefit derived from the regular use of a well-formulated fluoride-containing oral hygiene product. Several comprehensive clinical reviews, which include a Cochrane systematic review, have shown that regular brushing with a 1000 ppm fluoride toothpaste reduces the development of coronal cavities by approximately 25% compared to brushing with a non-fluoride toothpaste. Efficacy increases with increasing fluoride level, there being an approximately 5% benefit for a 1500 ppm over a 1000 ppm fluoride toothpaste, and also with a higher frequency of use.[88-93] Additionally, a recent systematic review and meta-analysis of data from pre-school children has affirmed the effectiveness of 1000–1500 ppm fluoride toothpaste in reducing caries in primary teeth.[94] Further benefits over 1500 ppm have also been shown for prescription levels, such as 5000 ppm fluoride.[95,96] However, the evidence regarding the anticaries efficacy of lower fluoride levels (450–550 ppm) remains equivocal.[93] Fluoride toothpastes are also clinically proven to reduce the development and progression of root caries.[37,97]

Daily-use toothpastes vary in their fluoride source and in other functional ingredients, such as the cleaning and polishing agent and the flavor. These differences are largely driven by consumer preference, especially mouth feel and taste. The fluoride source itself is not critical to its effectiveness in cavity prevention. The Cochrane systematic review, which analyzed the evidence from 70 clinical studies, concluded that there is no evidence for a significant difference in efficacy of toothpastes formulated with different forms of fluoride.[93]

The benefits of regular and effective use of fluoride toothpaste, and other topical fluoride products, in the long-term prevention and reduction of caries on a population basis have far exceeded expectations based upon the results of clinical efficacy studies. This observation suggests that anticaries benefits propagate over time as compared with the benefit estimates from short-term clinical experience. Based upon risk/benefit and compliance, it is evident that toothpaste is the ideal vehicle to deliver fluoride on a routine daily basis.[10] This reinforces the importance of regular and continued daily tooth brushing with a fluoride toothpaste to prevent dental caries in children, adolescents, and adults alike.

In summary, topical fluoride products, including fluoride dentifrices, reduce the risk of dental caries by targeting the host tissue, reducing the susceptibility of the tooth surface to acid attack. They do this by arresting the caries process, reducing demineralization, and increasing remineralization of demineralized enamel and dentin. Thus, they help to restore caries balance by providing an important protective factor. Topical fluoride products do, however, have inherent limitations under highly pathogenic conditions, such as a high plaque load and frequent sugar intake. Topical fluoride products do not target dental plaque, which is arguably the primary modifiable pathological factor in dental caries. Specifically, they do not reduce pathological factors either by reducing dental plaque levels, by promoting microbial homeostasis in sites with dental plaque, by preserving the dynamic balance in favor of non-pathogenic organisms, or by preventing environmental perturbations that lead to an overgrowth of acid-producing bacteria, such as *S. mutans*. Because of their primary mode of action, topical fluoride products help to control, but they cannot completely prevent, dental caries.[12]

A New Technology with a Step-Change Improvement in Everyday Caries Prevention

While fluoride products have dramatically reduced dental caries, the fact that caries remains a prevalent oral health and public health problem calls for new strategies to supplement

existing measures to reduce caries risk and improve dental health in individuals and in populations on a global basis.[25,98]

Over the past decade, there has been a noticeable trend toward conservative dental therapy and minimal intervention.[4,72,99-101] Researchers and the dental profession appreciate that new strategies and methods to intervene earlier in the caries process are an important next step to elevate conservative therapy and minimal intervention to a new level. Indeed, this is an expressed unmet need of the dental profession, who are looking for new technologies that are proven to be effective in high risk children and adults.[72,99-101]

It is also widely recognized that the diagnosis and detection of lesions in their earliest stages is critical to changing the paradigm in caries prevention.[1,10-12,20,21,25,72,98,99] Bowen noted that traditional caries clinical methods (that solely detect cavities) are an impediment to the introduction of innovative new technologies, and that enhanced methods of detecting pre-clinical enamel loss would accelerate the conduct of clinical trials and would, almost certainly, give more meaningful clinical results in respect of the caries continuum and, in particular, the arrest and reversal of early lesions. This was attributed to the fact that current caries clinical methods do not inform about the effects of an agent (or product) on the disease, *i.e.*, caries, but solely on the end point, *i.e.*, cavities.[1] Toward this end, advanced and more discerning techniques, such as Quantitative Light-induced Fluorescence (QLF) and the Electrical Caries Monitor (ECM) have been developed, and their use in clinical trials has been refined and optimized to enable the detection and assessment of pre-cavitated lesions over time.[102-104]

Any new strategy should recognize and complement the effects of fluoride. As fluoride's benefits are focused on the host tissue as a means of damage control after the caries process has been initiated and is in progress, combining fluoride with an agent that targets plaque pathogenicity and prevents the caries process would have potential to deliver a step-change improvement in caries prevention.

In reviewing the literature on potential routes to reduce plaque pathogenicity, it is immediately apparent that this is an active area of research, and has been so for several decades. A published review summarizes conceptual approaches, and provides a detailed review of specific research routes that have been validated or under investigation in "proof of concept" studies.[10] Despite sustained research activity in this field, remarkably few conceptual approaches have progressed beyond basic research, through translational research, *i.e.*, product development and validation in appropriately designed and conducted caries clinical trials to become cost-effective interventions.[10,11]

Of those avenues that have shown promise, a new technology, which comprises arginine, bicarbonate, and an insoluble calcium compound, and exploits a pre-biotic approach to caries prevention, is of special interest.[105] The principle underlying this technology is to modulate plaque pH by utilizing the arginine deiminase pathway in non-pathogenic, arginolytic organisms, such as *S. sanguis*. These arginolytic organisms are able to break down arginine to ammonia, which can neutralize plaque acids directly within the plaque matrix and, thus, stabilize undisturbed plaque biofilms.[31,106] The ongoing production of ammonia can elevate the resting pH of dental plaque to drive the process of remineralization. It can also mediate drops in pH resulting from sugar metabolism to reduce the process of demineralization at the biofilm-tooth interface. Thus, this new technology can prevent ecological shifts to acid-producing bacteria such as *S. mutans*, can help maintain a "healthy" plaque when challenged with dietary sugars, and, thereby, help to prevent caries.

Much progress has been made since Kleinberg's early work in understanding the molecular genetics and the physiological aspects of ammonia generation and its relationship to caries and health.[107,108] Several studies have shown that loss of alkali-generating potential in dental plaque through loss of urease activity has a positive relationship with dental caries experience.[109,110] More importantly, clinical studies have demonstrated that the *in situ* production of ammonia, from arginine naturally present in saliva, via the ADS in dental plaque is positively associated with reduced caries experience. When the relative enzymatic activity of ammonia-producing pathways (both ADS and urease activities) in dental plaque was compared for caries-free (DMFT=0), caries-experienced (DMFT ≥ 4, no active caries for 12 months), and caries-active (DMFT ≥ 4 with active caries) subjects, it was found that the caries-active subjects demonstrated reduced capability to generate ammonia. The results of this study demonstrate that caries status is correlated with both ADS activity and urease activity.[111] Similar observations have also been reported in an independent study.[112] A proof of concept clinical study has shown that an exogenous source of arginine can influence ADS activity in both caries-free and caries-active subjects. In this study, fluoride-free toothpaste with 1.5% arginine plus calcium carbonate was compared to a 1100 ppm fluoride toothpaste (silica /NaF) as a positive control which has been clinically proven to prevent cavity formation. After four weeks of twice-daily brushing, the arginine-containing toothpaste group had significantly increased ADS activity. Importantly, the ADS activity increase was most significant for the caries-active subjects. This indicates that exogenous arginine delivered during tooth brushing can reduce caries risk by increasing ADS activity.[113] Most importantly, another proof of concept clinical study has demonstrated that the delivery of exogenous arginine, to modulate bacterial metabolism, translates into a significant and clinically meaningful cavity prevention benefit. The same fluoride-free arginine plus calcium carbonate toothpaste and the same clinically proven positive control toothpaste, 1100 ppm NaF/silica, were evaluated in a two-year, caries clinical trial among 11- to 12-year-old Venezuelan children. After two years, the fluoride-free, arginine-containing toothpaste demonstrated equivalent efficacy to the 1100 ppm NaF/silica positive control toothpaste. This clearly indicates that the effect of the arginine-containing toothpaste on plaque metabolism translates into a significant and clinically meaningful cavity prevention benefit.[105]

A review of the scientific literature suggests that this new arginine-based technology is particularly noteworthy, as no other fluoride-free toothpaste technology has been clinically proven to deliver comparable efficacy to that of a 1100 ppm sodium fluoride toothpaste. In a subsequent clinical study, the benefit of chewing sugarless mints containing this new arginine-based technology after routine, twice-daily tooth brushing was compared to that of a placebo mint. The study showed that, after one-year's use of the mints

as an adjunct to normal oral hygiene, the arginine-containing mint reduced the formation of cavities significantly more effectively than the placebo mint, further validating the technology.[114]

Based upon the mechanism of action of arginine, which is complementary to the well-known mechanism of action of fluoride, arginine has the potential to significantly enhance the caries preventive benefits of traditional fluoride dentifrices. For this reason, a next generation dentifrice technology based upon 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride has been developed and clinically validated.

Three coronal caries studies, using QLF to measure changes in early caries lesions in children, have each shown that the new dentifrice containing 1.5% arginine and 1450 ppm fluoride in a calcium base is significantly more effective in arresting and reversing coronal caries lesions than a dentifrice containing 1450 ppm fluoride alone.[115-117] In one study, the new dentifrice was compared to two control dentifrices; a matched positive control containing 1450 ppm fluoride alone, and a matched fluoride-free negative control. After six months of product use, improvements from baseline in the representative parameter ΔQ (lesion volume) were 50.7%, 32.3%, and 11.4% for the new arginine-containing dentifrice, the positive control dentifrice, and the negative control dentifrice, respectively. The differences between the negative control and the two fluoride containing dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.003$), were statistically significant.[115]

In a second study, the new dentifrice was compared to two control dentifrices; a positive control containing 1450 ppm fluoride as sodium fluoride in a silica base, and a matched fluoride-free negative control. After six months of product use, improvements from baseline in the parameter ΔQ (lesion volume) were 50.6%, 34.0%, and 13.1% for the new arginine-containing dentifrice, the positive control dentifrice, and the negative control dentifrice, respectively. Once again, the differences between the negative control and the two fluoride-containing dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.008$), were statistically significant.[116]

In a third study, the new dentifrice was compared to a matched positive control dentifrice containing 1450 ppm fluoride alone. After six months of product use, improvements from baseline in the parameter ΔQ (lesion volume) were 44.6% and 28.9% for the new arginine-containing dentifrice and the positive control dentifrice, respectively. The difference between the new dentifrice and the positive control was statistically significant ($p < 0.001$).[117]

Two root caries studies in adults have each shown that the new dentifrice containing 1.5% arginine and 1450 ppm fluoride in a calcium base is significantly more effective in arresting and reversing root caries lesions than a dentifrice containing 1450 ppm fluoride alone.[118,119] In one study, the new dentifrice was compared to two control dentifrices; a positive control containing 1450 ppm fluoride as sodium fluoride in a silica base, and a matched fluoride-free negative control. After six months of product use, clinical hardness measures showed that only one lesion (0.7%) was worse in the new dentifrice group compared to 9.0% and 18.2% in the positive and negative control groups, respectively. In addition, 61.7%, 56.0%, and 27.0% of the lesions showed improvement for the new arginine-containing dentifrice, the positive control dentifrice, and the negative control dentifrice, respectively. The differences in the distribution of lesion change scores between the negative control and the two fluoride-containing dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.006$) were statistically significant.[118] In the second study, the new dentifrice was compared to a matched positive control containing 1450 ppm fluoride. After six months of product use, 70.5% of root caries lesions improved for subjects using the new dentifrice compared to 58.1% for subjects in the positive control group. The difference in the number of root caries lesions being hardened in the new dentifrice and positive control groups was statistically significant ($p < 0.05$).[119]

Finally, a two-year conventional caries clinical study has proven that two dentifrices containing 1.5% arginine and 1450 ppm fluoride in a calcium base, one with di-calcium phosphate and the other with calcium carbonate, are significantly more effective in preventing the formation of cavitated caries lesions than a dentifrice containing 1450 ppm fluoride alone. Three trained and calibrated dentists examined the children at baseline and after one and two years using the National Institute of Dental Research Diagnostic Procedures and Criteria. The number of decayed, missing, and filled teeth (DMFT) and surfaces (DMFS) for the three study groups were very similar at baseline, with no statistically significant differences among groups. After one year, there were no statistically significant differences in caries increments among the three groups. After two years, the two groups using the dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm F had statistically significantly ($p < 0.02$) lower DMFT increments (21.0% and 17.7% reductions, respectively) and DMFS increments (16.5% and 16.5%) compared to the control dentifrice. The differences between the two groups using the new dentifrices were not statistically significant. The results of this pivotal clinical study support the conclusion that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provide superior protection against caries lesion cavitation to dentifrices containing 1450 ppm fluoride alone.[120]

This Special Issue of *The Journal of Clinical Dentistry* reports one coronal and one root caries study,[115,118] together with *in vivo* and *in situ* mechanism of action studies that demonstrate that this new technology works by targeting dental plaque to modulate bacterial metabolism and raise plaque pH which, in turn, reduces demineralization and enhances remineralization of early caries lesions.[121,122] A Special Issue of the *Journal of Dentistry* reports two additional coronal and one additional root caries clinical studies.[116,117,119]

The following two papers in this Special Issue will describe the results of clinical studies which demonstrate the effectiveness of an innovative new dentifrice technology, based upon 1.5% arginine in combination with an insoluble calcium compound and 1450 ppm fluoride, in arresting and reversing the caries process and, thereby, providing superior caries prevention to a regular fluoride dentifrice. The final two papers report the evaluation of the enhanced effects of this new dentifrice technology on *in situ* demineralization and remineralization of dental enamel as com-

pared to a regular fluoride dentifrice, and the results of plaque metabolism studies showing the effects of this new dentifrice technology on plaque metabolism and plaque pH.

Summary and Conclusions

This paper provides an overview of modern concepts of dental caries, including its etiology, prevalence, and risk factors. The multifactorial nature of the disease is reviewed and the concept of reducing caries progression by reducing pathological factors and restoring caries balance is discussed. In addition, the role and efficacy of fluoride in reducing and preventing caries is highlighted, demonstrating its successes and limitations. Finally, a new innovative dentifrice technology, based upon arginine in combination with fluoride, is introduced.

In summary, the following scientific concepts are critical to the future of caries research and the development of new preventive measures. First, dental plaque is a complex oral biofilm which displays unique behavior that is quite different from the behavior of its constituent planktonic species. Second, dental caries results from an ecological shift in dental plaque from a healthy to a pathogenic flora. Third, dental caries is a process, not an endpoint, which can be monitored using advanced and discerning caries detection methods and, up to the point of cavitation, may be arrested and reversed. Fourth, the caries process is a dynamic balance between pathological and protective factors; it can progress if the pathological factors are dominant and can be reversed if the protective factors prevail.

These concepts are discussed in the broad context of the diversity and metabolic integrity of dental plaque, the role of fermentable dietary sugars and cariogenic bacteria in the development of plaque and the production of acid at the tooth surface, adaptive mechanisms which help balance plaque pH and microbial homeostasis, the chemistry of de- and remineralization, and caries lesion development over time.

In discussing risk factors and the concept of reducing risk, the susceptible host tissue and the cariogenic bacteria in the plaque biofilm are identified as targets for preventive therapy.

Fluoride toothpaste targets the tooth by delivering low levels of fluoride to the mouth to reduce demineralization and enhance remineralization. Thus, fluoride functions as a protective factor by helping to arrest and reverse the caries process. However, it does not prevent initiation of the caries process. Fluoride does not directly target dental plaque, which suggests that a dual approach of combining an agent which targets dental plaque to control plaque pathogenicity and virulence with fluoride to target the host tissue, would have potential to deliver a step-change improvement in caries prevention.

Researchers and the dental profession are looking for new technologies that will promote remineralization of early caries lesions, and reverse the caries process at the earliest possible stage in order to take conservative therapy and minimum intervention to the next level. They also recognize that conventional clinical methods have limitations in detecting and assessing early caries lesions and the caries process itself. State-of-the-art methods, such as QLF and ECM, which have been developed, refined, and optimized to enable detection and assessment of pre-cavitated lesions over time, offer advantages in this regard.

A novel technology, based on arginine and an insoluble calcium compound in a fluoride-free toothpaste, has been identified as providing anticaries efficacy that is comparable to the efficacy of a 1100 ppm sodium fluoride toothpaste. This technology targets dental plaque to prevent initiation and progression of the caries process by reducing pathological factors. As the mechanisms of action of arginine and fluoride are highly complementary, a next generation dentifrice has been developed, which combines arginine and insoluble calcium with fluoride, and has been clinically proven, using both state-of-the-art and conventional detection methods, to provide superior caries prevention. Importantly, this new technology offers the potential to meet the unmet needs for early intervention.

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A Clinical Investigation Using Quantitative Light-Induced Fluorescence (QLF) of the Anticaries Efficacy of a Dentifrice Containing 1.5% Arginine and 1450 ppm Fluoride as Sodium Monofluorophosphate

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Abstract

- **Objective**: The purpose of this study was to assess the ability of a new dentifrice containing arginine, an insoluble calcium compound, and fluoride to arrest or reverse naturally occurring buccal caries lesions measured using Quantitative Light-induced Fluorescence (QLF).
- **Methods**: Three study groups used dentifrices which contained 1) 1.5% arginine and 1450 ppm fluoride as sodium monofluorophosphate (experimental), 2) 1450 ppm fluoride as sodium monofluorophosphate (positive control), and 3) no fluoride (negative control). All three dentifrices were formulated in the same calcium base. The study participants were from three schools in the city of Chengdu, Sichuan Province, China. A total of 446 of 450 recruited subjects completed the study. Of these, 147 were in the experimental, 148 in the positive control, and 151 in the negative control groups. The initial age of the children was 10–12 years (mean 11.4 ± 0.54); 47.5% were female.
- **Results**: Using QLF, assessments of buccal caries lesions were made at baseline and after three and six months of product use. For ΔQ, representing lesion volume, the baseline mean value for the three groups was 27.30, and at the three-month examination the mean values were 16.76, 19.25, and 25.89 for the experimental, positive, and negative control dentifrices, respectively. This represents improvements from baseline of 38.6%, 29.5%, and 5.2%. At six months, the ΔQ values for the three groups were 13.46, 18.47, and 24.18, representing improvements from baseline of 50.7%, 32.3%, and 11.4%. For all QLF metrics, ΔF (loss of fluorescence), area, and ΔQ, the differences between the negative control and both the experimental and positive control groups were statistically significant ($p \leq 0.01$). The differences between the experimental and positive control groups attained statistical significance for ΔQ ($p \leq 0.003$) at the six-month examination.
- **Conclusion**: It is concluded that both of the fluoride-containing toothpastes are significantly better at arresting and reversing buccal caries lesions than the non-fluoride toothpaste. Furthermore, it is concluded that the new dentifrice containing arginine, an insoluble calcium compound, and fluoride provides significantly greater anticaries benefit than a dentifrice containing fluoride alone.

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Introduction

Fluoride has been the cornerstone of caries prevention in dentistry since it first became widely available some 50 years ago. It is one of the great success stories in public health medicine. Indeed, the widespread use of fluoride-containing toothpastes is believed by most experts to be the most significant contributor to the dramatic decline in dental caries seen in many parts of the world.[1] In spite of this success, caries experience is neither consistently declining around the world, nor is it declining to zero. Data from established market economies show that the decline in dental caries prevalence and severity has slowed, and in some cases appears to have halted, whereas data from many developing

market economies show that caries experience has increased. The introductory paper in this Special Issue provides a review of these data, and concludes that dental caries remains a prevalent condition in childhood, adolescence, and throughout adulthood.[2]

Today, there is wide recognition that new strategies are needed to supplement existing measures to reduce caries risk and improve the dental health of individuals and populations globally.[2-4] Furthermore, it is well appreciated that a paradigm change in caries prevention is timely and appropriate.[2,5,6] The development of new strategies and methods to promote remineralization of caries lesions and, as a corollary, the use of advanced and discerning clinical methods that allow detection of such lesions at an early stage is an important next step.[7,8] Among the variety of approaches that may make this possible, new and more effective oral hygiene products, especially toothpastes that deliver superior caries prevention, have an important role to play if there is to be continued progress with the caries problem.[2,9]

Dental caries is a disease caused by the fermentation of sugars to acids by the acid-producing bacteria present in dental plaque overlying the tooth. These acids dissolve calcium and phosphate, causing demineralization of the tooth's enamel. Fluoride, which is clinically proven to be effective in reversing the caries process and reducing cavities, targets the tooth surface and works primarily by promoting remineralization of demineralized tooth enamel, and by reducing enamel demineralization.[2,10]

An alternative and complementary approach to preventing dental caries is to target dental plaque and reduce its pathogenicity and virulence. A review paper provides a broad overview of research approaches that had been validated or were under investigation at the time of writing.[11] The introductory paper in this issue provides a brief overview of new technologies to deliver a step-change improvement in everyday caries management, and identifies the approach of modulating plaque physiology to control plaque pH as being of special interest. [2]

A new and innovative technology, based upon a combination of arginine, an insoluble calcium compound, and fluoride has been developed and shown to have greater efficacy in preventing, arresting, and reversing the caries process than fluoride alone. Specifically, calcium-based toothpastes containing 1.5% arginine and either 1450 or 1000 ppm fluoride as sodium monofluorophosphate (MFP) have been shown in a series of *in situ* clinical studies to inhibit demineralization and enhance remineralization more effectively than calcium-based toothpastes containing the same level of fluoride alone.[12] Further, *in vivo* mechanism of action studies have indicated that the presence of arginine modulates plaque metabolism (producing ammonia to neutralize plaque acids) and plaque pH which, in turn, may help to control aciduric acid-producing organisms, such as *S. mutans*.[13]

Over the past several decades, the assessment and validation of the caries benefit of new oral care products have been conducted in randomized controlled caries clinical trials (RCTs). These have generally been conducted in children or adolescents, and have traditionally measured changes in cavitation over a two- to four-year time period. While RCTs have been the longstanding method for determining anticaries benefits, it is apparent from academic consensus meetings held during the past five to ten years that there is a significant opportunity to develop new clinical methods to study the early stages of the caries process, pre-cavitation, to complement and enhance the knowledge derived from conventional caries clinical methods.[3,4] In particular, the ability to detect the progression and reversal of caries lesions at the early stages would enhance our ability to assess and validate the caries benefits of new preventive strategies and measures, especially new technologies that intervene early in the caries process. Detection of lesions in the early stages enables monitoring of the progression of the disease through the various stages of the caries process rather than simply monitoring an endpoint, such as cavitation.

Recognition of the limitations of conventional RCTs has stimulated clinical research in both industry and academia, with the result that significant steps have been made toward improved detection and assessment of both cavitated and non-cavitated lesions.[4,14] The development and validation of Quantitative Light-induced Fluorescence (QLF) to detect and assess early caries lesions is of particular interest to the study reported in this paper. This, and other detection methods, were discussed at length at the "2005 Clinical Models Workshop: Remin-Demin, Precavitation, Caries," sponsored by the National Institute of Dental and Craniofacial Research (NIDCR) and the Task Force of Design and Analysis in Dental and Oral Research. Regarding QLF specifically, the meeting conclusions included that: 1) the validation of what QLF measures (the content validity) is excellent; QLF has been shown to correlate well with the "gold" standard for content validity, *i.e.*, Transverse Microradiography (TMR); 2) the strengths of QLF for use in *in situ* and *in vivo* studies are numerous; initial problems with the method have been resolved, including choice of surfaces, drying, and repositioning; and 3) demonstration of the ability of QLF to distinguish products of known efficacy will further validate the predictive power (the predictive validity) of the method.[15-17]

Subsequently, additional clinical studies comparing toothpastes with and without fluoride have served to further demonstrate the suitability of the QLF method to detect and monitor early caries lesions, and to validate the predictive validity of QLF by differentiating products of known anticaries efficacy previously demonstrated in randomized clinical trials using traditional methods.[18] Specifically, two 1450 ppm fluoride toothpastes, one containing sodium fluoride (NaF) and the other containing sodium monofluorophosphate (MFP), were compared to a non-fluoride control for their ability to arrest and reverse early caries lesions in children aged around 12 years old. Lesions were longitudinally monitored over time, and improvements in all three QLF lesion parameters (ΔF, area, and ΔQ) were seen for both of the two fluoride and the non-fluoride groups over the six months of the study. Statistically significant differences were seen between the non-fluoride toothpaste and the MFP toothpaste after three months, and between both fluoride-containing toothpastes and the non-fluoride groups after six months. No significant difference between NaF and MFP toothpastes was reported.[18] This result mirrors the effects of the use of fluoride toothpaste in reducing the formation of cavities seen in previous conventional RCTs[19] and, thereby, clearly demonstrates the predictive validity of the QLF method.

Likewise, the results of a study in which the effects in re-min-

eralizing white spot lesions of a non-fluoride control dentifrice were compared to the effects of a dentifrice containing 950 ppm fluoride, further validate the predictive value of the QLF method. At three, six, and twelve months, the fluoride dentifrice group demonstrated statistically significantly greater reductions in lesion area and fluorescence loss than the non-fluoride control group. Thus, the study demonstrated that regular use of the fluoride toothpaste resulted in the arrest and reversal of white spot lesions, whereas regular use of the non-fluoride toothpaste did not.[20] The study investigators concluded that "since the impact of fluoride dentifrices has been clinically demonstrated on numerous occasions using the conventional caries detection methods, these data indicate the ability of QLF to quantify this effect using a relatively small panel of subjects and a reduced period."[20]

QLF is a method of longitudinally imaging teeth to measure the area and degree of demineralization of the tooth structure by taking advantage of the fluorescent properties of the teeth. It works using the principle that dentin fluoresces green when illuminated with blue light, and by blocking out the blue light using a band pass filter, the amount of green fluorescence can be assessed. When a carious lesion is present, a dark patch is observed due to loss of fluorescence caused by both the illuminating blue light and the fluorescent green light being scattered within the lesion. By comparing the amount of fluorescence in the demineralized area to that of the surrounding sound enamel, the fluorescence loss (ΔF) can be calculated. By applying a threshold to the image, based on the degree of fluorescence loss, the area of the lesion can be quantified. A further parameter known as ΔQ, calculated from the fluorescence loss and the area of the lesion, indicates the volume of the demineralized lesion.[21]

The purpose of this study was to assess the ability of a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and fluoride to arrest or reverse naturally occurring buccal caries lesions in children using QLF. Three study groups used dentifrices which contained 1) 1.5% arginine and 1450 ppm fluoride as sodium monofluorophosphate (experimental), 2) 1450 ppm fluoride as sodium monofluorophosphate (positive control), or 3) no fluoride (negative control). All were formulated in the same calcium base.

Materials and Methods

This six-month study was a randomized, controlled, double-blind clinical trial in which an experimental dentifrice was compared with both positive and negative controls. The study received ethical approval from the Institutional Review Board of Sichuan Province Committee for Oral Health.

The study participants were children, aged 10–12 years at the start of the study, from three primary schools in the city of Chengdu, Sichuan Province, China. Schools were selected on the basis of their ability to provide supervised brushing to the participants. The communities served by these schools were similar in terms of socio-economic factors. Chengdu's water supply contains 0.3 ppm fluoride.

Participating subjects had to have at least one visible white spot lesion on the buccal surface of one of the upper six anterior teeth, and a signed consent form from a parent or guardian. Subjects meeting the screening criteria were randomly allocated to groups by the study administrator. There were three products used in this study:

1. Experimental dentifrice: 1.5% arginine and 1450 ppm fluoride as sodium monofluorophosphate in a calcium base (Colgate-Palmolive Company, New York, NY, USA);
2. Positive control: 1450 ppm fluoride as sodium monofluorophosphate toothpaste in a calcium base (Colgate-Palmolive Company, New York, NY, USA); and
3. Negative control: Non-fluoride toothpaste in a calcium base (Colgate-Palmolive Company, New York, NY, USA).

Two tubes of dentifrice and a Colgate® Extra Clean toothbrush (Colgate-Palmolive Company, New York, NY, USA) were supplied after the baseline, and at the three-month examinations. The tubes were individually labelled for each study participant and supplied in plain white laminated tubes to conceal the formulation. Only one type of toothpaste was assigned per family, and additional tubes of toothpaste were available on request from participants.

Participants were given oral hygiene instruction and advised to brush at least twice per day (morning and evening) with the toothbrush and dentifrice supplied. During school days, participants brushed in the afternoon with their allocated toothpaste for two minutes under the supervision of the school nurse.

A screening examination was conducted to assess if consenting subjects were suitable for inclusion in the study. For those subjects recruited into the study, assessments were performed at each of the three examination periods (baseline, three, and six months). These examinations were performed by the same examiner in the school nurse's office in a darkened room to control ambient light.

Between three and five images per subject were taken of the upper anterior teeth so that clear views of any lesions could be captured. The teeth were dried with compressed air for five seconds prior to being imaged. The images were captured using a custom camera system consisting of a high resolution 3 CCD camera (Jai M91P, Jai Corporation, Copenhagen, Denmark), a 16 mm f1.4 lens (Pentax, Slough, UK), and a long pass yellow filter (495 nm, Schott, Stafford, UK). The light source was a 150W fiber optic unit with variable output (Fiber Lite, PL-900, Dolan & Jenner, Boxborough, MA, USA) with a blue band pass filter (370 nm, Dolan & Jenner). This was connected to a fiber optic ring illuminator mounted onto the camera (MA3172, Dolan and Jenner). The camera and illuminator were mounted in a geometry stabilizing unit (Figure 1). This, together with video repositioning software, enabled subjects to be accurately repositioned at each visit. Images were acquired using QLF Patient software (Inspektor Research Systems BV, Amsterdam, The Netherlands). For the three- and six-month visits, a grab level of at least 0.95 was used with the video repositioning software. Images were analyzed to calculate lesion area and loss of fluorescence using QLF 2.00 software (Inspektor Research Systems BV). All images were taken by the same examiner.

Statistical Analysis

The QLF software was used to calculate the area (mm^2) and loss of fluorescence, ΔF (%), of the lesion using a threshold of difference of 5% from the reconstructed image. The ΔF parameter is a surrogate measure of lesion depth. ΔQ (mm^2%), which

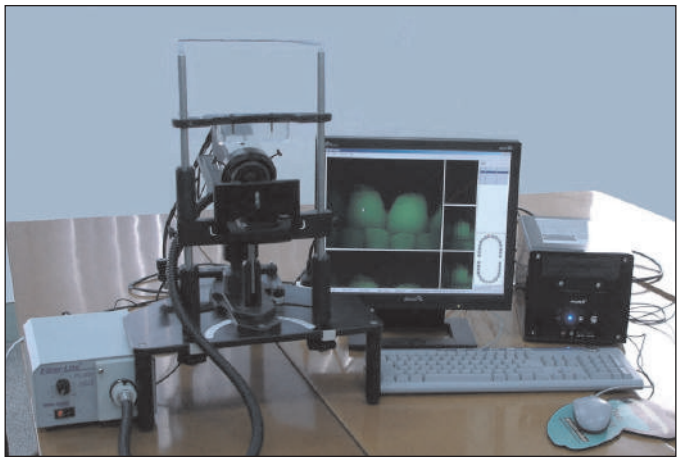

Figure 1. *Image capture and geometry stabilizing apparatus.*

represents the volume of the lesion, is calculated by multiplying ΔF by the lesion area.[21,22]

The primary outcome for this study was the mean subject ΔQ at the six-month examination. The three study groups, for all three outcomes (ΔF, area, and ΔQ), were compared using a linear model controlling for the baseline value and number of lesions per subject, and applying a Bonferroni adjustment to the pair-wise comparisons.

Results

Reproducibility

Prior to the baseline examination, 20 subjects were randomly selected for repeat examinations after 24 hours without using the video repositioning (Vidrep) function of the QLF software. After analysis, the ΔQ values of the same subjects were used to calculate an Intra-Class Correlation coefficient (ICC) which was 0.92. In addition, 40 subjects were randomly selected to be analyzed for repeat image analysis after a one-week interval. An ICC value of 0.86 was reached for the ΔQ values.

Disposition of Subjects

A total of 2,014 subjects consented to take part in the study and were screened. Of these subjects, four-hundred fifty (450) satisfied the inclusion and exclusion criteria and were recruited into the study, had a baseline examination, and were assigned study product. At the three-month examination, two subjects were lost from study, and by the six-month examination, a further two subjects were lost (Figure 2).

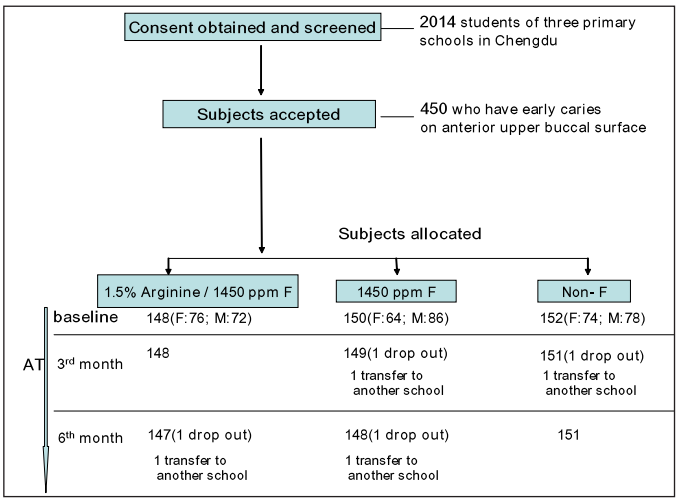

Figure 2. *Disposition of subjects.*

A total of 446 subjects completed the study. Of these, 147 were in the experimental, 148 in the positive control, and 151 in the negative control groups. The age of these subjects ranged from 10–12 years (mean 11.4 ± 0.54) and 47.5% were female. No adverse events were reported in any of the study groups during the course of the study.

Clinical Results

The baseline, three- and six-month subject mean values for ΔF, area, ΔQ, and number of lesions per subject are shown in Table I for the three study groups. The baseline mean loss of fluorescence was 9.34, with an average lesion area of 2.47 mm^2 and mean ΔQ of 27.30. The mean number of lesions per subject was 2.73, with a minimum of one lesion and maximum of six lesions per subject. There were no statistically significant differences between the three study groups for any of the baseline measurements.

Three Months. The baseline-adjusted QLF metrics for the three study groups are shown in Table II, and the statistical significance of paired comparisons after applying a Bonferroni adjustment for multiple comparisons is shown in Table III.

For ΔF, the baseline mean value was 9.34, and at the three-month examination was 8.63, 8.72, and 9.43 for the experimental, positive, and negative control dentifrices, respectively. For area (mm^2), the baseline mean value was 2.47, and at the three-month examination the mean values improved for all three groups to 1.64, 1.82, and 2.29 for the experimental, positive, and nega-

Table I

Subject Mean (SD) Number of Lesions at Baseline and QLF Metrics (ΔF, Area, and ΔQ) at Baseline, and Three, and Six Months for the Three Study Groups

		Baseline			Three Months			Six Months		
Group	N Lesions/ Subject	ΔF (%)	Area (mm^2)	ΔQ (mm^2 %)	ΔF (%)	Area (mm^2)	ΔQ (mm^2 %)	ΔF (%)	Area (mm^2)	ΔQ (mm^2 %)
1.5% Arginine/ 1450 ppm fluoride	2.8 (1.3)	9.17 (2.14)	2.43 (2.10)	27.12 (28.59)	8.53 (1.91)	1.61 (1.53)	16.61 (19.13)	8.14 (1.48)	1.42 (1.13)	13.32 (12.60)
1450 ppm fluoride	2.6 (1.1)	9.38 (2.25)	2.57 (1.84)	27.49 (23.58)	8.73 (1.92)	1.89 (1.54)	19.40 (18.58)	8.67 (1.79)	1.86 (1.46)	18.63 (17.86)
Non-fluoride	2.8 (1.1)	9.46 (2.30)	2.42 (1.86)	27.28 (29.27)	9.52 (2.24)	2.25 (2.01)	25.88 (35.61)	9.20 (2.00)	2.18 (1.92)	24.15 (33.06)

Table II
Baseline-Adjusted Subject Mean (SE) QLF Metrics (ΔF, Area, and ΔQ) at Three and Six Months for the Three Study Groups with Number of Lesions at Baseline as Co-Variable

	Adjusted Three Months			Adjusted Six Months		
Group	ΔF (%)	Area (mm^2)	ΔQ (mm^2 %)	ΔF (%)	Area (mm^2)	ΔQ (mm^2 %)
1.5% Arginine/ 1450 ppm fluoride	8.63 (0.11)	1.64 (0.07)	16.76 (1.01)	8.21 (0.11)	1.45 (0.07)	13.46 (1.06)
1450 ppm fluoride	8.72 (0.11)	1.82 (0.07)	19.25 (1.01)	8.67 (0.11)	1.79 (0.07)	18.47 (1.06)
Non-fluoride	9.43 (0.11)	2.29 (0.06)	25.89 (1.00)	9.13 (0.11)	2.22 (0.07)	24.18 (1.04)
Baseline Mean	9.34	2.47	27.30	9.34	2.47	27.30

Table III
Pair-Wise Comparisons of Study Groups for the Mean Subject Level Outcome from Linear Model, Controlling for Baseline Values and Number of Lesions per Subject

	Three Months			Six Months		
Comparison	ΔF (%)	Area (mm^2)	ΔQ (mm^2 %)	ΔF (%)	Area (mm^2)	ΔQ (mm^2 %)
Arginine/1450 ppm fluoride to 1450 ppm fluoride	1.00	0.14	0.25	0.01	0.002	0.003
Arginine/1450 ppm fluoride to Non-fluoride control	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
1450 ppm fluoride to Non-fluoride Control	< 0.001	< 0.001	< 0.001	0.01	< 0.001	< 0.001

tive control dentifrices, respectively. For ΔQ, the baseline mean value was 27.3, and at the three-month examination the baseline mean values were 16.76, 19.25, and 25.89. This represents improvements from baseline of 38.6%, 29.5%, and 5.2% for the experimental, positive, and negative control dentifrices, respectively. For all QLF metrics, the differences between the non-fluoride, negative control dentifrice group and both the experimental and positive control groups were statistically significant ($p < 0.001$). The differences between the experimental and positive control groups did not attain statistical significance ($p > 0.05$) at the three-month time point.

Six Months. The baseline-adjusted ΔF values at the six-month examination were 8.21, 8.67, and 9.13, and for area 1.45, 1.79, and 2.22 for the experimental, positive, and negative control dentifrices, respectively. For ΔQ, compared to the baseline mean value of 27.3, the values were 13.46, 18.47, and 24.18. This represents improvements from baseline of 50.7%, 32.3%, and 11.4% for the experimental, positive, and negative control dentifrices, respectively. The results for the baseline-adjusted ΔQ at both three and six months are shown in Figure 3. The improvement seen for the experimental group at three months is comparable to that seen at six months for the fluoride-containing positive control dentifrice. For all QLF metrics, the differences between the non-fluoride group and both the experimental and positive

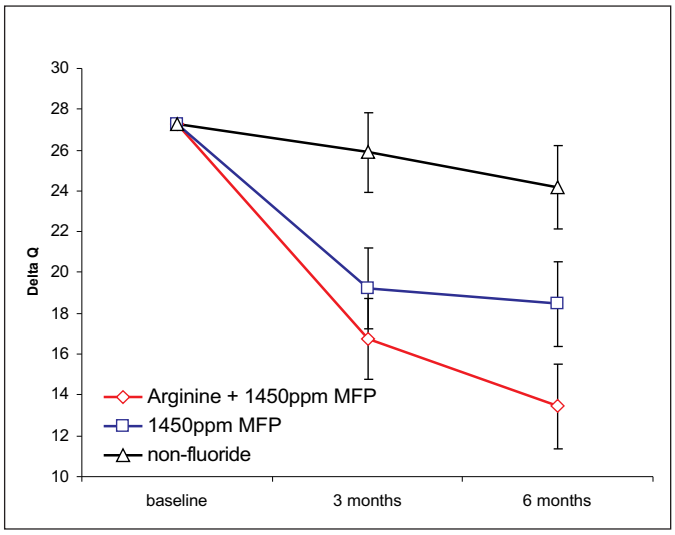

Figure 3. *Mean ΔQ (representing lesion volume in mm2 %) outcomes at baseline, three, and six months with 95% confidence intervals. Data source: Table II.*

control groups were statistically significant ($p \leq 0.01$). The differences between the experimental and positive control groups also attained statistical significance for all QLF metrics ($p \leq 0.003$) at the six-month time point. Figure 4 plots the percentage of subjects with mean improvements from baseline as a function of the percentage mean improvement. It can be seen that the average lesion size was reduced by 50% or more in 45% of subjects for the experimental group, compared to only 23% ($p < 0.001$) in the positive control and 13% ($p < 0.001$) for the non-fluoride negative control group.

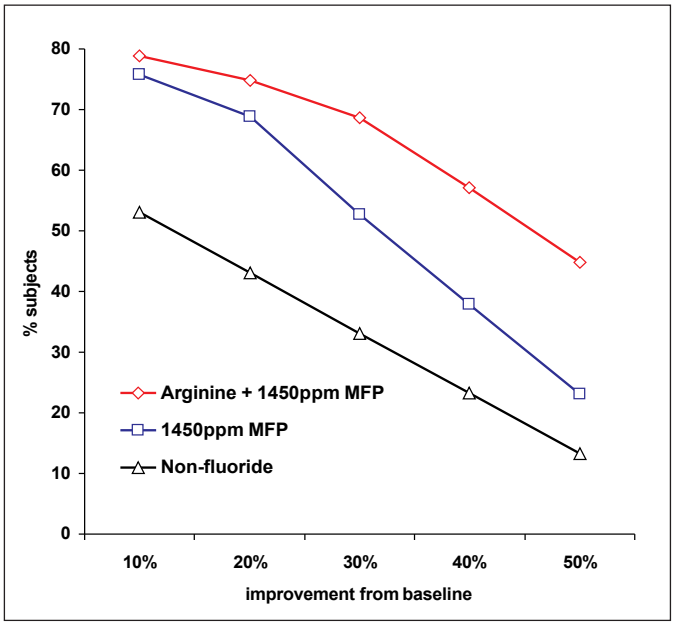

Figure 4. *Distribution of subjects who experienced a reduction of 10–50% in average volume (mm^2 %) at six months for the three study groups.*

Discussion

In the current study, use of the products resulted in improvements in ΔQ from baseline of 38.6%, 29.5%, and 5.2% after

three months, and 50.7%, 32.3%, and 11.4% after six months for the experimental, positive control, and negative control dentifrices, respectively. Figure 5 illustrates an example of QLF images, their respective QLF analyses, and the accompanying clinical photographs of teeth with lesions as they improved over the six-month study period. At the three- and six-month examinations, the differences between the negative control group and both the experimental and positive control groups were statistically significant ($p \leq 0.01$). The differences between the experimental and positive control groups attained statistical significance for all QLF metrics ($p \leq 0.01$) after six months of product use. Further, for the primary outcome variable, ΔQ, the differences between the negative control and the two fluoride-containing dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.003$), were statistically significant after six months of use.

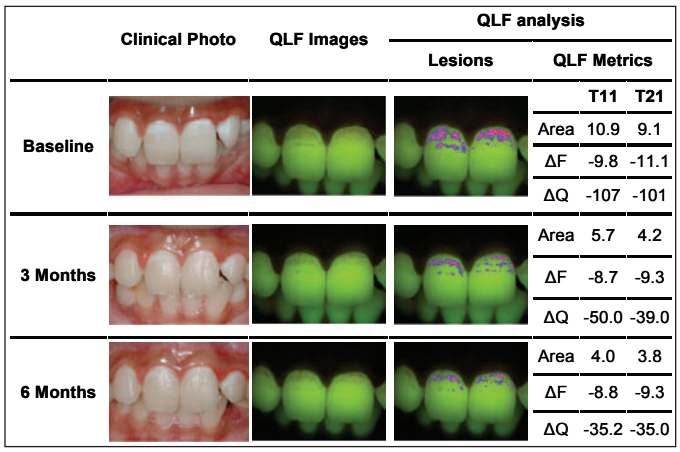

Figure 5. *Quantitative Light-induced Fluorescence (QLF) images at baseline, three, and six months with accompanying clinical photographs,*

These results indicate that regular tooth brushing with a dentifrice can help to arrest and reverse early caries lesions through plaque control, there being a small improvement observed in the non-fluoride group after both three and six months. These results also demonstrate that the inclusion of fluoride in a dentifrice significantly improves the remineralization of early caries lesions, over and above that afforded by tooth brushing alone. Thus, these results confirm the results of the previous QLF study which showed a fluoride toothpaste to be more effective than a non-fluoride toothpaste.[18] These results also reinforce the consistency of the effects of the use of fluoride toothpaste on early lesions, seen in QLF studies, and the effects of the use of fluoride toothpaste in reducing the formation of cavities, seen in previous conventional RCTs.[19] Most importantly, it can be seen that the addition of arginine to a calcium-based fluoride dentifrice provided an additional caries prevention benefit by improving the mean reversal of early lesions from 32.3% to 50.7%. Clearly, the magnitude of the benefit achieved from the addition of the arginine over and above the effects of fluoride alone, is comparable to the magnitude of the benefit achieved by the addition of 1450 ppm fluoride over and above the effects of tooth brushing with non-fluoride toothpaste. Furthermore, because the combination of arginine, insoluble calcium, and fluoride functions both to prevent and to arrest the caries process early in its development, these results indicate that this new and innovative dentifrice technology has the potential to deliver a step change improvement in preventing cavitation.

It could, perhaps, be argued that there is a contribution to the reversal of early lesions from abrasion of the surface of the lesion by the action of tooth brushing with the dentifrice, and, thus, the results seen in this study are not solely due to remineralization of lesions. While this is a theoretical possibility, the literature suggests that any effects due to abrasion over the course of this study are likely to be small. Specifically, normal brushing with a toothpaste, in the absence of an erosive challenge, has been shown to have minimal effects on enamel wear, and is expected to cause insignificant dentin wear during a lifetime of such normal use.[23] Nonetheless, all the dentifrices used in this study used the same base formulation, demonstrating that the effects of both the fluoride and the combination of arginine and fluoride, are in addition to that afforded by tooth brushing.

A previously conducted study in Venezuela compared the anticaries effects of a fluoride-free toothpaste containing arginine bicarbonate and calcium carbonate to a silica-based toothpaste containing 1100 ppm fluoride as sodium fluoride. A total of 726 children, aged between 10 and 11 years at baseline, with a DMFT between 3 and 6, were included in the study. The mean DMFS at baseline was 6.8. After one year, the mean DMFS of the fluoride group was 7.9 compared to 5.7 in the arginine group. After two years, the corresponding mean DMFS values were 7.7 and 7.2, respectively. The data show that the fluoride-free arginine-containing dentifrice is highly effective in reducing the formation of cavities, being comparable in efficacy to that of a 1100 ppm sodium fluoride dentifrice.[24] This study is significant in demonstrating that a non-fluoride technology can be as effective in reducing caries as a 1100 ppm fluoride toothpaste.

Two additional coronal caries studies, using QLF to measure changes in early caries lesions in children, have also shown that dentifrices containing 1.5% arginine and 1450 ppm fluoride in a calcium base are significantly more effective in arresting and reversing coronal caries lesions than dentifrices containing 1450 ppm fluoride alone.[25,26] In one of these studies, the new dentifrice was compared to two control dentifrices; a positive control containing 1450 ppm fluoride as sodium fluoride in a silica base and a matched fluoride-free negative control. After six months of product use, improvements from baseline in the parameter ΔQ (lesion volume) were 50.6%, 34.0%, and 13.1% for the new arginine-containing dentifrice, the positive control dentifrice, and the negative control dentifrice, respectively. Once again, the differences between the negative control and the two fluoride-containing dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.008$), were statistically significant.[25] In the other of these studies, the new dentifrice was compared to a matched positive control dentifrice containing 1450 ppm fluoride alone. After six months of product use, improvements from baseline in the parameter ΔQ (lesion volume) were 44.6% and 28.9% for the new arginine-containing dentifrice and the positive control dentifrice, respectively. The difference between the new dentifrice and the positive control was statistically significant ($p < 0.001$).[26]

Two root caries studies have also been conducted in adults to

evaluate the efficacy of dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride in arresting and reversing root caries lesions, and have shown the arginine-containing dentifrices to be significantly more effective than dentifrices containing 1450 ppm fluoride alone.[27,28] In one study, the new dentifrice was compared to two control dentifrices; a positive control containing 1450 ppm fluoride as sodium fluoride in a silica base, and a matched fluoride-free negative control. After six months of product use, clinical hardness measures showed that only one lesion (0.7%) was worse in the new dentifrice group compared to 9.0% and 18.2% in the positive and negative control groups, respectively. In addition, 61.7%, 56.0%, and 27.0% of the lesions showed improvement for the new arginine-containing dentifrice, the positive control dentifrice, and the negative control dentifrice, respectively. The differences in the distribution of lesion change scores between the negative control and the two fluoride-containing dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.006$), were statistically significant.[27] In the second study, the new dentifrice was compared to a matched positive control containing 1450 ppm fluoride. After six months of product use, 70.5% of root caries lesions improved for subjects using the new dentifrice compared to 58.1% for subjects in the positive control group. The difference in the number of root caries lesions being hardened in the new dentifrice and positive control groups was statistically significant ($p < 0.05$).[28]

Finally, a two-year conventional caries clinical study has proven that two dentifrices containing 1.5% arginine and 1450 ppm fluoride in a calcium base, one with di-calcium phosphate and the other with calcium carbonate, are significantly more effective in preventing the formation of cavitated caries lesions than a dentifrice containing 1450 ppm fluoride alone. Three trained and calibrated dentists examined the children at baseline and after one and two years using the National Institute of Dental Research Diagnostic Procedures and Criteria. The number of decayed, missing, and filled teeth (DMFT) and surfaces (DMFS) for the three study groups were very similar at baseline, with no statistically significant differences among groups. After one year, there were no statistically significant differences in caries increments among the three groups. After two years, the two groups using the dentifrices containing 1.5% arginine, an insoluble calcium compound and 1450 ppm F had statistically significantly ($p < 0.02$) lower DMFT increments (21.0% and 17.7% reductions, respectively) and DMFS increments (16.5% and 16.5%) compared to the control dentifrice. The differences between the two groups using the new dentifrices were not statistically significant. The results of this pivotal clinical study support the conclusion that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provide superior protection against caries lesion cavitation to dentifrices containing 1450 ppm fluoride alone.[29]

The dentifrice tested in the current study, containing arginine in combination with an insoluble calcium compound and either 1450 or 1000 ppm fluoride, has also been shown in a series of *in situ* clinical studies to inhibit demineralization and enhance remineralization of demineralized enamel more effectively than dentifrices containing an insoluble calcium compound and the same level of fluoride alone.[12] Further, *in vivo* mechanism of action studies indicate that the presence of arginine modulates plaque metabolism (pH, and ammonia and lactic acid production) which, in turn, may help to control aciduric, acid-producing organisms such as *S. mutans*.[13]

From the current study, it can be concluded that both the toothpaste containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride, and the toothpaste containing 1450 ppm fluoride alone are significantly better at arresting or reversing buccal caries lesions than a non-fluoride toothpaste. Furthermore, it can be concluded that the arginine-containing toothpaste provides a significantly greater anticaries benefit than does toothpaste containing 1450 ppm fluoride alone.

Taken together, the results of all the studies conducted on this new and innovative dentifrice show that the combination of 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride works in complementary ways to both prevent and arrest the caries process early in its development. Furthermore, it has been proven to more effectively prevent the progression of caries to cavitation than a dentifrice with fluoride alone. These results further suggest that regular, twice-daily use of this new and innovative dentifrice technology on a global basis could deliver a highly significant impact on the prevalence and incidence of dental caries.

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A Clinical Investigation of the Efficacy of a Dentifrice Containing 1.5% Arginine and 1450 ppm Fluoride, as Sodium Monofluorophosphate in a Calcium Base, on Primary Root Caries

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Abstract

- **Objective:** The purpose of this six-month study was to assess the ability of a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride, as sodium monofluorophosphate, to arrest and reverse primary root caries lesions in adults.
- **Methods:** Three test groups used dentifrices which contained either: 1) 1.5% arginine and 1450 ppm fluoride as sodium monofluorophosphate in a calcium base (experimental); 2) 1450 ppm fluoride as sodium fluoride in a silica base (positive control); or 3) no fluoride in a calcium base (negative control). The study participants were residents of the city of Chengdu, Sichuan Province, China. In order to take part, subjects had to have at least one non-cavitated primary root caries lesion. A total of 412 subjects completed the study. They were aged from 50 to 70 years (mean age 64 ± 4.1 years) and 53.6% were female. Efficacy for arresting and reversal of primary root caries was assessed by clinical hardness measures and through the use of the Electrical Caries Monitor.
- **Results:** After three months of product use, clinical hardness measures showed that 27.7%, 24.6%, and 13.1% of lesions had improved in the experimental, positive, and negative control groups, respectively, and 0.7%, 4.5%, and 16.8% had become worse, respectively. The differences in the distribution of lesion change between the negative control group and both the experimental ($p < 0.001$) and positive control ($p = 0.001$) were statistically significant. The Electrical Caries Monitor was also used as an objective measure of lesion severity. The end values increased from baseline to the three-month examinations, but none of the differences between the groups attained statistical significance. After six months, clinical hardness measures showed that only one lesion (0.7%) was worse than at the baseline examination-in the experimental group compared to 9.0% and 18.2% in the positive and negative control groups, respectively. In addition, 61.7%, 56.0%, and 27.0%, respectively, showed improvement for the three groups. The differences in the distribution of lesion change scores between the negative control group and both the experimental ($p < 0.001$) and positive control ($p < 0.001$) were statistically significant, as was the difference between the experimental group and the positive control ($p = 0.006$). The Electrical Caries Monitor end values for the experimental, positive, and negative control groups at the six-month examination were 7.9, 1.9 mega Ωs, and 387 kilo Ωs, respectively. The differences between the negative control group and both the experimental ($p < 0.001$) and positive control ($p < 0.001$) were statistically significant. The difference between the experimental and positive control groups was also statistically significant ($p = 0.03$).
- **Conclusion:** It is concluded that the new toothpaste containing 1.5% arginine and 1450 ppm fluoride, as sodium monofluorophosphate in a calcium base, provided greater anticaries benefits than a conventional toothpaste containing 1450 ppm fluoride. Both fluoride toothpastes demonstrated greater benefits than non-fluoride toothpaste.

(J Clin Dent 2013;24[Spec Iss A]:23–31)

Introduction

Caries research and caries preventive measures have, to a large extent, been focused on children and adolescents. Nonetheless, it is clear from global prevalence data that the elderly dentate population has increased dramatically in many parts of the world as a result of increased life expectancy and prolonged retention of teeth. Not surprisingly, this change in population demographics has been accompanied by a significant increase in caries experience in older adults and, most especially, in the prevalence and incidence of root caries lesions.[1] Interestingly, in the US it has been reported that increases in root caries incidence with increasing age are substantially higher than the corresponding increases in the incidence of coronal caries.[2]

The impact of dental caries in adults, at least in the developed world, is evident in the cost of dental services. In an analysis of the distribution of dental costs across life stages in the US, total costs increased substantially across the age range from adolescents to 50–59 years of age, mainly due to major restorative dentistry costs, and remained at a plateau until age 80–84.[3] In particular, root caries lesions are often difficult and expensive to manage restoratively, and impose a significant burden on dental services.[4]

Root caries is caused by the fermentation of sugars to acids by acid-producing bacteria present in dental plaque overlying exposed root surfaces, and also within the demineralized root caries lesion. These acids dissolve calcium and phosphate causing demineralization of the dentin.[1] The major risk factors for primary root caries include root exposure, reduced saliva flow, inadequate oral hygiene, frequent sugar intake, and the presence of partial dentures, all of which are common in older adults.[5] To date, strategies to remineralize root caries lesions have focused primarily on improved plaque removal and use of fluoride-containing oral care products.

Fluoride is clinically proven to be effective in reversing the caries process and arresting and reversing root caries.[6,7] It works primarily by promoting remineralization and reducing demineralization of enamel and dentin.[1] This reinforces the importance of regular twice-daily tooth brushing with fluoride toothpaste for the prevention of dental caries throughout life.

Root caries sites may be easily cleaned of retentive plaque so plaque removal can help reduce the cariogenic challenge, reducing demineralization and enhancing remineralization. On the other hand, at a given pH dentin is more soluble than enamel, which suggests that under an equivalent cariogenic challenge, dentin will exhibit more demineralization than enamel. For this reason, it has been suggested that more effective measures may be required to enhance fluoride and better protect dentin as compared to enamel.[6,8] Thus, it appears that new approaches are required to supplement existing measures of plaque removal and fluoride, to address the needs of the aging population, and to provide more complete protection against caries.[1]

A paradigm shift in caries prevention is, therefore, both timely and appropriate.[1,9,10] Alternative strategies and methods to promote remineralization of caries lesions and, as a corollary, the use of advanced and discerning clinical methods to assess lesions at an early stage are important next steps.[11,12] New, more effective oral hygiene products, especially toothpastes that deliver superior prevention of dentin caries, have an important role to play if there is to be continued progress with the caries problem and increased focus on the prevention of caries in adults.[1]

An alternative and complementary approach to preventing dental caries is to target dental plaque and reduce its pathogenicity and virulence. The introductory paper in this issue provides a brief overview of new technologies to deliver a step change improvement in everyday caries management. The knowledge that acid-producing bacteria play an important role in invading root caries lesions, and that plaque pH changes may have greater impact on exposed dentin demineralization than on enamel, suggests that modulating plaque physiology to control plaque pH is of special interest to caries prevention in older adults.[1]

A new and innovative technology, based upon arginine in combination with an insoluble calcium compound and fluoride, has been identified and shown in a series of *in situ* clinical studies to deliver greater efficacy in reducing demineralization and enhancing remineralization of demineralized enamel than fluoride alone.[13] This technology has an advantage over fluoride alone because it not only provides fluoride for protection of the tooth surface, it also provides arginine for control of plaque pathogenicity. *In vivo* mechanism of action studies have shown that the presence of arginine results in the modulation of plaque metabolism (enhanced ammonia production to help neutralize plaque acids and restore neutral pH) which, in turn, may help to control acid-producing organisms such as *S. mutans*.[14]

This new technology has been validated in three coronal caries studies using Quantitative Light-induced Fluorescence (QLF) to measure changes in early caries lesions in children. Each of these studies has shown that the new dentifrice containing 1.5% arginine and 1450 ppm fluoride in a calcium base is significantly more effective in arresting and reversing coronal caries lesions than a dentifrice containing 1450 ppm fluoride alone.[15-17] In one study, the new dentifrice was compared to two control dentifrices; a matched positive control containing 1450 ppm fluoride alone and a matched fluoride-free negative control. After six months of product use, improvements from baseline in the representative parameter ΔQ (lesion volume) were 50.7%, 32.3%, and 11.4% for the new arginine-containing dentifrice, the positive control dentifrice, and the negative control dentifrice, respectively. The differences between the negative control and the two fluoride-containing dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.003$) were statistically significant.[15] In a second study, the new dentifrice was compared to two control dentifrices; a positive control containing 1450 ppm fluoride as sodium fluoride in a silica base, and a matched fluoride-free negative control. After six months of product use, improvements from baseline in the parameter ΔQ were 50.6%, 34.0%, and 13.1% for the new arginine-containing dentifrice, the positive control dentifrice, and the negative control dentifrice, respectively. Once again, the differences between the negative control and the two fluoride-containing dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.008$), were statistically significant.[16] In a third study, the new dentifrice was compared to a matched positive control dentifrice containing 1450 ppm fluoride alone. After six months of product use,

improvements from baseline in the parameter ΔQ were 44.6% and 28.9% for the new arginine-containing dentifrice and the positive control dentifrice, respectively. The difference between the new dentifrice and the positive control was statistically significant ($p < 0.001$).[17]

On the basis of the results of the *in situ* clinical studies on the effects in preventing and arresting the caries process,[13] it was anticipated that this new and innovative technology, with 1.5% arginine and 1450 ppm fluoride in a calcium base, might also deliver superior anticaries benefits to a dentifrice with fluoride alone in an older population with root caries.

Root caries clinical study protocols have been used with great success in the evaluation of anticaries efficacy of oral care products because they offer a well-validated method for assessment of effects in adult populations that complements the assessment of effects in children in conventional coronal caries clinical trials. Root caries lesions 1) are amenable to remineralization and so they can be arrested and reversed with topical fluoride and other products, 2) are generally accessible for clinical assessment, and 3) improvement in caries status can often be seen in as little as three months in studies involving relatively small numbers of subjects.[18]

In a number of studies,[18-21] the visual/tactile assessment of change in status of root caries lesions, based on their hardness, has been supplemented by use of the Electrical Caries Monitor (ECM). The ECM is an objective method of assessment, providing data on a continuous measurement scale, that indicates the severity of a lesion with a high degree of sensitivity and specificity.[19] The method is based on the principle that sound dentin has a high resistance to electricity. As dentin demineralizes, the tissue becomes porous to water and acts as a relatively good conductor of electricity, reducing the resistance of the tissue. When a lesion remineralizes, less water is present in the lesion and so the resistance increases. By using the ECM to monitor changes in lesion resistance over time, the process of remineralization or demineralization can be monitored and the efficacy of oral care products assessed effectively, side-by-side, with visual/tactile measures.

One of the first studies to combine visual/tactile assessments of lesion hardness with a dental probe and the ECM was reported by Baysan and colleagues.[18] The study assessed the anticaries efficacy of a high fluoride (5000 ppm) compared to a conventional fluoride (1100 ppm) toothpaste using adult subjects, each with at least one primary root caries lesion. At the six-month examination, 56.9% of the subjects using the high fluoride toothpaste had one or more lesions that had become hardened, compared to 28.6% of the group using conventional toothpaste. The difference was statistically significant. The ECM results after six months showed that there was little change in the mean log resistance for the conventional toothpaste compared to an increase of 0.56 for the high fluoride toothpaste ($p < 0.001$).[18]

Root caries protocols have also been used to compare the efficacy of conventional fluoride to non-fluoride toothpaste. Statistically significant differences were seen between the conventional fluoride and non-fluoride products after six months using both visual/tactile and ECM measures.[20,21] These root caries studies clearly demonstrate that the root caries clinical protocol is able to differentiate the efficacy of products previously differentiated in conventional coronal caries clinical trials.[22,23] The advantage of the root caries protocol is that it can assess anticaries efficacy in a short time period using a relatively small number of subjects with a high degree of sensitivity.

Two root caries studies have been conducted in adults to evaluate the efficacy of the new dentifrice containing 1.5% arginine and 1450 ppm fluoride in a calcium base in arresting and reversing root caries lesions. Each of these studies showed that the new arginine-containing dentifrice is significantly more effective than a dentifrice containing 1450 ppm fluoride alone. The first of these studies is reported in this paper. In the second study, the new dentifrice was compared to a matched positive control containing 1450 ppm F. After six months of product use, 70.5% of root caries lesions improved for subjects using the new dentifrice compared to 58.1% for subjects in the positive control group. The difference in the number of root caries lesions being hardened in the new dentifrice and positive control groups was statistically significant ($p < 0.05$).[24]

The purpose of this study was to assess the ability of the new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride to arrest and reverse primary root caries lesions in an adult population. Three test groups used dentifrices containing 1) 1.5% arginine and 1450 ppm fluoride as sodium monofluorophosphate in a calcium base (experimental), 2) 1450 ppm fluoride as sodium fluoride in a silica base (positive control), and 3) no fluoride in a calcium base (negative control).

Materials and Methods

This six-month study was a randomized, controlled, double-blind clinical trial in which an experimental dentifrice was compared to both positive and negative controls. The study received ethical approval from the Institutional Review Board of Sichuan Province Committee for Oral Health.

The study participants were residents of the city of Chengdu, Sichuan Province, China. In order to take part, subjects had to sign an informed consent, have at least one non-cavitated, primary root caries lesion amenable to examination, and have at least 10 natural uncrowned teeth without evidence of advanced periodontal disease. Participants were excluded from the study if they had taken part in another dental study in the previous three months. Subjects meeting the screening criteria were recruited and randomly allocated to groups by the study administrator, independent of the examination team. A stratification based on the number of root caries lesions present (< 3 and 3 or more) and gender was employed to help maintain group balance.

For each subject, one non-cavitated primary root caries lesion was selected and followed throughout the study. Lesions with clearly demarcated borders on the buccal surfaces of incisor, canine, or premolar teeth were chosen to facilitate the clinical examinations. In cases where multiple similar lesions were present in an individual, the most anterior lesion was selected.

Test Products

There were three products used in this study:

1. Experimental dentifrice: 1.5% arginine and 1450 ppm

fluoride as sodium monofluorophosphate in a calcium base (Colgate-Palmolive Company, New York, NY, USA);
2. Positive control: 1450 ppm fluoride as sodium fluoride toothpaste in a silica base (Colgate-Palmolive Company, New York, NY, USA);
3. Negative control: Non-fluoride toothpaste in a calcium base (Colgate-Palmolive Company, New York, NY, USA).

The sodium fluoride toothpaste in a silica base was chosen as the positive control because this technology is widely used in toothpastes to provide cavity prevention benefits on a global basis. Four tubes of dentifrice and a toothbrush were supplied to each subject at the baseline and three-month examinations. The tubes were individually labeled for each study participant, and supplied in plain white containers to conceal the identity of the product. Only one type of toothpaste was assigned per family, and additional tubes of toothpaste were available upon request from participants.

Participants were instructed in appropriate oral hygiene procedures, and advised to brush at least twice per day (morning and evening) with the toothbrush and toothpaste supplied. To help ensure the products were being used by the study participants, at both the three- and six-month examinations, empty and partially used tubes were returned to the study site before additional toothpaste was supplied.

Examinations

Examinations were performed at baseline, and after three and six months by an experienced examiner. The clinical assessment commenced with an examination of the soft and hard tissues of the mouth. A detailed assessment of the selected root caries lesion was then performed using the methods described by Baysan, *et al.*[18]

Dental Plaque

The amount of plaque overlying each lesion was measured using the Plaque Index of Silness and Löe[25] and scored as follows:

0 = no plaque
1 = a thin film of plaque adhering to the gingival margin and adjacent area of the tooth. The plaque may be seen *in situ* only after using the probe on the tooth surface
2 = moderate accumulation of soft deposits within the gingival pocket or on the tooth and gingival margin which can be seen with the naked eye
3 = abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Dental plaque scores were recorded at the baseline examination to ensure that the groups were similar at baseline with respect to this variable.

Lesion Height, Width, and Distance from the Gingival Margin

The maximum height and width of the lesions were measured to the nearest 0.5 mm using a standard periodontal probe with 1 mm markings (PCPUNC15, Hu Friedy, Chicago, IL, USA). The product of these two values was calculated and used to estimate the "area" of the lesion which was used in the statistical analysis. The distance from the gingival margin to the gingival border of the lesion was also measured to the nearest 0.5 mm.

Hardness

The hardness of each lesion was defined as follows: a soft lesion is one which permitted a sharp probe (No. 5 Sharp Explorer, Shang Hai Dental Instrument Co., China) to penetrate with ease, and there was no resistance to its withdrawal; a leathery lesion permitted a sharp probe to penetrate the surface, but there was some resistance to its withdrawal; a hard lesion is comparable in hardness to the surrounding sound root dentin.

Electrical Caries Monitor Assessment

The ECM IV (Lode Diagnostics BV, Groningen, The Netherlands) was used to measure the electrical resistance of each carious lesion (Figure 1). The ECM measures the electrical resistance of a site on the tooth during controlled drying. The electrical resistance (ohms) was measured at 23.3 Hz and < 0.3 mA while drying the tooth for five seconds at an air flow rate of 5 L/minute. During the ECM readings, the measuring probe was applied to a lesion while the subject held the reference electrode. Measurements were made at the lesion center, and on sound dentin surrounding the lesion, to check the integrity of the lesion measurement. The value at the end of the drying period (end value) for the center of the lesion was recorded and used in the statistical analysis.

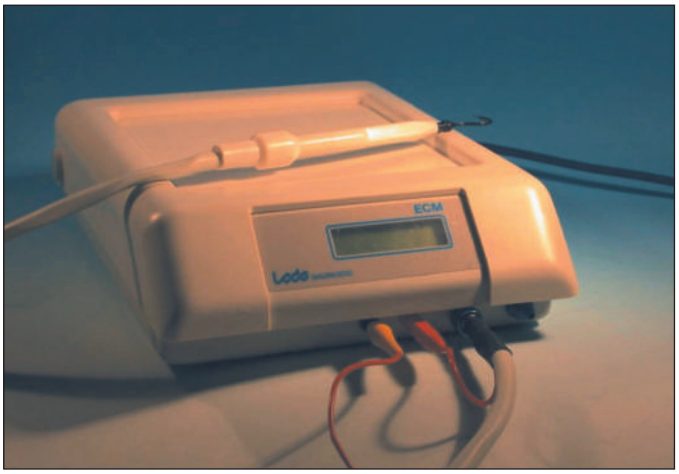

Figure 1. *Electrical Caries Monitor.*

Statistical Analysis

Hardness. Scores of 0, 1, and 2 were assigned to the lesions categorized as hard, leathery, and soft, respectively. The three- and six-month examination scores were compared to baseline. Lesions with scores that reduced were considered to have reversed, and those that increased were considered to have progressed. The frequency distribution of lesions classified as reversing, remaining the same, and progressing were compared using a chi-square test for each of the three possible paired comparisons for the three study groups. The change in lesion hardness scores was the primary outcome for the study.

Electrical Caries Monitor. The ECM resistance values were transformed using the $\log_{10}$ function to ensure a normal distribution and to help equalize the variance among the groups. A linear model with the baseline ECM value as a covariate was used to assess differences between groups, with a Bonferroni adjustment[23] applied to the paired comparisons.

Calibration of Examiner. Before the clinical study started, a reproducibility study was conducted for ten subjects, with repeat measures recorded after 24 hours. The Kappa value[24] for intra-examiner reproducibility for the hardness scores was 0.92. The Intra Class Correlation Coefficient was calculated from the $\log_{10}$ ECM value of lesions and sound tissues around lesions. The ICC values were 0.89 for lesions and 0.92 for sound tissues.

Disposition of Subjects

A total of 3,248 subjects consented to take part in the study and were screened. Of these subjects, 444 satisfied the inclusion and exclusion criteria, had a baseline examination, and were recruited into the study. At the three-month examination, sixteen subjects were lost from the study, and by the six-month examination, a further sixteen subjects were lost (Figure 2). A total of 412 subjects completed the study and formed the basis for the statistical analysis. Of these, 141 were in the experimental, 134 in the positive control, and 137 in the negative control groups. The subjects were aged from 50 to 70 years (mean 64 ± 4.1) and 53.6% were female.

Comparability of the Subjects (Lesions) at Baseline

The frequency distributions of the baseline plaque scores and distance of the lesion from the gingival margin are shown in Table I. The mean plaque scores were balanced and ranged from 2.1 to 2.2 (± 0.9) for the three study groups. The groups were also balanced with regard to the distance of the lesions from the gingival margin, with means of either 0.5 or 0.6 mm (± 0.8), and lesion area which ranged from 6.6 (± 4.7) to 5.5 (± 3.8) mm2. Lesions were classified as either leathery or soft at baseline. The percentage of leathery lesions varied from 60% to 58% for the three groups (Table II). The mean Electrical Caries Monitor end values ranged from 217 to 253 kΩs at baseline, with log10 transformed values from 4.93 to 4.95 (Table III).

Overall these data demonstrate excellent baseline balance between the study groups, with no statistically significant differences identified for any of the paired comparisons ($p > 0.05$).

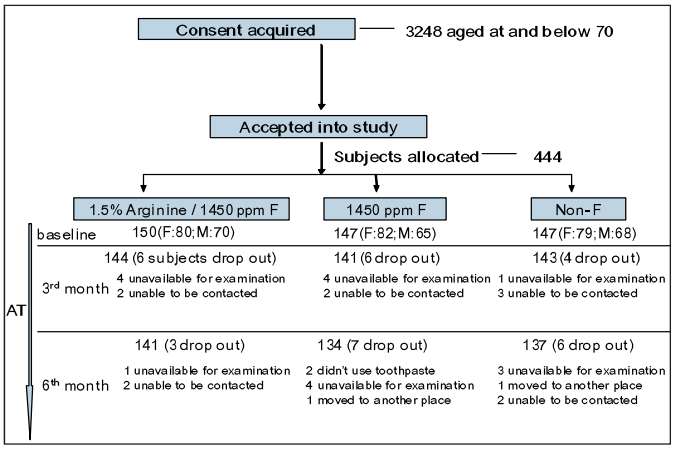

Figure 2. *Disposition of subjects.*

Table I
Baseline Plaque Score and Distance of the Lesion from the Gingival Margin for the Three Study Groups

	Plaque Score				Distance from the Gingival Margin (mm)					
Group	0	1	2	3	0	0.5	1	1.5	2	>2
1.5% Arginine and	4	36	48	53	78	20	27	3	7	6
1450 ppm F as MFP	2.8%	25.5%	34.0%	37.6%	55.3%	14.2%	19.1%	2.1%	5.0%	4.3%
in a Calcium Base										
N = 141										
1450 ppm F as NaF	5	36	35	58	75	13	23	7	10	6
in a Silica Base	3.7%	26.9%	26.1%	43.3%	56.0%	9.7%	17.2%	5.2%	7.5%	4.5%
N = 134										
Non-F Control with	3	31	34	69	81	18	21	1	12	4
a Calcium Base	2.2%	22.6%	24.8%	50.4%	59.1%	13.1%	15.3%	0.7%	8.8%	2.9%
N = 137										

Table II
Cross Tabulation of Baseline Hardness with the Three- and Six-Month Hardness Scores for the Three Study Groups

	Baseline	Three Months			Six Months		
Group	Hardness	Hard	Leathery	Soft	Hard	Leathery	Soft
1.5% Arginine and	Leathery	20	63	1	55	28	1
1450 ppm F as MFP	N = 84 (60%)	23.8%	75.0%	1.2%	65.5%	33.3%	1.2%
in a Calcium Base	Soft	0	19	38	8	24	25
N = 141	N = 57 (40%)	0.0	33.3%	66.7%	14.0%	42.1%	43.9%
1450 ppm F as NaF	Leathery	17	56	6	47	20	12
in a Silica Base	N = 79 (59%)	21.5%	70.9%	7.6%	59.5%	25.3%	15.2%
N = 134	Soft	0	16	39	0	28	27
	N = 55 (41%)	0.0%	29.1%	70.9%	0.0%	50.9%	49.1%
Non-F Control	Leathery	8	49	23	20	35	25
with a	N = 80 (58%)	10.0%	61.3%	28.8%	25.0%	43.8%	31.3%
Calcium Base	Soft	0	10	47	0	17	40
N = 137	N = 57 (42%)	0.0%	17.5%	82.5%	0.0%	29.8%	70.2%

Three Months

For the experimental, positive, and negative control groups, respectively, clinical hardness measures showed that 27.7%, 24.6%, and 13.1% of lesions had improved, and 0.7%, 4.5%, and 16.8% had become worse (Figure 3). The differences in the distribution of lesion change between the negative control group and both the experimental ($p < 0.001$) and positive control ($p = 0.001$) were statistically significant (Table IV).

In all three groups, the mean Electrical Caries Monitor resistance values increased from the baseline to three-month examinations, with the greatest improvement seen in the experimental group (Table III). None of the differences between the groups attained statistical significance ($p > 0.05$).

Six Months

For the experimental, positive, and negative control groups, respectively, clinical hardness measures showed that 61.7%, 56.0%, and 27.0% of lesions had improved, and 0.7%, 9.0%, and 18.2% had become worse (Figure 3). The differences in the distribution of lesion change between the negative control group and both the experimental ($p < 0.001$) and positive control ($p < 0.001$) were statistically significant, as was the difference between the experimental group and the positive control ($p = 0.006$; Table IV). It

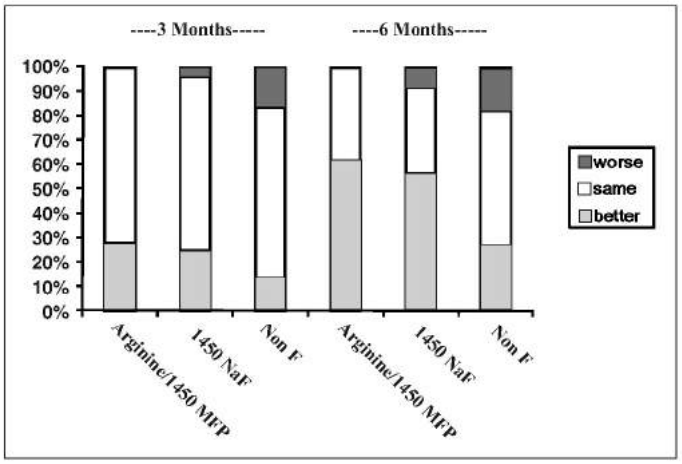

Figure 3. *Percentage of lesions getting better, staying the same, or getting worse for the three study groups after three and six months.*

Table III

Resistance Values for Lesions at Baseline and Three and Six Months for the Three Study Groups

	ECM End Value (Kilo Ωs) Mean (SD)			Log_{10} ECM End Value Mean (SD)			Baseline-Adjusted Log_{10} ECM End Value Mean (SE)	
Group	Baseline	Month 3	Month 6	Baseline	Month 3	Month 6	Month 3	Month 6
1.5% Arginine and 1450 ppm F as MFP in a Calcium Base	217 (991)	862 (2,905)	7,896 (32,505)	4.93 (0.40)	5.13 (0.64)	5.49 (0.99)	5.13 (0.04)	5.50 (0.06)
1450 ppm F as NaF in a Silica Base	249 (673)	407 (1,178)	1,861 (6,389)	4.95 (0.48)	5.10 (0.53)	5.29 (0.79)	5.10 (0.04)	5.28 (0.06)
Non-F control in a Calcium Base	253 (985)	363 (1,450)	387 (1,317)	4.93 (0.47)	5.01 (0.48)	4.99 (0.53)	5.02 (0.04)	5.00 (0.06)
Three Months		Six Months						
All paired comparisons	ns	1.5% Arginine * 1450	p = 0.033					
		1.5% Arginine * non F	p < 0.001					
		1450 * non F	p = 0.003					

Statistical comparison based on linear model using baseline measurement as covariable and applying a Bonferroni adjustment to the paired comparisons to take account of the multiple (3) *post hoc* comparisons. Covariates evaluated at baseline log ECM value of 4.94

Table IV

Change in the Baseline Hardness Status of Lesions after Three and Six Months for the Three Study Groups

	Status after Three Months			Status after Six Months		
Group	Better	Same	Worse	Better	Same	Worse
1.5% Arginine and 1450 ppm F as MFP in a Calcium Base	39 27.7%	101 71.6%	1 0.7%	87 61.7%	53 37.6%	1 0.7%
1450 ppm F as NaF in a Silica Base	33 24.6%	95 70.9%	6 4.5%	75 56.0%	47 35.1%	12 9.0%
Non-F Control with a Calcium Base	18 13.1%	96 70.1%	23 16.8%	37 27.0%	75 54.7%	25 18.2%
Chi-square Test						
Three Months		Six Months				
1.5% Arginine * 1450	ns	1.5% Arginine * 1450	p = 0.006			
1.5% Arginine * non F	p < 0.001	1.5% Arginine * non F	p < 0.001			
1450 * non F	p = 0.001	1450 * non F	p < 0.001			

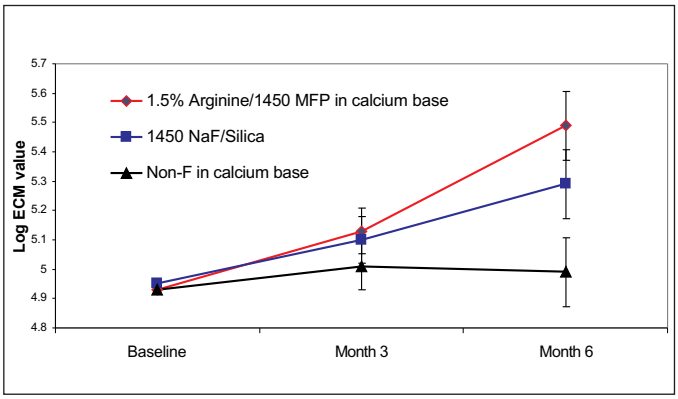

Figure 4. *Log ECM values for lesions at baseline, three, and six months for the three study groups with 95% confidence intervals to mean values*

should be noted that these values are unadjusted for multiple comparisons. With a conservative probability adjustment, achieved by multiplying the p-value by a factor of three (three *post hoc* comparisons), the differences remain statistically significant.

The Electrical Caries Monitor resistance values for the experimental and positive control groups at the six-month examination were 7.9 and 1.9 mega Ωs, respectively, compared to only 387 kilo Ωs for the negative control group (Table III). The differences between the negative control group and both the experimental ($p < 0.001$) and positive control ($p < 0.001$) groups were statistically significant. The difference between the experimental and positive control groups was also statistically significant ($p = 0.033$), the experimental group demonstrating a greater improvement in resistance (Figure 4) that reflects superior efficacy in arresting and remineralizing the root caries lesions than the positive control group.

Discussion

In the current study, clinical hardness measures showed that after three months of product use, improvements were observed in 27.7%, 24.6%, and 13.1% of subjects, whereas 0.7%, 4.5%, and 16.8% became worse for the arginine-containing toothpaste, and the positive and negative control toothpaste groups, respectively (Figure 3). The differences between the non-fluoride control and the fluoride-containing toothpastes were statistically significant ($p = 0.001$; Table IV). After six months, clinical hardness measures showed that 61.7%, 56.0%, and 27.0% of subjects showed improvement, whereas 0.7%, 9.0%, and 18.2% became worse for the arginine-containing toothpaste, and the positive and negative control toothpaste groups, respectively (Figure 3). The differences between the non-fluoride toothpaste group and the two fluoride toothpaste groups were statistically significant ($p < 0.001$), as was the difference between the arginine-containing toothpaste and the toothpaste containing fluoride only ($p = 0.006$; Table IV). These differences were confirmed by the results from the Electrical Caries Monitor (Figure 4), which also showed, after six months of product use, that both fluoride toothpastes were statistically significantly different to the non-fluoride control, and that the arginine-containing dentifrice was statistically significantly different compared to the positive control toothpaste containing 1450 ppm fluoride alone.

These results show that regular tooth brushing with toothpaste can help arrest and reverse root caries through plaque control, there being a small overall improvement observed in the non-fluoride group after six months. The results further demonstrate that the inclusion of fluoride in a dentifrice can significantly improve the remineralization of active root caries lesions, over and above the effects of tooth brushing with toothpaste alone. This result is in good agreement with previously published results showing the superior efficacy of a conventional fluoride compared to a non-fluoride dentifrice. Most importantly, this study shows that the addition of arginine to a fluoride dentifrice with a calcium base provided an additional root caries benefit by improving the mean reversal of root caries lesions from 56% to 61.7%, and by improving the mean progression of root caries lesions from 9% to 0.7%. In terms of clinical outcomes, the reversal and arrest of progression of lesions in this way may be of significant clinical importance, reducing the need for restorative intervention.

A previously conducted study in Venezuela compared the anticaries effects of a fluoride-free toothpaste containing arginine bicarbonate and calcium carbonate to a silica-based toothpaste containing 1100 ppm fluoride as sodium fluoride. A total of 726 children, aged between 10 and 11 years at baseline, with a DMFT between 3 and 6, were included in the study. The mean DMFS at baseline was 6.8. After one year, the mean DMFS of the fluoride group was 7.9 compared to 5.7 in the arginine group. After two years, the corresponding mean DMFS values were 7.7 and 7.2, respectively. The data show that the fluoride-free arginine-containing dentifrice is highly effective in reducing the formation of cavities, being comparable in efficacy to that of a 1100 ppm sodium fluoride dentifrice.[28]

The dentifrice tested in the current study, containing arginine in combination with an insoluble calcium compound and 1450 ppm fluoride, has also been shown in a series of *in situ* clinical studies to inhibit demineralization and enhance remineralization of demineralized enamel more effectively than a calcium-based 1450 ppm fluoride toothpaste alone.[13] It has also been shown through *in vivo* mechanism of action studies that the presence of arginine results in the modulation of plaque metabolism (pH, ammonia and lactic acid production) which, in turn, may help to control acid-producing organisms, such as *S. mutans*.[14]

The dentifrice tested in the current study, containing arginine in combination with an insoluble calcium compound and 1450 ppm fluoride, has also been evaluated in three separate studies which used QLF to assess early buccal caries lesions, for its effectiveness in arresting and reversing early caries lesions in children. Each of these studies has shown that the new dentifrice containing 1.5% arginine and 1450 ppm fluoride in a calcium base is significantly more effective in arresting and reversing coronal caries lesions than a dentifrice containing 1450 ppm fluoride alone.[15-17] In one study, after six months of product use, improvements from baseline in the representative parameter ΔQ (lesion volume) were 50.7%, 32.3%, and 11.4% for the new arginine-containing dentifrice, the matched positive control dentifrice with 1450 ppm fluoride alone, and the matched fluoride-free negative control dentifrice, respectively. The differences between the negative control and the two fluoride-containing

dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.003$), were statistically significant.[15] In a second study, after six months of product use, improvements from baseline in the parameter ΔQ were 50.6%, 34.0%, and 13.1% for the new arginine-containing dentifrice, the positive control dentifrice containing silica and 1450 ppm fluoride as sodium fluoride, and the matched, fluoride-free negative control dentifrice, respectively. The differences between the negative control and the two fluoride-containing dentifrices ($p < 0.001$), as well as the differences between the new dentifrice and the positive control ($p = 0.008$), were also statistically significant.[16] In a third study, after six months of product use, improvements from baseline in the parameter ΔQ were 44.6% and 28.9% for the new arginine-containing dentifrice and the matched positive control dentifrice with 1450 ppm fluoride alone, respectively. The difference between the new dentifrice and the positive control was statistically significant ($p < 0.001$).[17]

From the current study, it can be concluded that both the arginine-containing toothpaste and the 1450 ppm fluoride toothpaste are significantly better at arresting and reversing root caries lesions than a non-fluoride toothpaste. Furthermore, it can be concluded that the arginine-containing toothpaste provides a significantly greater root caries benefit than a toothpaste containing just 1450 ppm fluoride as sodium fluoride.

The results of the current study have been augmented by a second root caries study, in which the new dentifrice was compared to a matched positive control containing 1450 ppm fluoride alone. After six months of product use, 70.5% of root caries lesions improved for subjects using the new dentifrice compared to 58.1% for subjects in the positive control group. The difference in the number of root caries lesions being hardened in the new dentifrice and positive control groups was statistically significant ($p < 0.05$).[24]

Finally, a two-year conventional caries clinical study has proven that two dentifrices containing 1.5% arginine and 1450 ppm fluoride in a calcium base, one with di-calcium phosphate and the other with calcium carbonate, are significantly more effective in preventing the formation of cavitated caries lesions than a dentifrice containing 1450 ppm fluoride alone. Three trained and calibrated dentists examined the children at baseline and after one and two years using the National Institute of Dental Research Diagnostic Procedures and Criteria. The number of decayed, missing, and filled teeth (DMFT) and surfaces (DMFS) for the three study groups were very similar at baseline, with no statistically significant differences among groups. After one year, there were no statistically significant differences in caries increments among the three groups. After two years, the two groups using the dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm F had statistically significantly ($p < 0.02$) lower DMFT increments (21.0% and 17.7% reductions, respectively) and DMFS increments (16.5% and 16.5%) compared to the control dentifrice. The differences between the two groups using the new dentifrices were not statistically significant. The results of this pivotal clinical study support the conclusion that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provide superior protection against caries lesion cavitation compared to dentifrices containing 1450 ppm fluoride alone.[29]

Taken together, the results of all the studies conducted on this new and innovative dentifrice, reported in this Special Issue publication and a Special Issue of the *Journal of Dentistry*, show that a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride works in complementary ways to both prevent and arrest the caries process early in its development. Furthermore, it has been proven to more effectively prevent the progression of caries to cavitation than a dentifrice with fluoride alone. These results further suggest that regular, twice-daily use of this new and innovative dentifrice technology on a global basis could deliver a significant impact on the prevalence and incidence of dental caries.

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In Situ Clinical Effects of New Dentifrices Containing 1.5% Arginine and Fluoride on Enamel De- and Remineralization and Plaque Metabolism

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Abstract

- **Objective**: The primary objective of the three studies reported in this paper was to evaluate the effects of new dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride for their ability to promote remineralization of demineralized enamel, and to prevent mineral loss from sound enamel specimens. A secondary objective was to determine the effects on plaque metabolism with respect to the conversion of arginine to ammonia and sucrose to lactic acid.
- **Methods**: In Study 1, an intraoral remineralization/demineralization clinical model was used to assess the ability to promote remineralization of enamel of two dentifrices containing 1.5% arginine and 1450 ppm fluoride, as sodium monofluorophosphate (MFP), relative to a positive control with dicalcium phosphate dihydrate (Dical) and 1450 ppm fluoride, and a negative control with Dical and 250 ppm fluoride. One of the arginine-containing dentifrices contained Dical, and the other contained calcium carbonate as the source of insoluble calcium. Microradiography and image analysis were used to measure mineral changes. The study used a double-blind crossover design with a two-week treatment period. Each treatment period was preceded by a one-week washout period. Each product was used twice a day for two weeks. In the two other studies, the ability of dentifrices containing 1.5% arginine and fluoride to prevent demineralization of sound enamel blocks was assessed using an intraoral demineralization/remineralization clinical model and a double-blind crossover design with a five-day treatment period. A one-week minimum washout period preceded each treatment phase. Microhardness was used to assess mineral changes. Cariogenic challenges were administered by dipping each intraoral retainer into a 10% sucrose solution four times per day. Each product was used twice per day during the treatment period. Plaque was harvested from the specimens to measure the ability of the plaque to convert arginine to ammonia (Studies 2 and 3) and sucrose to lactic acid (Study 3) at the end of each treatment period. In Study 2, a dentifrice containing 1.5% arginine, Dical, and 1450 ppm fluoride as MFP was compared to a matched positive control containing 1450 ppm fluoride and to a matched negative control containing 250 ppm fluoride. In Study 3, a dentifrice containing 1.5% arginine, calcium carbonate, and 1000 ppm fluoride as MFP was compared to a matched positive control containing 1000 ppm fluoride and to a matched negative control containing 0 ppm fluoride.
- **Results**: In Study 1, the percent mineral changes were +18.64, +16.77, +4.08, and –24.95 for the 1.5% arginine/Dical/1450 ppm fluoride, the 1.5% arginine/calcium carbonate/1450 ppm fluoride, the positive control, and negative control dentifrices, respectively. Study validation was successfully achieved by showing that the positive control was statistically significantly better that the negative control in promoting remineralization ($p = 0.0001$). The two arginine-containing test products were statistically significantly better than the positive control ($p < 0.05$). No significant difference was observed in efficacy between the two arginine-containing products, indicating that efficacy in promoting remineralization was independent of the choice of Dical or calcium carbonate as the source of insoluble calcium. In Study 2, the percent demineralization values were –8.50, +1.67, and +12.64 for the 1.5% arginine/ Dical/1450 ppm fluoride, the positive control, and negative control dentifrices, respectively. Study validation was successfully achieved by showing that the positive control was statistically significantly better at preventing demineralization than the negative control ($p < 0.0001$). The arginine-containing dentifrice was shown to be statistically significantly better at preventing enamel demineralization than the positive control ($p < 0.0001$). Plaque metabolism measures for plaque exposed to the three treatments gave the following values for ammonia production after an arginine-sucrose challenge, expressed in nanomoles per milligram plaque: 162.7; 105.4; and 115.9 for the 1.5% arginine/Dical/1450 ppm fluoride, positive control, and negative control dentifrices, respectively. No statistically significant differences were observed between the three treatments, but the arginine-based dentifrice showed directionally higher ammonia production than both the positive and negative controls. In Study 3, the percent demineralization values were +1.16, +4.96, and +15.34, for the 1.5% arginine/calcium carbonate/1000 ppm fluoride, the positive control, and negative control dentifrices, respectively. Study validation was successfully achieved by showing that the positive control was statistically significantly better at preventing demineralization than the negative control ($p < 0.0001$). The arginine-containing dentifrice was shown to be statistically significantly better at preventing enamel demineralization than the positive control ($p < 0.05$). Plaque metabolism measures for plaque exposed to the three treatments gave the following values for ammonia production after an arginine-sucrose challenge, expressed in nanomoles per milligram plaque: 99.6; 56.2; and 42.2 for the 1.5% arginine/calcium carbonate/1000 ppm fluoride, the positive control, and negative control dentifrices, respectively. Plaque treated with the arginine-

containing dentifrice produced significantly more ammonia than the positive and negative control dentifrices ($p < 0.05$). No significant difference in ammonia production was observed between the two controls. Lactic acid production after a sucrose challenge gave the following values, expressed as nanomoles per milligram plaque: 4.06; 5.12; and 4.64 for the 1.5% arginine/calcium carbonate/1000 ppm fluoride, the positive control, and negative control dentifrices, respectively. No significant difference was observed between the three treatments, but the arginine-based treatment showed directionally lower lactic acid production.

- **Results**: The results of these three studies show that dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride have a significantly improved ability to promote remineralization and prevent demineralization of enamel relative to dentifrices containing the same level of fluoride alone. Two different sources of insoluble calcium were evaluated, Dical and calcium carbonate. Dentifrices with Dical and with calcium carbonate, each in combination with 1.5% arginine and fluoride, provided superior efficacy as compared to matched dentifrices with fluoride alone, and the two products demonstrated comparable efficacy in promoting remineralization. The results of these studies demonstrate that the addition of 1.5% arginine to Dical-and calcium carbonate-based fluoride dentifrices provides superior efficacy in preventing demineralization and promoting remineralization, and, further, indicate that the arginine-containing dentifrices enhance the ability of plaque to metabolize arginine to ammonia.

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Introduction

Dental caries is a prevalent and ubiquitous oral health problem. In simplest terms, dental caries involves the loss of tooth mineral as a result of attack by acids produced through the fermentation of dietary sugars by acid-producing (cariogenic) bacteria. Caries can affect children at a very early age[1-3] and will continue to afflict most individuals throughout adolescence and adulthood,[4-6] thus presenting a significant oral health and public health concern on a global basis.[7-9] Many decades of scientific research have greatly increased our understanding of dental caries, and the application of this knowledge has led to the successful implementation of fluoride-based therapies to help prevent and arrest caries development and progression.[10] Nonetheless, dental caries remains a prevalent disease, largely because of its complex etiology and multi-factorial nature. The existence of dental caries, however, does not alone merit it as a major health issue. The disease must have a measurable impact or cost to society if it is to be elevated to the level of a major healthcare issue. The US Surgeon General's report in 2000 clearly identified the importance of good oral health as an integral part of general health and well being.[11] Healthy and strong teeth are important attributes of good oral health.

There are various ways of expressing the costs of caries to society, the most tangible, of course, being those costs associated with dental restorations. Roughly 60% of dental healthcare costs to dental insurers in the US are associated with dental restorations, and this translates into roughly $45 billion per year.[12] This does not take into account people without dental insurance. For populations of the world who cannot afford or do not have ready access to a dentist the cost is less tangible, but nevertheless important. Pain and suffering associated with caries is a true cost because it diminishes the quality of life. The global prevalence and associated costs have not escaped the attention of academia, government health organizations, the dental profession, and companies associated with developing oral care products. As a result, developing more effective strategies for the prevention of caries remains a key area of interest.

In the area of preventative treatments, most caries preventive regimens utilize fluoride which is, without question, a highly successful caries preventive agent. The dramatic decline in caries prevalence and severity observed over the last several decades has been attributed to fluoride's widespread use.[12] Indeed, the widespread use of fluoride dentifrices has been widely acknowledged by academic experts, the dental profession, and professional health organizations to be the single most important factor contributing to the decline observed in caries over the past several decades.[13,14]

Caries is a disease that is caused by prolonged contact of dental plaque with the tooth surface, accompanied by frequent ingestion of dietary sugars. The caries process is a cyclical and dynamic process with biological origins. Frequent ingestion of sugar, along with incomplete plaque removal associated with poor oral hygiene habits or improper brushing, are key to the progression of caries.[7,15] Cariogenic bacteria that reside naturally in dental plaque, as part of the bacterial community, utilize sugars for energy and use the acid by-product of sucrose fermentation to proliferate in the biofilm and gain a competitive advantage, as many non-pathogenic communal bacteria do not contain protective mechanisms to survive prolonged and frequent exposure to acids.[16] The acidic environment is also harmful to the tooth. Normally, the fluid in contact with the tooth is neutral in pH and supersaturated with respect to enamel. When the pH falls following a sucrose challenge and the consequent formation of acid at the plaque-tooth surface interface, the plaque fluid becomes undersaturated with respect to enamel and the tooth mineral begins to dissolve. Saliva plays a protective role by serving as a source of calcium and phosphate, and helps restore the plaque pH to a more neutral state after a cariogenic challenge so that repair processes may commence.[17-19] Frequent ingestion of sugar creates a shift in the plaque community from one supporting a healthy tooth environment to a more pathogenic state favoring the cariogenic bacteria. This exposes the tooth to longer periods of undersaturation, and shifts the mineral balance in favor of net mineral loss from the tooth.

The mode of action of fluoride has a favorable benefit on the mineral balance.[20,21] Its main role in preventing caries is to modulate the calcium phosphate chemistry at the tooth surface, but it does not influence the biological origin of caries. Specifically, it helps prevent demineralization of the tooth surface under acidic conditions, and helps promote remineralization at neutral pH when the caries challenge is no longer present.[22-28] A limitation of fluoride is that it does little to influence the primary

cause of caries, *i.e.*, acid production by cariogenic bacteria in dental plaque. Because of this, fluoride, as well as other technologies that rely solely on protective-repair mechanisms of the tooth mineral, cannot be expected to provide complete protection.

A promising approach to enhancing the efficacy of fluoride is to combine it with a technology that targets the cause of caries; that is, one that is capable of reducing the overall cariogenic potential of dental plaque. The cariogenic potential of plaque has both chemical and biological components. Nature provides a blueprint on how to lessen the cariogenic potential of plaque. Saliva is a key natural defense system used by the oral cavity to help protect against caries. Aside from saliva's ability to wash away and dilute acids, it contains both chemical and biological protective factors that help modulate the cariogenic potential of plaque. As noted, saliva is neutral in pH and rich in calcium and phosphate, which helps maintain supersaturation with respect to tooth mineral to aid in remineralization and prevention of demineralization. From a biological perspective, saliva is a key source of nitrogen-based metabolites, such as arginine and urea, which are derived from the breakdown of peptides and proteins by salivary enzymes.[17-19] Arginine is metabolized by arginolytic bacteria using the arginine deiminase system to produce energy in the form of adenosine triphosphate, and ammonia and carbon dioxide.[29] The important feature of this pathway is the production of ammonia which neutralizes acids and promotes a more alkaline pH that is unfavorable to cariogenic bacteria. Thus, by utilizing arginine as a survival mechanism against acidic conditions created by cariogenic bacteria, the arginolytic bacteria help to maintain a neutral pH, a condition in which cariogenic bacteria, such as *S. mutans*, are poor competitors in the biofilm, and their ability to dominate the plaque community and cause harm to the tooth is reduced.

Kleinberg has developed a highly effective fluoride-free anticaries technology based on the protective benefits provided by saliva. This technology is based upon a combination of arginine, calcium carbonate, and a cariostatic anion, such as bicarbonate, to deliver anticaries benefits.[19,30] This technology has been proposed to reduce the cariogenic potential of plaque by providing calcium to help maintain supersaturation under conditions of acid challenge, bicarbonate for buffering capacity, and arginine as a metabolic substrate for alkali production. Colgate-Palmolive has broadened the scope of this patented technology by combining arginine with an insoluble calcium compound and fluoride to provide a dentifrice with clinically proven superior anticaries benefits.[31,32]

This article summarizes the results of three intraoral caries clinical studies that demonstrate the enhanced efficacy of dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride in promoting remineralization and preventing demineralization of enamel as compared to matched dentifrices with fluoride alone. Complementary measurements of the ability of arginine-containing dentifrices to enhance arginine metabolism of plaque provide further insight into how this new technology may work to provide an improved benefit when combined with fluoride.

Materials and Methods

Table I provides an overview of the study details for the three studies reported in this article. This overview includes the study design, subject characteristics, study location, test dentifrices, appliance type, enamel specimen type, intraoral treatment, outcome measures, and statistics. Based upon previous remin/demin and demin/remin studies, it has been established that these study designs have residual standard deviations of 30 units and 10 units, respectively. The studies reported in this article were powered to detect differences among treatments of one standard deviation with an 80% probability, which requires minimum sample sizes of 30 and 10 subjects for the remin/demin and demin/remin studies, respectively. In all studies, each treatment phase was preceded by a minimum of a one-week washout period, during which subjects brushed their teeth twice daily with a Colgate adult soft bristle toothbrush and non-fluoride silica-based dentifrice. The duration of this washout period was based upon previous studies which showed no evidence of carryover effects. Each study utilized a randomized, double-blind, crossover design, with a balanced order presentation to further minimize potential carryover effects. Treatment times were based upon previous studies in which clinically relevant product differences were differentiated. All of the dentifrices were over-wrapped and coded to blind the studies. In each study, subjects were required to meet all the inclusion and exclusion criteria outlined in the protocol, and to read and sign an informed consent prior to starting the study. Each study protocol was reviewed by, and received ethical approval from, the appropriate Institutional Review Board.

Study 1

Design. Study 1 utilized an intraoral remineralization/ demineralization clinical model in which subjects wore an intraoral appliance consisting of a lower partial mandibular denture (Figure 1). The intraoral appliances each contained enamel-thin sections that had an artificially induced caries lesion. Mineral changes in the enamel-thin sections were measured by microradiography and image analysis to determine the percent mineral change or net remineralization of the artificially induced lesions. This study was conducted to determine if dentifrices containing 1.5% arginine, an insoluble calcium compound as either calcium carbonate or Dical, and 1450 ppm fluoride as MFP, significantly enhance remineralization, as compared to a dentifrice containing an insoluble calcium compound and 1450 ppm fluoride alone, and to determine if the two arginine-containing dentifrices were equally effective. This study included four treatment periods to test the following dentifrices: 1.5% arginine with 1450 ppm fluoride as MFP in a Dical base (test dentifrice); 1.5% arginine with 1450 ppm fluoride as MFP in a calcium carbonate base (test dentifrice); 1450 ppm fluoride as MFP in a matched Dical base (positive control); and 250 ppm fluoride as MFP in a matched Dical base (negative control).

Procedure for Preparing Enamel-Thin Sections. Details of the preparation and use of enamel-thin sections have been given in several previous publications.[33,34] Enamel blocks approximately 3 mm in width were cut from sound extracted human molars or canine teeth that were free of large cracks, white spots, or dis-

Table I
Summary of Study Details

Study Number	Study Design	Subject Characteristics	Study Location	Test Dentifrice	Appliance Type	Enamel Specimens	Intraoral Treatment	Outcome Measures	Statistics
1	Four cell, double-blind, randomized crossover	30 healthy male and female subjects; aged 18–70; minimum of 20 natural uncrowned teeth (excluding third molars); lower partial mandibular denture with enough space to fit 2 specimens	Colgate-Palmolive Global Technology Center, Mumbai, India	1. 1.5% arginine with 1450 ppm fluoride as MFP in calcium carbonate base 2. 1.5% arginine with 1450 ppm fluoride as MFP in dicalcium phosphate base 3. 1450 ppm fluoride as MFP in dicalcium phosphate base 4. 250 ppm fluoride as MFP, dicalcium phosphate base	Lower partial mandible denture	Acid demineralized human enamel-thin sections	Twice-daily brushing with dentifrice treatment for 2 weeks	% Mineral change 100*(before – after)/before from microradiography	Two-factor (subject and treatment) ANOVA to determine if significant differences exist; Tukey's multiple comparison test for pair-wise differences of treatments
2	Three cell, double-blind, randomized crossover	16 healthy male and female subjects; aged 18–65; minimum of 20 natural uncrowned teeth (excluding third molars)	Colgate-Palmolive Global Technology Center, Piscataway, NJ, USA	1. 1.5% arginine with 1450 ppm fluoride as MFP in dicalcium phosphate base 2. 1450 ppm fluoride as MFP in dicalcium phosphate base 3. 250 ppm fluoride as MFP in dicalcium phosphate base	Upper palatal retainer	Sound bovine enamel	Twice-daily brushing with dentifrice; treatment for 5 days; 4 × daily *ex vivo* sucrose challenge	Primary: % change in enamel micro-hardness = 100* (before – after)/ before Knoop indentation before and after treatment) Secondary: Ammonia production from harvested plaque	Two-factor (subject and treatment) ANOVA to determine if significant differences exist; Tukey's multiple comparisons test for pair-wise differences of treatments
3	Three cell, double-blind, randomized crossover	18 healthy male and female subjects; aged 18–65; minimum of 20 natural uncrowned teeth (excluding third molars)	Colgate-Palmolive Global Technology Center, Piscataway, NJ, USA	1. 1.5% arginine with 1000 ppm fluoride as MFP in calcium carbonate base 2. 1000 ppm fluoride as MFP in calcium carbonate base 3. 0 ppm fluoride in calcium carbonate base	Upper palatal retainer	Sound bovine enamel	Twice-daily brushing with dentifrice; treatment for 5 days; 4 × daily *ex vivo* sucrose challenge	Primary: % change in enamel micro-hardness = 100* (before – after)/ before) (Knoop indentation before and after treatment) Secondary: Ammonia and lactic acid concentrations from harvested plaque	Two-factor (subject and treatment) ANOVA to determine if significant differences exist; Tukey's multiple comparisons test for pair-wise differences of treatments

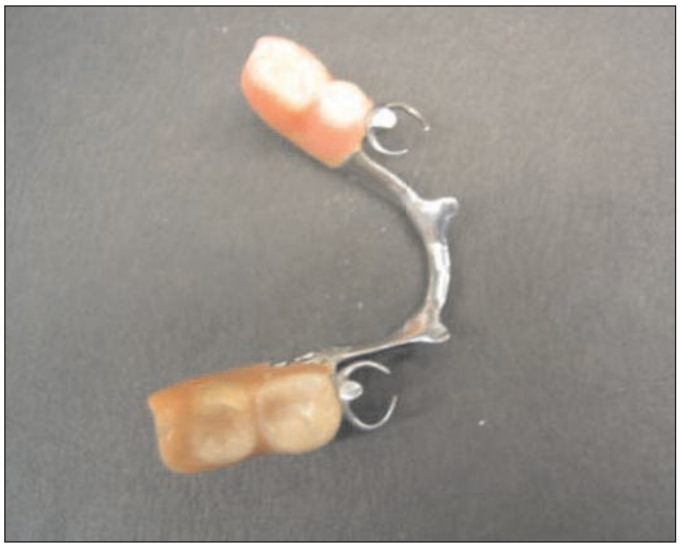

Figure 1. *Intraoral lower mandible partial denture appliance used in remineralization/demineralization study (Study 1).*

coloration. They were then cleaned by scrubbing with a toothbrush and a diluted liquid detergent, and sterilized for four hours using ethylene oxide. The blocks were then mounted in a specimen holder using cyanoacrylate adhesive, and were sectioned with a Leica 1600 Microtome saw (Leica, Bannockburn, IL, USA) to a 150 micron thickness. The thin sections were then embedded in a polyester film together with a nickel-plated marker to ensure consistent area measurement throughout the study. Specimens were stored at room temperature during preparation. In the last step of the preparatory phase, caries-like lesions were formed in the exposed enamel edges of the thin sections by immersing them in 0.1 N acetic acid (Sigma, St. Louis, MO, USA), pH 4.6, for 48 hours at 37°C. The enamel-thin sections were then removed from the demineralizing solution, rinsed with de-ionized water, and air dried at room temperature.

Microradiography and Measurement of Mineral Density Changes of Enamel-Thin Sections. Microradiography was used on the enamel-thin sections to obtain mineral density changes. Mineral density changes were measured from radiographs of

the enamel-thin sections before lesion formation (sound), after lesion formation (untreated), and after treatment with the dentifrice being tested (treated). Image analysis was used to obtain the mineral density profiles. Lesion areas before and after treatment were calculated by subtracting the sound profile from the profile of the untreated and treated profiles to generate difference profiles. The area under the curve of a difference profile represents the lesion area. A custom-designed program was used to overlay the profiles and measure the lesion areas. Mineral changes are expressed as percentage change from the initial lesion size after treatment, as given by the formula below:

Mineral Change = Lesion area before treatment
– Lesion area after treatment
% Mineral Change = Mineral change/
(Lesion area before treatment) X 100

Placement of Enamel-Thin Sections into Lower Partial Mandibular Denture. Depending on the available space for the enamel-thin sections, holes were drilled into the left or right side of the lower partial mandibular denture, slightly larger than the size of the specimen. Two enamel-thin sections were then mounted at this site and held in place by use of a light-cured, non-fluoride dental composite.

Clinical Procedure. Thirty healthy subjects in Mumbai, India, aged 18–70 years, with at least 20 natural teeth and a partial mandibular denture, were recruited into this study. After the one-week washout period, the subjects placed the lower partial denture with the implanted enamel-thin sections into their mouths. The subjects were instructed to keep the appliance in the mouth for 24 hours a day during the two-week treatment phases. With the appliance in the mouth, the subjects were instructed to brush their teeth and the specimens with the assigned dentifrice twice per day (morning and evening before going to bed), for one minute each time, followed by a ten-second rinse with tap water. Subjects were allowed to remove the appliance after meals to clean them. Cleaning was permitted by rinsing the appliance under tap water only. No other oral care product was used during the course of the clinical study.

After each experimental two-week treatment period, the subjects returned the appliances and the specimens were removed for analysis. The subjects then began a one-week washout period before the next two-week treatment period.

Statistical Analysis. The primary measured response was the change in lesion area (% mineral change) before and after treatment. A two-factor ANOVA with the subject and treatment as factors was performed. A difference among treatments was considered significant if a 95% confidence level was achieved. If a significant difference was detected, a Tukey's multiple comparison test was used to validate the study (comparison of positive versus negative control). If the test method was validated, a second two-factor ANOVA, excluding the negative control, was conducted to compare the two test products versus the positive control. A difference among treatments was considered significant if a 95% confidence level was achieved. If a significant difference was detected, a Tukey's multiple comparison test was used to determine which treatments were significantly different from each other.

Study 2

Design. Study 2 utilized an intraoral demineralization/remineralization clinical model in which mineral changes (before and after each treatment) were measured by microhardness. Subjects wore an upper palatal retainer (Figure 2) containing four prepared bovine enamel specimens. These enamel blocks were covered with metal wire mesh to accumulate plaque during the treatment period. This study was conducted to determine if a dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride prevents demineralization significantly more effectively than a matched dentifrice containing 1450 ppm fluoride alone. This study included three treatment periods to test the following dentifrices: 1.5% arginine with 1450 ppm fluoride as MFP in a Dical base (test dentifrice); 1450 ppm fluoride as MFP in a Dical base (positive control); and 250 ppm fluoride as MFP in a Dical base (negative control).

Procedure for Preparing Bovine Enamel Blocks. To prepare the bovine enamel specimens, each bovine tooth was cut into blocks with a final measurement of approximately 5 mm by 5 mm. The blocks were then cleaned, rinsed with distilled water, and sterilized by ethylene oxide for four hours. When not in use during the preparatory phase, blocks were stored in distilled water in a refrigerator.

In the grinding step, the blocks were flattened using a variable speed grinder/polisher with three retaining rings and 15 μ diamond polishing disc (Buehler, Lake Bluff, IL, USA). The polishing disc was wetted with water, and three specimen carriers, which are capable of holding 49 blocks each, were placed on the disc. The blocks were placed dentin-side down in the specimen carriers, and the dentin was ground flat for 2.5 minutes at 100 rpm as approximately three liters of water were poured slowly onto the center of the disc. After grinding, the blocks were removed and visually examined for flatness, and the procedure was repeated until the blocks appeared flat by visual inspection. After this procedure, the blocks were placed enamel-side down in the specimen carriers, and the enamel was ground flat for five minutes at 100 rpm as approximately four liters of water were

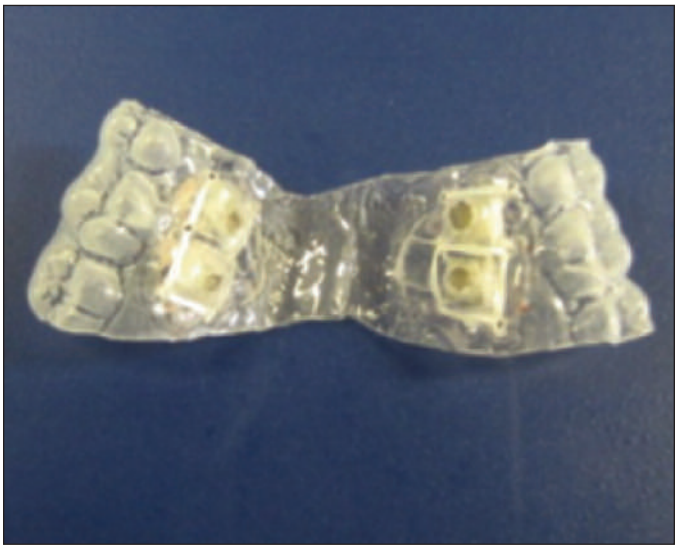

Figure 2. *Intraoral retainer used in demineralization/remineralization studies (Studies 2 and 3).*

poured slowly onto the center of the disc. After grinding, the blocks were removed and visually examined for flatness.

The blocks that passed the visual inspection were then polished using the same apparatus. A polishing cloth was fitted to the polishing disc, and a diamond suspension, METADI® 6 µ (Buehler, Lake Bluff, IL, USA), was sprayed to evenly cover the cloth. Blocks were placed enamel-side down, as previously described, and the system was run for ten minutes at 100 rpm, adding more diamond suspension as needed. The blocks and the polishing cloth were then rinsed with two liters of water over a two-minute time period. The blocks were then polished for another ten minutes, using the same procedure and second polishing suspension, Masterprep 0.05 µ (Buehler, Lake Bluff, IL, USA). The blocks were then washed by pouring 25 ml of 10% w/w Alconox solution (VWR, West Chester, PA, USA) onto the polishing cloth and running it for one minute before the blocks had a final rinse using four liters of water over another five-minute time period. The blocks were then removed and sonicated with distilled water for ten minutes, which was repeated until all appearance of foaming, suds, and cloudiness was gone. The final height of the blocks was determined by a micrometer.

Microhardness Testing of Bovine Enamel Blocks. The microhardness of the enamel blocks was determined using a Micromet 5101 Micro-hardness Tester with Knoop Diamond Indenter and a 50 gram load (Buehler, Lake Bluff, IL, USA). Baseline indents were required to be symmetrical, and readings no greater than 55–60 microns. Blocks were gently buffed with a dry microcloth before testing to both dry the block and remove any surface contaminant. The stage micrometers were zeroed and a block was located with a corner at 0,0. Under low-power magnification, the block was set in place in order to find a clean area. Once found, the indenter was placed over the block and released. After 15 seconds, the indenter was picked up, the micrometer was adjusted in the x direction another 0.01 mm, and another indent was dropped. This was repeated until five indents were made. The indents were then measured using a higher power lens, and the average was obtained for baseline microhardness (M1). After each five-day treatment period, the blocks were reassessed for microhardness (M2) as described above. Percent changes in indentation length 100*(M2 – M1)/M1 were used to determine changes in enamel hardness, as they are directly correlated with mineral content.

Preparation of Retainers for the Intraoral Study. Customized retainers were prepared by first casting an impression of the upper maxillary palate of each subject. Once the impressions were made, a sheet of 0.020 cm thick vacuum forming plastic material (Buffalo Dental Mfg. Syosset, NY, USA) was molded to fit across each subject's roof of the mouth, and to form to molar teeth on either side of the mouth.

Placement of Bovine Enamel Blocks into Retainer. Holes were punched into each of the retainers in order to expose the surface of the bovine enamel blocks to the treatment being used. Two blocks, measuring approximately 4 mm by 4 mm by 1 mm, were placed on both the right and left side of the retainer. They were secured into the retainers by drilling several small holes on the sides of the punched out holes, and stitching dental floss in back of the blocks. A thin sheet of soft dental orthodontic tray wax (Kerr, Romulus, MI, USA) was placed across back of the blocks to secure them into place. Before placing the blocks into the retainer, they were covered with a sheet of wire mesh. The wire mesh was used to accumulate plaque over the five-day treatment period.

Total Ammonia Production from *In Situ* Formed Plaque Samples. The plaque that accumulated on the blocks was collected before the microhardness measurement after treatment (M2), pooled, and stored at –20°C until analysis. The plaque assay procedure was adapted from a previously published method.[35] In the procedure, plaque was kept on ice and the concentration was normalized to 1 mg/ml in 1 phosphate buffered saline, pH 7.4 (Gibco, Grand Island, NY, USA). The samples were mixed with a vortex and then sonicated to break up plaque clusters and homogenize the sample. Each plaque sample was then challenged with sucrose (VWR, West Chester, PA, USA) and arginine (Sigma, St. Louis, MO, USA) to give a final concentration of 0.1% and 5 millimoles, respectively. The samples were incubated in a 37°C shaking water bath for 30 minutes before ammonia production was analyzed. A diagnostic ammonia assay kit (Diagnostic Chemicals Limited, Oxford, CT, USA) was used to quantify the ammonia produced in the plaque.

Clinical Procedure. Sixteen healthy subjects from an established pool of subjects in a clinical database in Piscataway, New Jersey, USA, aged 18-65, with a minimum of 20 natural teeth, were recruited into this study. After the one-week washout period, the subjects placed the custom retainer with the four bovine enamel blocks onto the upper maxillary palate, and they were instructed to brush their teeth, and not the retainer containing the bovine enamel blocks, with the assigned dentifrice twice per day (morning and evening before going to bed) for one minute each time, followed by a 10-second rinse with tap water. Subjects were also instructed to dip their appliances into a 10% sucrose solution four times per day at approximately 9:00 a.m., 11:00 a.m., 4:00 p.m., and 7:00 p.m. for ten minutes each time. The upper retainer was worn for 24 hours per day for each five-day treatment period. Subjects were allowed to remove the appliance only during meal time and to clean them. Cleaning was permitted by rinsing the retainer under tap water only. No other oral care product was used during the course of the clinical study.

After each experimental five-day treatment period, subjects returned their retainers, and the bovine enamel blocks were removed for analysis. The subjects then began a nine-day washout period before the next five-day treatment period.

Statistical Analysis. For the microhardness measurements, the primary response was the percent change in indentation length before and after treatment. The secondary outcome was the ammonia production. A two-factor ANOVA, with the subject and treatment as factors, was conducted on both the primary and secondary outcomes to determine whether significant differences existed between treatments. A difference was considered significant if a 95% confidence level was achieved. If a significant difference was detected, a Tukey's multiple comparison test was used to validate the study outcome (comparison of positive versus negative control) and determine which treatments were significantly different from each other.

Study 3

Design. This study followed the intraoral demineralization/remineralization clinical model used in Study 2. The only difference in the analyses of the samples was that plaque that accumulated on the enamel blocks was analyzed for lactic acid production, in addition to ammonia production. This study was conducted to determine if a dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1000 ppm fluoride prevents demineralization and delivers anticaries benefits significantly more effectively than a matched dentifrice containing 1000 ppm fluoride alone. This study included three treatment periods to evaluate the following dentifrices: 1.5% arginine with 1000 ppm MFP in a calcium carbonate base (test dentifrice); 1000 ppm MFP in a calcium carbonate base (positive control); and 0 ppm MFP in a calcium carbonate base (negative control). Eighteen healthy subjects from the established pool in the clinical database in Piscataway, New Jersey, USA, aged 18–65, with similar age and oral profiles, were recruited into this study.

Total Lactic Acid Production from *In Situ* Formed Plaque Samples. The plaque that accumulated on the blocks was collected before the microhardness measurement after treatment, pooled, and stored at –20°C until analysis. The plaque sample was first resuspended in ice cold 0.03% trypticase soy broth (TSB; Difco, Becton, Dickinson and Company, Sparks, MD, USA) to a final concentration of approximately 0.03–0.04 mg of plaque per ml of TSB. Sucrose then was added to each plaque sample to a final concentration of 10% before incubation for ten minutes at 37°C with mild shaking. After the ten-minute incubation time, the samples first were heated to 80°C for five minutes to kill the bacteria and to release all acids, then cooled on ice water for an additional five minutes. After this cooling, the samples were centrifuged and the supernatant was filtered. The lactate (lactic acid anion) concentration in the supernatant was measured using capillary electrophoresis.

The conditions used to analyze the plaque samples using the capillary electrophoresis were adapted from a previously published method.[36] The separations were carried out on a fused-silica capillary with a 50 cm effective length X 50 µm internal diameter. The optimized buffer system consisted of 20 mM 2,6-pyridine dicarboxylic acid and 0.5 mM hexadecyltrimethyl ammonium bromide, pH 5.66 (Sigma, St. Louis, MO, USA). Because organic acids have little or no ultraviolet (UV) absorbance, detection was accomplished by using 2, 6-pyridine dicarboxylic acid as a background electrolyte (BGE). In this indirect detection method, the BGE has strong UV absorptive properties and produces a high background absorption in the UV detector. In the absence of non-absorbing analytes, the background signal is constant. When ionic analytes are introduced, they displace UV-absorbing additive ions on a charge-to-charge basis, resulting in a negative peak relative to the high UV absorption baselines. With the analysis, the sample was injected by pressure for ten seconds at 0.5 psi. The separation was performed at –25 kV, and the capillary was thermostated at 25°C. The wavelength for indirect UV detection was selected at 254 nm, and the signal with negative peaks was inverted to obtain a more familiar electropherogram to integrate and process.

To correct for injection errors, each sample was run with the incorporation of a 1.5 mM sodium nitrate internal standard, and a calibration curve was constructed using sodium lactate standards (Sigma, St. Louis, MO, USA). The concentration of lactate present in the plaque sample was determined based upon the ratio of lactate/nitrate peak area and the initial plaque weight.

Statistical Analysis. For the microhardness measurements, the primary response was the percent change in indentation length before and after treatment. The secondary outcomes were the ammonia and lactic acid concentrations. A two-factor ANOVA with the subject and treatment was conducted on both the primary and secondary outcomes to determine whether significant differences existed between treatments. A difference was considered significant if a 95% confidence level was achieved. If a significant difference was detected, a Tukey's multiple comparison test was used to determine which treatments were significantly different from each other.

Results

Study 1

This was an intraoral remineralization/demineralization study comparing four dentifrices: 1.5% arginine with 1450 ppm fluoride in a calcium carbonate base; 1.5% arginine with 1450 ppm fluoride in a Dical base; 1450 ppm fluoride in a Dical base (positive control); and 250 ppm fluoride in a Dical base (negative control)

Twenty-nine of thirty panelists successfully completed the study. The inability of one subject to complete the study was not related to product use, rather a result of personal reasons unrelated to the study.

The results of the study are summarized in Table II. Use of all three dentifrices containing 1450 ppm fluoride as MFP resulted in positive mineral changes in the enamel-thin sections, demonstrating that the enamel was remineralized. This is in contrast

Table II

The Effect of Two Dentifrices Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1450 ppm Fluoride on Remineralization of Demineralized Enamel, Expressed as Average Percent Mineral Change, Compared to Control Dentifrices Containing 1450 ppm and 250 ppm Fluoride, Respectively (Study 1)

Products Tested	Product Designation	Percent Mineral Change (± SD)
Dical toothpaste with 250 ppm fluoride as MFP	Negative control	– 24.6 ± 58.2[a]
Dical toothpaste with 1450 ppm fluoride as MFP	Positive control	+ 4.1 ± 29.3[a,b]
Calcium carbonate toothpaste with 1450 ppm fluoride as MFP and 1.5% arginine	Test Dentifrice 1	+16.8 ± 26.6[a,b]
Dical toothpaste with 1450 ppm fluoride as MFP and 1.5% arginine	Test Dentifrice 2	+18.6 ± 37.2[a,b]

[a] The negative control was statistically significantly different from the positive control and from the two test products ($p < 0.0001$).

[b] The two test products were statistically significantly different from the positive control ($p < 0.05$).

to the 250 ppm fluoride (as MFP) negative control where two weeks of product use resulted in net mineral loss in the enamel-thin sections.

Using the full dataset, a two-factor ANOVA using the subject and treatment as factors indicated that the treatment effect was highly significant ($p < 0.0001$). In order to validate the study, the results for the positive control were compared to those of the negative control using a Tukey's multiple comparison test. The positive control was shown to be significantly better than the negative control at promoting remineralization ($p = 0.0001$), which demonstrates study validity. With the study validated, a second two-factor ANOVA was conducted on a dataset excluding the negative control. This analysis indicated that the treatment effect was highly significant ($p = 0.01$). The two test products were compared to the positive control using a Tukey's multiple comparison test. Both of the dentifrices containing arginine (calcium carbonate and Dical variants) were shown to be significantly better than the positive control at remineralizing the enamel-thin sections ($p < 0.05$). There was no statistically significant difference with respect to remineralization of the enamel-thin sections between the two dentifrices containing 1.5% arginine.

Study 2

This intraoral demineralization/remineralization study compared three dentifrices: 1.5% arginine with 1450 ppm fluoride in a Dical base; 1450 ppm fluoride in a Dical base (positive control); and 250 ppm fluoride in a Dical base (negative control).

Twelve of the originally recruited sixteen subjects successfully completed the three treatment phases in this study. The other four subjects did not complete the study for reasons related to inconvenience or discomfort in wearing the upper palatal retainer for the entire course of the study. Product use was not a reason for discontinuing the study.

The results in Table III show that the dentifrice containing 1450 ppm fluoride as MFP in a Dical base (positive control) was significantly better than the dentifrice containing 250 ppm fluoride as MFP in a Dical base (negative control) at preventing demineralization ($p < 0.0001$), thus demonstrating that the study was successfully validated. In this study, the larger the percent demineralization value, the greater is the amount of demineralization or mineral loss. Compared to the positive control dentifrice containing 1450 ppm fluoride alone, the arginine-containing dentifrice was significantly better in preventing demineralization ($p < 0.0001$). In addition, the arginine-containing dentifrice was the only dentifrice that had a net mineral gain or remineralization, which indicates an increase in enamel hardness after use. These results demonstrate that the new dentifrice containing 1.5% arginine and 1450 ppm fluoride in a Dical base is significantly more effective in preventing enamel loss than a matched dentifrice with 1450 ppm fluoride alone.

Table IV shows the results for the amount of ammonia produced from the collected plaque samples following the arginine-sucrose challenge. While there was a numerical increase in ammonia produced by plaque collected after use of the arginine-containing test dentifrice compared to the matched positive control dentifrice containing 1450 ppm fluoride alone, the result was not statistically significant. No difference in ammonia production was observed between the dentifrices containing 250 ppm fluoride (negative control) and 1450 ppm fluoride (positive control).

Study 3

This was an intraoral demineralization/remineralization study comparing three dentifrices: 1.5% arginine with 1000 ppm fluoride in a Dical base; 1450 ppm fluoride in a Dical base (positive control); and 250 ppm fluoride in a Dical base (negative control).

All eighteen of the subjects successfully completed the study. This intraoral demineralization/remineralization study was also validated by demonstrating that the positive control dentifrice,

Table III

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1450 ppm Fluoride in Preventing Enamel Demineralization, Expressed as Average Percent Demineralization, Compared to Control Dentifrices Containing 1450 ppm and 250 ppm Fluoride, Respectively (Study 2)

Products Tested	Product Designation	Percent Demineralization (± SD)[c]
Dical toothpaste with 250 ppm fluoride as MFP	Negative control	12.6 ± 12.5[a]
Dical toothpaste with 1450 ppm fluoride as MFP	Positive control	1.7 ± 7.7[a,b]
Dical toothpaste with 1450 ppm fluoride as MFP and 1.5% arginine	Test	–8.5 ± 5.6[b]

[a] The negative control was statistically significantly different from the positive control ($p < 0.0001$).

[b] The test product was statistically significantly different from the positive control ($p < 0.0001$).

[c] A negative value indicates a net mineral gain or remineralization.

Table IV

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1450 ppm Fluoride on Total Ammonia Production from *In Situ* Plaque Samples Formed During a Demineralization/Remineralization Study (Study 2) Compared to Control Dentifrices Containing 1450 and 250 ppm Fluoride, Respectively

	Product Designation	Ammonia (nmol per mg Plaque) (Mean ± SD)
Dical toothpaste without 250 ppm fluoride	Negative control	115.9 ± 67.5
Dical toothpaste with 1450 ppm fluoride as MFP	Positive control	105.4 ± 76.2
Dical toothpaste with 1450 ppm fluoride as MFP and 1.5% arginine	Test	162.7 ± 92.8[a]

[a] The test product was not statistically significantly different from the positive control ($p = 0.13$).

containing 1000 ppm fluoride as MFP in a calcium carbonate base, was significantly more effective at preventing demineralization ($p < 0.0001$) than the negative control dentifrice containing 0 ppm fluoride in a calcium carbonate base (Table V). The results also demonstrate that the arginine-containing dentifrice with 1000 ppm fluoride in a calcium carbonate base was significantly more effective in preventing demineralization than the matched positive control dentifrice with fluoride alone ($p < 0.05$).

Table VI shows the amount of ammonia produced from the *in situ* formed plaque samples following the arginine-sucrose challenge. After tooth brushing with the new arginine-containing dentifrice, plaque samples produced significantly more ammonia than plaque samples after brushing with the matched positive control dentifrice containing 1000 ppm fluoride alone ($p < 0.05$). As observed in Study 2, there were no significant differences in ammonia production from plaque samples after brushing with the dentifrice without fluoride (negative control) and with the dentifrice containing 1000 ppm fluoride (positive control). The results suggest that fluoride level does not impact the arginolytic activity of plaque.

In addition to determining the production of ammonia, the study determined the amount of lactic acid produced by *in situ* formed plaque samples. Although the difference in lactic acid production was not statistically significant compared to the positive control or the negative control, the arginine-containing dentifrice produced the least amount of lactic acid (Table VII).

Discussion

In these three studies, intraoral caries clinical models were used to test the ability of a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and fluoride to promote remineralization and prevent demineralization of enamel. Two formula variants were assessed, one using Dical and the other using calcium carbonate as the source of the insoluble calcium compound. The arginine level used in these formulations was 1.5%, which is the same level of arginine used in a non-fluoride dentifrice containing arginine, bicarbonate, and calcium carbonate that was shown to be as effective as a sodium fluoride dentifrice in preventing cavity formation in a two-year conventional caries clinical trial.[30] Further, *in situ* dose response studies, using similar intraoral clinical protocols to those described here, have shown that increasing the arginine level above 1.5% does not provide an additional caries benefit.[37]

Intraoral caries clinical models were used to evaluate this new dentifrice because the technology is designed to work on multiple steps of the complex caries process to deliver its caries protection benefits. Intraoral caries models are the methods of choice for evaluating dental formulations with complex modes of action, because the efficacy of such products is reliant on both the chemical and biological dynamics of the oral environment. The scientific literature suggests that intraoral models have distinct advantages over *in vitro* methods in predicting efficacy outcomes, such as the outcome of conventional caries clinical trials, because they are better able to capture real life

Table V

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1000 ppm Fluoride in Preventing Enamel Demineralization, Expressed as Average Percent Demineralization, Compared to Control Dentifrices Containing 1000 ppm and 0 ppm Fluoride, Respectively (Study 3)

Products Tested	Product Designation	Percent Demineralization (± SD)
Calcium carbonate toothpaste without fluoride	Negative control	15.3 ± 15.6[a]
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP	Positive control	5.0 ± 6.3[a,b]
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP and 1.5% arginine	Test	1.2 ± 5.3[b]

[a] The negative control was statistically significantly different from the positive control ($p < 0.0001$).
[b] The test product was statistically significantly different from the positive control ($p < 0.05$).

Table VI

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1000 ppm Fluoride on Total Ammonia Production from *In Situ* Plaque Samples Formed During a Demineralization/Remineralization Study (Study 3) Compared to Control Dentifrices Containing 1000 ppm and 0 ppm Fluoride, Respectively

Products Tested	Product Designation	Ammonia (nmol per mg Plaque) (Mean ± SD)
Calcium carbonate toothpaste without fluoride	Negative control	42.2 ± 32.6
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP	Positive control	56.2 ± 36.3[a]
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP and 1.5% arginine	Test	99.6 ± 69.0[a]

[a] The test product was statistically significantly different from the positive control ($p < 0.05$).

Table VII

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1000 ppm Fluoride on Total Lactic Acid Production from *In Situ* Plaque Samples Formed During a Demineralization/Remineralization Study (Study 3) Compared to Matched Control Dentifrices Containing 1000 ppm and 0 ppm Fluoride, Respectively

Product Tested	Product Designation	Lactate (nmol per mg Plaque) (Mean ± SD)
Calcium carbonate toothpaste without fluoride	Negative control	4.6 ± 5.2
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP	Positive control	5.1 ± 7.4
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP and 1.5% arginine	Test	4.1 ± 6.7

conditions, such as product usage, the effect of saliva and its flow, and the bacterial/dental plaque component of caries.[33,34,38,39] This point is critically important to the evaluation of this new arginine-containing dentifrice because: 1) the fluoride component, MFP, relies on the dynamics of the mouth and the action of salivary enzymes to generate free fluoride ion, which is the active form of fluoride; 2) the arginine component relies on the dynamics of the mouth and the action of dental plaque, with its diversity of interdependent bacterial species, to generate ammonia and modulate plaque pH; and 3) the calcium component relies on the dynamics of the mouth to influence the degree of saturation of plaque fluid with respect to enamel. While *in vitro* methods can play a role in product testing, these critical efficacy factors cannot be adequately accounted for in *in vitro* methods limiting their value.

Two different intraoral caries models were used to assess the performance of dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride. The first model used is often referred to as the intraoral remineralization/demineralization model because it measures the relative ability of dentifrices to promote remineralization of partially demineralized thin sections of enamel. The second model is referred to as the intraoral demineralization/remineralization model. In this model, the relative ability of a dentifrice to prevent mineral loss from sound enamel blocks is measured. Real plaque and real saliva are present in the mouth to create and modulate the pH fluctuations in both models. The severity of the caries challenge and how it is initiated differs in the two models. The intraoral remineralization/demineralization model relies solely on each panelist's normal diet to create the cariogenic challenge during the clinical phase of the study. In the intraoral demineralization/remineralization model, the caries challenge is increased by using an *ex vivo* 10% sucrose rinse, four times a day, to simulate a high sugar diet and high caries risk situation. These two models, therefore, determine how different dentifrices affect the processes of remineralization and demineralization of enamel under different conditions. Because real dental plaque is retained on the enamel specimens in the demineralization/remineralization model, this model also provides an opportunity to harvest the plaque and determine if specific treatments have resulted in any changes in the plaque. In both intraoral demineralization/remineralization studies, plaque was collected and the metabolic potential of plaque to convert arginine to ammonia was measured. In one study, the ability of plaque to convert sucrose into lactic acid was also measured. The purpose of conducting the plaque metabolism measures was to gain insight into the mechanisms driving observed differences in enamel re- and demineralization between the products with and without arginine.

In the intraoral remineralization/demineralization clinical study, Study 1, two dentifrices containing 1.5% arginine and 1450 ppm fluoride as MFP were compared. These formulations differed in the source of calcium, *i.e.*, one used Dical and the other used calcium carbonate. The intraoral remineralization/demineralization model used in this study has been previously validated and shown to be predictive of the results of conventional caries clinical trials.[33,34] Two Dical dentifrices containing 1450 ppm fluoride and 250 ppm fluoride were used as the positive and negative controls, respectively. As the results clearly show that the positive control was statistically significantly more effective than the negative control in remineralizing demineralized enamel, this study is validated. This is consistent with the known fluoride dose response of this model. Dentifrices with no or low levels of fluoride have previously been shown to result in net demineralization, whereas fluoride products (1000–1450 ppm fluoride), with clinically proven anticaries efficacy, remineralize the enamel. The results of this study showed that the dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride resulted in enhanced remineralization relative to the positive fluoride control. This was true for both calcium variants, which showed no discernable difference in efficacy.

The enhanced remineralization potential of the arginine-containing dentifrices observed in this study is consistent with the findings of six-month coronal and root caries studies, as well as the results of a traditional two-year caries clinical study measuring effects on cavitation. Specifically, three coronal caries studies, using Quantitative Light-induced Fluorescence (QLF) to measure changes in early caries lesions in children, have each shown that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride are significantly more effective in arresting and reversing coronal caries lesions than dentifrices containing 1450 ppm fluoride alone.[40-42] Likewise, two root caries studies in adults have each shown that the new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride is significantly more effective in arresting and reversing root caries lesions than a dentifrice containing 1450 ppm fluoride alone.[43,44] Finally, a two-year conventional caries clinical study has proven that two dentifrices containing 1.5% arginine and 1450 ppm fluoride in a calcium base, one with di-calcium phosphate and the other with calcium carbonate, are significantly more effective in preventing the formation of cavitated caries lesions than a dentifrice containing 1450 ppm fluoride alone. Three trained and calibrated dentists examined the children at baseline and after one and two years using the National Institute of Dental Research Diagnostic Procedures and Criteria. The number of decayed, missing, and filled teeth (DMFT) and surfaces (DMFS) for the three study groups were very similar at baseline, with no statistically significant differences among groups. After one year, there were no statistically significant differences in caries increments among the three groups. After two years, the two groups using the dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm F had statistically significantly ($p < 0.02$) lower DMFT increments (21.0% and 17.7% reductions, respectively) and DMFS increments (16.5% and 16.5%) compared to the control dentifrice. The differences between the two groups using the new dentifrices were not statistically significant. The results of this pivotal clinical study support the conclusion that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provide superior protection against caries lesion cavitation to dentifrices containing 1450 ppm fluoride alone.[45]

Because plaque metabolic measures were not assessed in the

remineralization/demineralization study (Study 1), it is not possible to determine with certainty what is driving the improved remineralization performance. However, results from separate plaque metabolism studies support that the arginine-containing dentifrice creates a plaque environment that is more favorable for remineralization than a dentifrice with fluoride alone.[46,47] The results of one of these studies are presented in the next article in this Special Issue. In summary, brushing for two weeks with a dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride was shown to significantly increase plaque's ability to convert arginine to ammonia relative to brushing with a control dentifrice containing silica and 1450 ppm fluoride as NaF. The group who used the arginine-containing dentifrice also had a significantly higher resting pH than the group using the control product. The resting pH is plaque's natural pH in the absence of a sucrose challenge. It typically ranges from 6.8 to 7. Under resting pH conditions, plaque is supersaturated with respect to enamel and there is a positive driving force favoring remineralization. An increase in resting pH, such as that observed in the arginine-containing dentifrice group in the plaque metabolism study, increases the degree of saturation of the plaque fluid with respect to enamel, and increases the driving force for enamel remineralization.

In Studies 2 and 3, the intraoral demineralization/remineralization model was used to separately assess the ability of two dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride to prevent demineralization of sound enamel specimens. In this model, the enamel specimens undergo a strong cariogenic challenge with the result that they lose mineral during the course of the study. The most effective treatment loses the least amount of mineral. In Study 2, a dentifrice containing 1.5% arginine, Dical, and 1450 ppm fluoride as MFP was compared to matching positive and negative controls with 1450 ppm and 250 ppm fluoride, respectively. Net mineral loss was experienced for both positive and negative controls. The observation that the positive control was statistically significantly more effective in preventing mineral loss than the negative control validates the study. No net mineral loss was experienced following use of the arginine-containing dentifrice; enamel specimens actually showed an increase in hardness after the treatment period. Importantly, the arginine-containing dentifrice was shown to be statistically significantly more effective than the matched positive control dentifrice in preventing demineralization of enamel. This indicates that arginine is playing a significant role in the enhanced efficacy of this product. While the plaque metabolism results of Study 2 did not reach statistical significance, the numeric data indicate that use of the arginine-containing dentifrice increases the ability of plaque to convert arginine into ammonia relative to the fluoride controls.

In Study 3, a dentifrice containing 1.5% arginine, calcium carbonate, and 1000 ppm fluoride as MFP was compared to matching positive and negative controls with 1000 ppm and 0 ppm fluoride, respectively. The results are consistent with the results of Study 2. Specifically, the study was successfully validated by showing that the positive control was statistically significantly more effective than the negative control in preventing demineralization. Furthermore, the arginine-containing dentifrice was statistically significantly more effective than the positive control in preventing demineralization. Use of the arginine-containing dentifrice was shown to result in a statistically significant increase in arginine catabolism to ammonia. In this study, the ability of plaque to convert sucrose into lactic acid was also measured. There was a numerical decrease in lactic acid production for the arginine-containing dentifrice, but the result was not statistically significant. As caries is a dynamic process, differences in acid production at a given time point may be too small to measure, yet they may reduce the driving force for demineralization sufficiently to collectively add up over time to a measurable benefit on enamel. From the perspective of designing a clinical protocol, it is difficult to capture such an effect. With this noted, the plaque metabolism results support the findings of the two demineralization studies. The addition of 1.5% arginine to a dentifrice containing an insoluble calcium compound and fluoride creates a less cariogenic plaque environment which enhances the protective effects of fluoride, and translates into better overall protection against mineral loss.

The results of the three intraoral studies reported in this article provide strong evidence that dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride are significantly more effective than dentifrices with fluoride alone in both promoting remineralization and preventing demineralization. These improved effects on re- and demineralization were observed for both Dical and calcium carbonate dentifrices. In addition, the results on plaque metabolism measures support that both Dical and calcium carbonate dentifrices exhibit the same mode of action. The results support the hypothesis that dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride promote the breakdown of arginine to ammonia by the action of arginolytic bacteria.[29] The ammonia production neutralizes plaque acids to help maintain a pH-neutral environment, creating conditions that favor a healthy plaque community. Cariogenic bacteria utilize analogous mechanisms, in this case of acid production, to create a more acidic plaque that favors their survival at the expense of the nonpathogenic bacteria. The crucial difference between these two processes is the end effect on tooth mineral. Catabolism of arginine helps to create and maintain neutral pH conditions and a high level of supersaturation with respect to enamel that supports a healthy tooth structure and the remineralization process. Metabolism of sugars and production of acids, on the other hand, create conditions of undersaturation, facilitating damage to the teeth.

In summary, dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride provide superior caries protection than dentifrices with fluoride alone. Two dentifrices have been evaluated, one with Dical and the other with calcium carbonate as the source of calcium. Both of these variants provided enhanced caries protection. The complex mechanism of action of these dentifrices results from the effects of the arginine on plaque, which are distinct from, yet complementary to, the effects of fluoride on the tooth tissue. This new denti-

frice technology represents a major advancement in caries protection, and a paradigm shift in the approach to improving the efficacy of fluoride dentifrices.

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In Vivo Effects of a New Dentifrice Containing 1.5% Arginine and 1450 ppm Fluoride on Plaque Metabolism

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Abstract

- **Objective**: This paper presents the results of a clinical study assessing the *in vivo* effects on plaque metabolism of a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride compared to a commercially available dentifrice containing 1450 ppm fluoride alone.
- **Methods**: A four-week, parallel, randomized, double-blind clinical study using 54 subjects was conducted at the New York University College of Dentistry Bluestone Center for Clinical Research. Two study groups used the following products for two weeks: 1) a dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as sodium monofluorophosphate (MFP; test); and 2) a commercial silica dentifrice with 1450 ppm fluoride as sodium fluoride (NaF; control). In the following two-week period, all subjects used the control product. The effects of product use on plaque metabolism *in vivo* were assessed by conducting *ex vivo* analyses at baseline, after two weeks of assigned product use, and after two weeks of control product use. These plaque analyses comprised pH measurements before and after an *in vivo* sucrose rinse, and measurements of ammonia production and lactate production.
- **Results**: The study showed that subjects using the test dentifrice, containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride, had significantly higher plaque pH values before the sucrose challenge than those using the commercially available control dentifrice ($p \leq 0.01$). Plaque samples from subjects using the arginine-containing dentifrice also produced significantly higher levels of ammonia ($p \leq 0.01$). Subjects using the arginine-containing dentifrice also had a directionally higher plaque pH after the sucrose challenge, and their plaque samples produced a directionally lower level of lactate during the two-week treatment period compared to subjects using the control dentifrice. Following two weeks of subsequent use of the control product, there were no significant differences in plaque metabolism measures between groups.
- **Conclusion**: A new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride has been shown in this study to modulate plaque metabolism, increasing ammonia production and decreasing lactate production, thereby increasing plaque pH to help restore a pH-neutral environment.

(J Clin Dent 2013;24[Spec Iss A]:45–54)

Introduction

Dental caries is a multi-factorial disease that involves loss of tooth mineral resulting from acids produced by certain types of bacteria. These bacteria, often referred to as cariogenic or acidogenic bacteria, produce acids through the fermentation of carbohydrates, primarily sucrose, in the diet. The bacteria responsible for carbohydrate breakdown are predominantly gram positive microorganisms, with the species *Streptococcus mutans* attributed a major role in the initiation process.[1-4] With high sucrose availability, the acidogenic bacteria produce lactic acid and some acetic acid, and when sucrose is less available, other acids, such as propionic, formic, and succinic acids, are produced.[5] Lactic acid is, however, the main organic acid produced by plaque after a sucrose challenge, and largely contributes to the acidification of the plaque.[6-9] Sucrose is also utilized by cariogenic strains to produce extracellular polysaccharides which facilitate their adherence to the tooth surface and contribute to the pathogenicity of these organisms.[10] In addition, these cariogenic pathogens have the ability to maintain a stable internal pH, which is required for important intracellular processes to occur in a hostile acidic environment, so they are also acid-tolerant or aciduric.[2,11] Many species of bacteria cannot survive or thrive under hostile acidic conditions; therefore, frequent consumption of dietary sugars creates a competitive advantage for cariogenic bacteria in the plaque biofilm. The caries process enables cariogenic bacteria to produce organic acids, firmly adhere to the tooth surface, and perpetuate in an acidic environment which is advantageous to them, but not to other bacterial species fighting for survival in the dynamic dental plaque biofilm. The caries process is, thus, initiated and progresses when frequent ingestion of dietary sugars shifts the plaque biofilm environment from one favoring the commensal bacteria at a neutral pH, to a pathogenic state favoring the cariogenic bacteria and a more acidic pH, and results in continued and cyclic demineralization of the tooth surface.

Although plaque bacteria and dietary sugars are the engine and fuel for initiating and driving the caries process, the tooth is the substrate or target for caries. Tooth enamel is very similar in

chemical composition to hydroxyapatite, $Ca_{10}(PO_4)_6OH_2$. Under normal physiological conditions, for most healthy adults and children, the pH of the fluids in direct contact with the tooth, namely saliva and plaque fluid, is close to neutral, and the activities of calcium and phosphate support super-saturation with respect to the tooth mineral. This means that at neutral pH, the tooth mineral is stable and conditions favor remineralization. In contrast, when acid is produced by bacteria within dental plaque, the pH of the plaque fluid drops and the fluid becomes undersaturated with respect to tooth mineral, and so demineralization occurs. The demineralization process will continue until the pH rises again, conditions of saturation are met, and the tooth mineral solid phase becomes stable.[12,13]

The introduction of fluoride, especially in the form of toothpaste, has had a major impact on the incidence of caries globally, and is often cited as an example of a true public health success.[14] Despite the success of fluoride in reducing the incidence of caries, the disease remains a major oral health problem and is costly, not only from a monetary, but also from a quality of life perspective. Fluoride's primary mode of action is protection of the tooth against the effects of acids. It helps to prevent caries by making the tooth more resistant to acid and by promoting repair of the tooth surface that has been damaged by plaque acids. Fluoride, however, does little to influence the ecological and physiological environment of plaque, which is the source of caries. It is because of this limitation that fluoride alone cannot provide 100% daily protection against caries, and this is part of the reason why caries still remains a problem in spite of fluoride's widespread use.[15]

It is clear that fluoride toothpaste should continue to be part of any caries-preventive regimen given its long track record of success. However, there is a clear need to improve the efficacy of existing fluoride toothpaste, especially given the continued trend in increased sugar consumption.

In seeking to develop technologies to improve upon and complement fluoride's efficacy, one need look no further than the salivary defense system and the approach used by nature to protect against dental caries. Saliva plays several roles in defending the tooth against caries and helping to maintain and restore a healthy balance. First, it helps physically clear and neutralize acids formed in plaque after sugar consumption to help restore a neutral pH. Second, it contains calcium and phosphate to help maintain super-saturation with respect to tooth mineral. Additionally, saliva contains a mixture of salivary proteins and peptides that serve a variety of non-immune host defense functions. One important aspect of these salivary proteins and peptides is that they contain arginine, a basic amino acid. As salivary proteases break down these proteins and peptides, arginine is released and can be utilized by a class of beneficial bacteria as an energy source through use of the arginine deiminase system.[16] The important feature of the arginine deiminase system is the stoichiometric breakdown of arginine into two molecules of ammonia, which neutralizes acids and promotes neutral pH, which is less favorable to cariogenic bacteria, but favorable to the remineralization process.[16,17] A range of bacteria, including *S. sanguis*, *S. gordonii*, *S. parasanguis*, *S. rattus*, *S. milleri*, *S. anginosus*, *S. faecalis*, *A. naeslundii*, *A. odontolyticus*, *L. cellobiosus*, *L. brevis*, and *L. fermentum* have been identified as bacteria capable of utilizing arginine as a means of their own survival in the presence of acid-producing bacteria.[18-21] Under conditions of arginine utilization and alkali production by arginolytic bacteria, cariogenic bacteria, such as *S. mutans*, are poor competitors in the biofilm and their propensity to proliferate by acidifying the environment is attenuated.

Colgate-Palmolive has developed a breakthrough technology which enhances the natural protective effects of saliva and disrupts the caries process to fight caries at its source. This new technology is based upon the pioneering work of Kleinberg on plaque metabolism,[21] and his development and validation of a fluoride-free toothpaste which combined arginine to modulate plaque metabolism through the production of ammonia, calcium carbonate, an insoluble source of calcium ions, and bicarbonate, an acid-buffering agent. This fluoride-free toothpaste was clinically validated and shown to reduce cavity formation as effectively as a regular 1000 ppm fluoride toothpaste.[22] Colgate has expanded the scope of Kleinberg's original work by combining arginine, an insoluble calcium compound, and fluoride in a dentifrice which has been clinically proven to provide superior anti-caries benefits.[23-28] The mechanism by which the technology delivers protection against caries, through the effects of the arginine on plaque metabolism and plaque pH, is complementary to the mechanism of action of fluoride.

This paper presents the results of a clinical study which compared the effects of brushing with a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as MFP on measures of plaque metabolism compared to those of a commercially available dentifrice containing 1450 ppm fluoride as sodium fluoride in a silica base. The plaque measures included chairside plaque pH measurements, before and after an *in vivo* sucrose challenge, and *ex vivo* assessments of ammonia and lactic acid production to determine base-and acid-producing capacity of the dental plaque biofilm on the tooth surfaces where remineralization or demineralization could occur. The results help shed insight on the mode of action of the new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride, with respect to its proven clinical benefits in arresting and reversing the caries process.

Materials and Methods

Study Design and Clinical Procedure

The study was conducted at the New York University College of Dentistry Bluestone Center for Clinical Research. It was a two-cell, parallel, randomized, double-blind, four-week study in which two weeks of use of the assigned test and control products was followed by two weeks of use of the control product by both groups. The test product was a dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as MFP (Colgate-Palmolive, New York, NY, USA), and the control was a dentifrice containing only 1450 ppm fluoride as NaF in a silica base, Crest Calcident (Procter & Gamble, Cincinnati, OH, USA). Before the start of the study, there was a one-week wash-out period in which all of the subjects used the 1450 ppm fluoride silica control dentifrice. Sixty subjects were recruited into the study, 30 in each treatment group. The subjects met all

the inclusion and exclusion criteria outlined in the protocol, and signed an informed consent prior to starting the study. The study protocol was reviewed by and received ethical approval from an Institutional Review Board. The design of the study is shown in Figure 1.

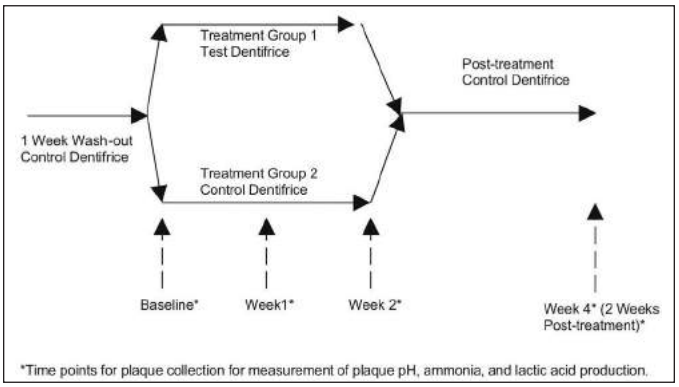

Figure 1. *Study design.*

Before beginning the wash-out period, each subject received a dental prophylaxis. Each subject then received the control dentifrice and a Colgate adult soft bristle toothbrush. Subjects were instructed to use the assigned dentifrice for one minute, brushing twice daily, in place of their normal dentifrice. They were also instructed not to use any other dental hygiene products for the duration of the study. After the one-week wash-out period, the subjects returned to the dental clinic for baseline plaque collection.

At baseline, subjects arrived at the dental clinic without morning oral hygiene and without eating or drinking since the previous evening. Fasting plaque samples were collected by the dentist. All harvested plaque was collected on ice in pre-weighed chilled vials, and stored at –20°C until analysis. One plaque sample per subject was collected by scraping the entire right side (both upper and lower arches) of the dentition and pooling the sample. The pH of this sample was immediately measured for an initial baseline value. This plaque sample was also used to assess ammonia production capacity. Each subject then rinsed with a 10% sucrose solution for two minutes using a method adapted from one previously described.[29] Eight minutes after this rinse, a second plaque sample was collected from each subject by scraping the entire left side (both upper and lower arches) of the dentition. The pH was immediately measured and recorded as the post-sucrose challenge value. This plaque sample was also used for lactic acid analysis. Plaque samples for subsequent assessments were alternated for each pair of measures between the right and left sides. After baseline collection, subjects were randomly assigned to either the test or control dentifrice, and were provided with a Colgate adult soft bristle toothbrush to use for the next two weeks. Subjects were instructed to use their assigned dentifrice for one minute, brushing twice daily, in place of their normal dentifrice. They were also reminded not to use any other dental hygiene products for the duration of the study.

After one week of use and again after two weeks of use of the test and control dentifrices, subjects returned to the clinic having observed the same restrictions prior to their appointment for plaque collection as described above. After the two-week plaque collection, all subjects were provided with the control dentifrice and a Colgate adult soft bristle toothbrush to use for the next two weeks, and were reminded of the brushing instructions. After two weeks of use of the control dentifrice, subjects returned to the clinic having observed the same restrictions prior to their appointment for their final plaque collection, after which the study was concluded.

Determination of Plaque pH

Plaque pH was determined by use of a pH microelectrode (Microelectrodes Inc., Bedford, NH, USA). All plaque pH values were taken immediately following collection at chairside. The plaque pH values measured before the sucrose rinse were termed "resting pH," and the plaque pH values measured after the sucrose rinse were termed "terminal pH."

Determination of Ammonia Production

The ammonia assay procedure was adapted from a previously published method.[30] During analysis, the plaque was kept on ice and the concentration of ammonia produced was normalized by weight to 1 mg/ml in phosphate buffered saline, PBS, pH 7.4 (Gibco, Grand Island, NY, USA). The samples were mixed with a vortex, and then sonicated to break up plaque clusters and homogenize the sample. Each plaque sample was then challenged with sucrose (VWR, West Chester, PA, USA) and arginine (Sigma, St. Louis, MO, USA) to give a final concentration of 0.1% and 5 mM, respectively. The samples were incubated in a 37°C shaking water bath for 30 minutes before ammonia production was analyzed. A diagnostic ammonia assay kit (Diagnostic Chemicals Limited, Oxford, CT, USA) was used to quantify the ammonia produced in the plaque.

Determination of Lactic Acid Production

Lactic acid is the primary acid formed when sucrose is metabolized by oral bacteria. For this reason, lactic acid was measured as a marker of acid production capability after a sucrose challenge. The method for determining lactic acid concentration in plaque samples was adapted from previously published methods.[29,31] Once the plaque weight had been measured and normalized, ice cold water was added to the samples. Then the samples were heated to 80°C for five minutes to kill the bacteria and release all acids, followed by cooling in ice water for an additional five minutes. The samples then were centrifuged, the supernatant was filtered, and the concentration of lactic acid in the supernatant was measured as the lactate anion using capillary electrophoresis.

The conditions used to analyze the plaque samples using capillary electrophoresis were adapted from a previously published method.[32] Separations were carried out on a fused-silica capillary with a 50 cm effective length X 50 µm internal diameter. The optimized buffer system consisted of 20 mM 2,6-pyridine dicarboxylic acid, and 0.5 mM hexadecyltrimethyl ammonium bromide, pH 5.66 (Sigma, St. Louis, MO, USA). Since organic acids have little or no ultraviolet (UV) absorbance, detection was accomplished using 2,6-pyridine dicarboxylic acid as a background electrolyte (BGE). In this indirect detection method, the BGE has strong UV absorptive properties and produces a high background absorption in the UV detector. In the absence

of non-absorbing analytes, the background signal is constant. When ionic analytes are introduced, they displace UV absorbing additive ions on a charge-to-charge basis, resulting in a negative peak relative to the high UV absorption baselines. With the analysis, the sample was injected by pressure for 10 seconds at 0.5 psi. The separation was performed at –25 kV and the capillary was thermostated at 25°C. The wavelength for indirect UV detection was selected at 254 nm, and the signal with negative peaks was inverted to obtain a more familiar electropherogram to integrate and process. To correct for injection errors, each sample was run with the incorporation of a 1.5 mM sodium nitrate internal standard, and a calibration curve was constructed using sodium lactate standards (Sigma, St. Louis, MO, USA). Based upon the ratio of lactate/nitrate peak area and the initial plaque weight, the concentration of lactate present in the plaque sample was determined.

Statistical Analysis

Data collected during the treatment phase (weeks 1 and 2) from subjects who completed the study were analyzed using repeated measures analysis (RMA). The model included treatment, time, and treatment*time interaction, with the baseline as covariate. Subject nested under treatment was included as a random effect. As previous intraoral clinical studies had demonstrated that the test dentifrice modulates plaque metabolism and enhances ammonia production,[32] one-sided upper tests were performed for pH and ammonia production, and one-sided lower tests were performed for lactic acid. Differences were considered significant for the treatment effect if a 95% confidence level was achieved. In addition to providing the statistical significance of any differences, the RMA provided an overall least squares mean for each treatment group for each plaque measure, *i.e.*, an "average" value across treatment time points (week 1 and week 2).

Data collected at the end of the post-treatment phase were analyzed using analysis of covariance (ANCOVA) with the baseline used as a covariate. A difference between groups post-treatment was considered significant if a 95% confidence was achieved in a two-tailed test.

Results

Fifty-one of the subjects successfully completed the four-week study, with 23 subjects in the group using the NaF control toothpaste and 28 in the group using the arginine/MFP test toothpaste during the two-week treatment phase. Nine subjects did not complete the study because of missed clinic appointments (unrelated to product use).

Plaque pH

The resting plaque pH data are summarized in Table I. Figure 2 shows plots of the mean resting pH values for the two treatment groups during the two-week treatment, and two-week post-treatment periods. The resting plaque pH values were balanced at baseline; there was no significant difference in baseline resting pH values between the test and control groups. During the two-week treatment period, the resting pH of the group using the test dentifrice was statistically significantly higher than the resting pH of the group using the control dentifrice ($p < 0.01$).

Table I

Means and Standard Deviations of Resting pH Values as a Function of Time for the Groups Using the Test Dentifrice and the Control Dentifrice During the Two-Week Treatment Period

Dentifrice Used During Treatment Period	Baseline	Week 1	Week 2	Week 4 (2 Weeks Post-Treatment)
Control (n = 23)	7.36 (0.21)	7.09 (0.37)	7.10 (0.32)	7.17 (0.44)
Test (n = 28)	7.34 (0.41)	7.26 (0.33)	7.31 (0.26)	7.26 (0.35)

Treatment Period (Repeated Measures Analysis): Treatment significant ($p \leq 0.01$); Least squares mean (standard error) resting pH values were 7.28 (0.048) and 7.09 (0.056) for the test and control groups, respectively; Time and Treatment*Time not significant ($p = 0.6$ and 0.7, respectively). Post-treatment (ANCOVA): Treatment not significant ($p = 0.4$).

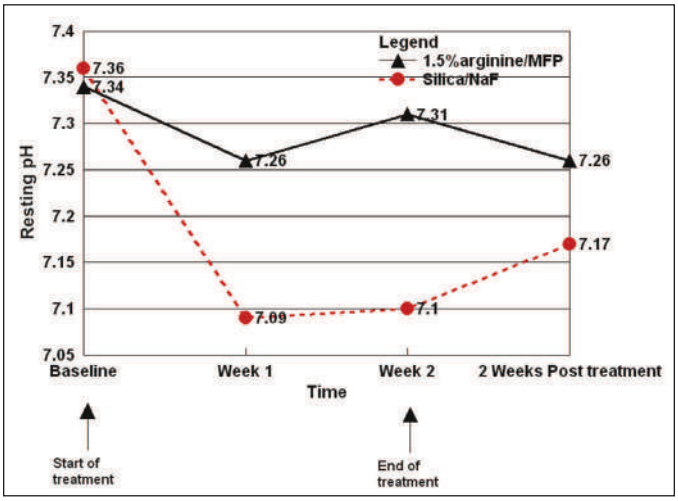

Figure 2. *Graph showing resting pH data for the groups using the test dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as MFP, and the control dentifrice containing silica and 1450 ppm fluoride as NaF, during the two-week treatment period.*

The least squares mean (standard error) resting pH values were 7.28 (0.048) and 7.09 (0.056) for the test and control groups, respectively. Time and treatment*time effects were not significantly different ($p = 0.6$ and $p = 0.7$, respectively).

After the two-week post-treatment phase, during which all subjects used the control dentifrice, the resting pH of the group who previously used the test dentifrice remained higher than that of the group who had used the control dentifrice, but the difference was not statistically significant ($p = 0.4$).

The terminal pH data are summarized in Table II. Figure 3 shows plots of the mean terminal pH values for the two treatment groups for the two-week treatment, and two-week post-treatment periods. There was a small numerical difference in baseline terminal pH values between the test and control groups, but this difference was not statistically significant. During the treatment period, the terminal pH of plaque from the test group was numerically higher (less acidic) than plaque from the control group. However, the statistical analysis showed the treatment effect was not significant ($p = 0.17$). The least squares mean (standard error) terminal pH values were 5.96 (0.078) and 5.84 (0.090) for the test and control groups, respectively. Time and treatment*time effects were not significantly different ($p = 0.2$ and $p = 0.9$, respectively).

Table II

Means and Standard Deviations of Terminal pH Values as a Function of Time for the Groups Using the Test Dentifrice and the Control Dentifrice During the Two-Week Treatment Period

Dentifrice Used During Treatment Period	Baseline	Week 1	Week 2	Week 4 (2 Weeks Post-Treatment)
Control (n = 23)	6.18 (0.59)	5.90 (0.55)	5.79 (0.54)	5.95 (0.58)
Test (n = 28)	6.08 (0.67)	6.03 (0.65)	5.88 (0.43)	5.77 (0.49)

Treatment Period (Repeated Measures Analysis): Treatment significant (p = 0.17); Least squares mean (standard error) terminal pH values were 5.96 (0.078) and 5.84 (0.090) for the test and control groups, respectively; Time and Treatment*Time not significant (p = 0.2 and 0.9, respectively). Post-treatment (ANCOVA): Treatment not significant (p = 0.3).

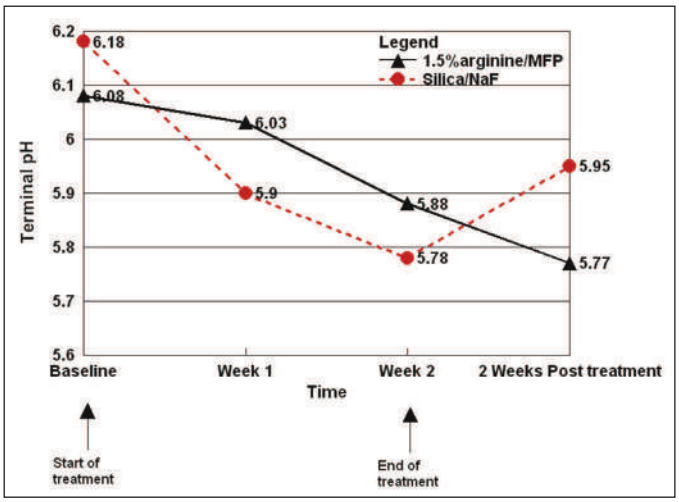

Figure 3. *Graph showing terminal pH data for the groups using the test dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as MFP, and the control dentifrice containing silica and 1450 ppm fluoride as NaF, during the two-week treatment period.*

After the post-treatment phase, the terminal pH values of the two groups switched; the terminal pH of plaque from the group previously using the test product was numerically lower (more acidic) than that from the control group. However, the difference between the two groups was not statistically significant (p = 0.3).

Ammonia Production

Table III shows the results for the total amount of ammonia produced by the plaque samples collected during the study. The baseline value for the group using the test dentifrice was higher than that for the control product. For this reason, the results are reported both as raw means and as baseline-adjusted changes in ammonia production from baseline (referred to as delta ammonia production with delta = post – BL). Figures 4 and 5, respectively, show plots of the raw mean and delta (differences from baseline) values for ammonia production for the two treatment groups after the *ex vivo* arginine-sucrose challenge. During the treatment period, ammonia production was increased compared to baseline in plaque harvested from the test group, whereas ammonia production was reduced compared to baseline in the control group. The difference between test and control groups during the treatment period was statistically significant (p ≤ 0.01). The least squares mean (standard error) ammonia production val-

Table III

Mean and Standard Deviation of Ammonia Production (Expressed Both as Raw Means and as Baseline-Adjusted Changes from Baseline [Delta Ammonia Production]) for the Groups Using the Test Dentifrice and the Control Dentifrice During the Two-Week Treatment Period

	Ammonia Production* (nmol/mg Plaque)		Delta Ammonia Production (nmol/mg Plaque)	
	Test (n = 28)	Control (n = 23)	Test (n = 28)	Control (n = 23)
Baseline	72.1 (46.2)	99.1 (92.2)	N/A	N/A
Week 1	108.6 (63.3)	81.0 (53.2)	+36.5 (66.6)	–21.8 (88.7)
Week 2	104.1 (49.2)	88.6 (65.9)	+32.0 (48.0)	–10.6 (109.0)
Week 4 (2 Weeks Post-Treatment)	102.6 (51.9)	100.3 (61.0)	+30.5 (64.7)	+1.1 (105.2)

*Treatment Period (Repeated Measures Analysis): Treatment significant (p ≤ 0.01); Least squares mean (standard error) values for ammonia production were 108.9 (7.4) and 81.5 (8.4) for the test and control groups, respectively; Time, and Treatment*Time not significant (p = 0.9 and 0.6, respectively). Post-treatment (ANCOVA): Treatment not significant (p = 0.8).

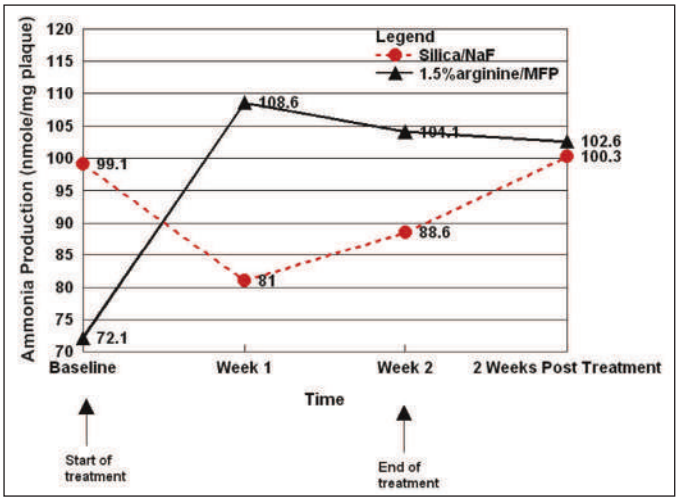

Figure 4. *Graph showing ammonia production for the groups using the test dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as MFP, and the control dentifrice containing silica and 1450 ppm fluoride as NaF, during the two-week treatment period.*

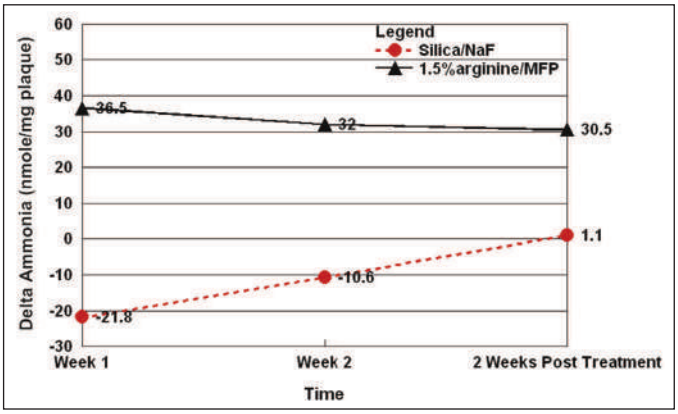

Figure 5. *Graph showing changes in ammonia production from baseline (delta ammonia production) for the groups using the test dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as MFP, and the control dentifrice containing silica and 1450 ppm fluoride as NaF, during the two-week treatment period.*

ues were 108.9 (7.4) and 81.5 (8.4) for the test and control groups, respectively. Time and treatment*time effects were not significantly different (p = 0.9 and p = 0.6, respectively).

After the post-treatment period, ammonia production remained elevated relative to baseline in the group previously using the test product, whereas ammonia production returned essentially to the baseline value in the group using the control product. The difference in ammonia production between the test and control groups was not statistically significant (p = 0.8).

Lactic Acid Production

The results for lactic acid production are summarized in Table IV. Lactate production at baseline was higher in plaque samples collected from the group that was randomly assigned to the test dentifrice, than in plaque samples collected from the group assigned to the control product. For this reason, lactate production at subsequent time points was expressed as raw means and as a difference from baseline (delta lactate production with delta = post – BL). Figures 6 and 7, respectively, show plots of the raw mean and delta (differences from baseline) values for lactic acid production for the two treatment groups after the *in vivo* sucrose challenge. Small differences in lactate production were observed during the treatment period, suggesting reduced lactate production in plaque collected from the test group relative to that of the control group; however, the product differences were not statistically significant (p = 0.2). The least squares mean (standard error) lactic acid production values were 4.03 (0.48) and 4.59 (0.51) for the test and control groups, respectively. For lactic acid, the time effect was significant (p = 0.03), but the treatment*time effect was not significantly different (p = 0.7).

After the post-treatment period, lactic acid production increased in both groups. However, the difference in lactic acid production between the test and control groups was not statistically significant (p = 0.7).

Table IV

Mean and Standard Deviation of Lactate Production (Expressed Both as Raw Means and as Baseline-Adjusted Changes from Baseline (Delta Ammonia Production) for the Groups Using the Test Dentifrice and the Control Dentifrice During the Two-Week Treatment Period

	Lactate Production* (nmol/mg Plaque)		Delta Lactate Production (nmol/mg Plaque)	
	Test	Control	Test	Control
Baseline	4.6 (2.9)	3.6 (2.6)	N/A	N/A
Week 1	3.4 (2.6)	3.6 (4.0)	–1.4 (2.7)	+0.1 (3.4)
Week 2	4.8 (2.6)	4.9 (3.3)	+0.2 (3.5)	+1.2 (3.9)
Week 4 (2 Weeks Post-Treatment)	5.5 (5.0)	5.8 (4.0)	+1.2 (4.9)	+2.2 (4.0)

*Treatment Period (Repeated Measures Analysis): Treatment not significant (p = 0.22); Least squares mean (standard error) values for lactic acid production were 4.03 (0.48) and 4.59 (0.51) for the test and control groups, respectively; Time significant (p = 0.03), and Treatment*Time not significant (p = 0.7). Post-treatment (ANCOVA): Treatment not significant (p = 0.7).

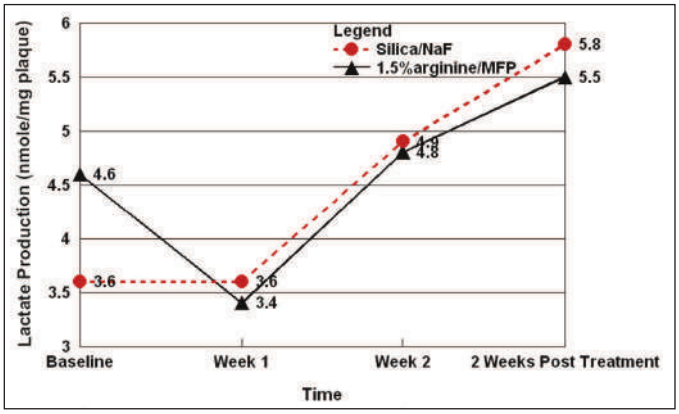

Figure 6. *Graph showing lactic acid production for the groups using the test dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as MFP, and the control dentifrice containing silica and 1450 ppm fluoride as NaF, during the two-week treatment period.*

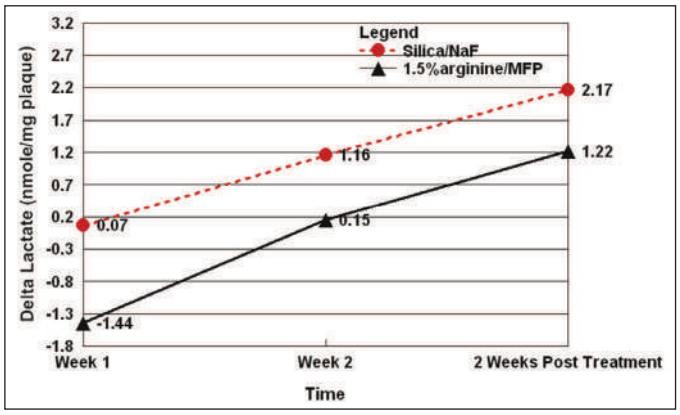

Figure 7. *Graph showing changes in lactic acid production from baseline (delta lactate production) for the groups using the test dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride as MFP, and the control dentifrice containing silica and 1450 ppm fluoride as NaF, during the two-week treatment period.*

Discussion

This paper presents the results of a clinical study which examined the effects on plaque metabolism of a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride, as MFP, to demonstrate the mechanism by which the new dentifrice provides its enhanced efficacy relative to dentifrices containing fluoride alone in intraoral caries remineralization and demineralization studies,[32] as well as in six-month caries clinical studies of the arrest and reversal of early lesions in enamel and root surfaces, and a two-year caries clinical study of cavitation.[23-28] The arginine, calcium, and fluoride are each important components of the dentifrice formula. This study focused solely on examining the role of an arginine-containing dentifrice in influencing beneficial shifts in *in vivo* plaque metabolism, as measured by pH (resting pH and terminal pH after sucrose challenge) and by ammonia and lactic acid production *ex vivo*.

Ammonia produced from arginine was shown to have a significant impact on resting plaque pH, defined as the pH of plaque when it has not been exposed to a sucrose challenge. The resting pH of normal healthy adults is typically reported to be in the range of 6.8 to 7.0. Under such conditions, enamel is safe and protected because the plaque environment is super-saturated with respect to the tooth mineral. In this study, the resting pH of the test and control groups was balanced at baseline, but

the values were slightly higher than those typically reported in the literature. During the two-week treatment period, the resting pH of the test group was significantly higher than that of the control group ($p < 0.01$). An elevated resting pH would be expected to result in increased remineralization because it elevates the degree of super-saturation of the plaque fluid with respect to enamel. Indeed, an apparently small elevation in resting pH, of *e.g.*, 0.1 pH units, over an extended period of twice-daily use would be expected to result in an increased and continued driving force for calcium and phosphate uptake into demineralized enamel and a significant cumulative effect on remineralization. An elevated resting pH would also be expected to afford greater protection against acids, because larger quantities of acids would be needed to cause under-saturation and demineralization. The results of this study are consistent with the results of the intraoral caries models, where enhanced remineralization and better protection against enamel demineralization were observed,[33] and with the results of the six-month enamel caries and root caries studies which showed enhanced efficacy in arresting and reversing early lesions.[23-27]

In order to determine what is driving the effect on resting pH, this study evaluated the effects on ammonia production. Plaque samples from the group treated with the test dentifrice produced significantly more ammonia than the group treated with the control product ($p < 0.01$). The results for resting pH and ammonia production are consistent; the greater level of alkali production observed in the test group compared to the control group during the treatment period would favor a higher resting pH in the test group. It is noteworthy that the effect of the test dentifrice on arginine metabolism by plaque is a lasting effect and not a transient one that quickly diminishes after brushing, as ammonia production was measured approximately 12 hours after the last brushing. In addition, measurements of ammonia production after the two-week post-treatment use of the control product demonstrated that the enhanced ability of the test group to metabolize arginine relative to baseline had persisted, and was numerically greater than that of the control group. In other words, the results of this study show that brushing with the arginine-containing test dentifrice enabled the flora to utilize endogenous arginine more effectively during the post-treatment period. In this way, endogenous arginine might help sustain the positive benefits of brushing with the arginine-containing dentifrice.

The enhanced effects of the arginine-containing dentifrice on resting pH and ammonia production would be expected to create a less favorable environment for cariogenic bacteria, and may help suppress their emergence and domination of the plaque biofilm through acidification of the plaque environment by the production of acids. In this study, terminal pH and lactic acid production were measured after an *in vivo* sucrose challenge to assess the acidogenic potential of the plaque. After ingesting carbohydrates, the pH of plaque fluid is reduced because of the production of plaque acids. The depth and duration of the pH drop is dependent on several factors, such as salivary flow rate, buffering capacity, and the relative amounts of acid-producing bacteria. The pH profile as a function of time is often referred to as the Stephan curve (Figure 8).[34] In this study, terminal pH

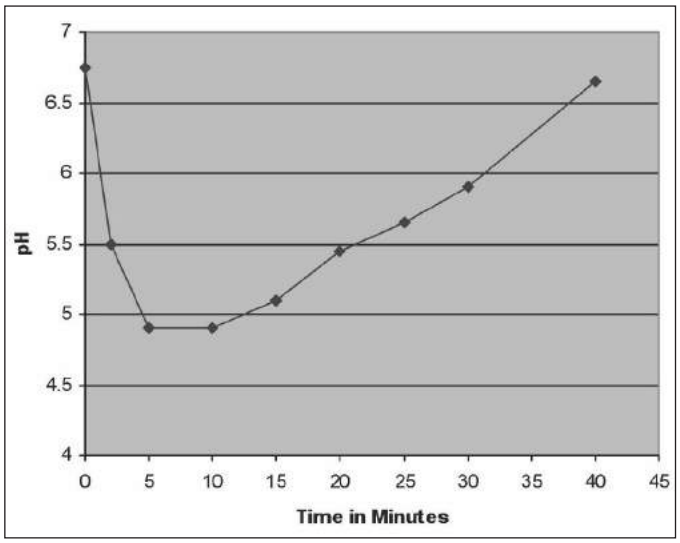

Figure 8. *Stephan curve showing plaque pH after rinsing with a 10% glucose solution.*

was measured eight minutes after the sucrose challenge, which represents a single time point on the Stephan curve and a single time point in the caries challenge. A lower terminal pH is indicative of a greater caries challenge, which would lead to more damage to the tooth surface. At baseline, the group assigned to the test dentifrice had a slightly more acidic terminal pH than the group assigned to the control. During the treatment phase, a higher terminal pH value was observed for the test group than for the control group, but this difference was not statistically significant. The pH data showed that the effect of the new arginine-containing dentifrice on plaque pH is more pronounced on resting pH than on terminal pH. This is both rational and intuitive. One would expect considerable variability in the Stephan curve profiles, both in terms of depth (pH drop) and breadth (time to return to "normal") of the curves, from individual to individual. This, in turn, would result in greater variability in the terminal pH values than in the resting pH values and this was, indeed, the observation in this study, shown by the standard deviations in Tables I and II. Further, and perhaps more importantly, the resting pH measured is a direct reflection of the resting pH in the mouth, whereas the terminal pH measured is a single surrogate for the repeated terminal pH values experienced in the mouth.

Lactic acid production was also measured as a means of determining the metabolic output of acid-producing bacteria following a sucrose challenge. Many bacteria have been cited to produce acids, *S. mutans* being one of the more prolific acid-producers and a primary pathogen for caries. During the two-week treatment period, lactic acid production was reduced relative to baseline in plaque from the test group, and slightly increased relative to baseline in plaque from the control group. These effects were only directional, yet they are consistent with the terminal pH data. While the effects of the arginine-containing dentifrice on acid production and terminal pH are not as strong as those observed on resting pH and ammonia production, intuitively, as indicated above, these observations make sense. Arginine influences alkali production directly, whereas it may only influence acid production indirectly, as a secondary effect. Additionally,

measurement of terminal pH and lactic acid production at a single time point after a single cariogenic challenge at each sampling visit, may be inadequate to fully assess the effects of the treatments on these two parameters, given the likely number of cariogenic challenges experienced during this study on a daily basis. A typical subject undergoes frequent cariogenic challenges throughout the course of a day. As the caries process is a continuum, caries progression depends on the cumulative effects of exposure of the tooth to acidic pH, rather than the effects at a single time point. In addition, small changes in pH can have a dramatic effect on rates of demineralization. Given the cumulative effect of these numerous challenges, the seemingly small reduction in acid production and small improvement in terminal pH experienced by the arginine-containing test group relative to the control group may translate into a much larger anticaries benefit than would be predicted based on a single measure in time, as used in the current study.

The results of this plaque metabolism study provide insight into factors driving the superior caries protection of the new dentifrice containing 1.5% arginine, an insoluble calcium compound, and fluoride, observed in the six-month enamel and root caries studies,[23-27] the two-year caries clinical study of cavitation,[28] and the intra-oral remineralization and demineralization clinical studies,[33] as compared to dentifrices containing fluoride alone. The primary mechanisms by which the arginine-based technology and the fluoride deliver their anticaries effects are independent and complementary to each other. Fluoride plays a defensive role by protecting the tooth mineral from acids, and helping to repair early demineralized lesions. The arginine-based technology plays a proactive role by directly impacting the source of caries, *i.e.*, dental plaque. While its mode of action is undoubtedly complex and multi-faceted, this study indicates that the arginine-based technology modulates the plaque environment to enhance utilization of the arginine deiminase system, a key pathway in certain bacteria through which ammonia is produced. Thus, use of the arginine-containing dentifrice shifts the metabolic capacity of plaque to more effectively utilize arginine, produce alkali to neutralize plaque acids, and elevate resting plaque pH compared to the use of dentifrices containing fluoride alone.

The results of this study, which support the improved anticaries efficacy of the arginine-containing test product, are consistent with the published literature suggesting that utilization of alkali production by bacteria may be a means of protecting against caries. The pioneering research by both Kleinberg and co-workers[18,21] and by Burne and Marquis[16] has highlighted the importance of metabolic balance between acid and base producers in the progression of caries. Alkali production from nitrogen-rich substrates, such as arginine and urea, has been identified as the protective mechanism used by commensal bacteria to survive acidic conditions. Because energy in the form of adenosine triphosphate is generated during the catabolism of arginine, supplementing the oral cavity with arginine from the arginine-containing dentifrice may afford the arginolytic bacteria a bio-energetic advantage, and help them better compete with the cariogenic bacteria for survival.

Much progress has been made since Kleinberg's early work in understanding the molecular genetics and the physiological aspects of ammonia generation and its relationship to caries and health.[35,36] Several studies have shown that loss of alkali-generating potential in dental plaque through loss of urease activity has a positive relationship with dental caries experience.[37,38] More importantly, clinical studies have demonstrated that the *in situ* production of ammonia, from arginine naturally present in saliva, via the ADS in dental plaque is positively associated with reduced caries experience. When the relative enzymatic activity of ammonia-producing pathways (both ADS and urease activities) in dental plaque was compared for caries-free (DMFT = 0), caries-experienced (DMFT ≥ 4, no active caries for 12 months), and caries-active subjects (DMFT ≥ 4 with active caries) subjects, it was found that the caries-active subjects demonstrated reduced capability to generate ammonia. The results of this study demonstrate that caries status is correlated with both ADS activity and urease activity.[39] Similar observations have also been reported in an independent study.[40] A proof of concept clinical study has shown that an exogenous source of arginine can influence ADS activity in both caries-free and caries-active subjects. In this study, fluoride-free toothpaste with 1.5% arginine plus calcium carbonate was compared to a 1100 ppm fluoride toothpaste (silica /NaF) as a positive control which has been clinically proven to prevent cavity formation. After four weeks of twice-daily brushing, the arginine-containing toothpaste group had significantly increased ADS activity. Importantly, the ADS activity increase was most significant for the caries-active subjects. This indicates that exogenous arginine delivered during tooth brushing can reduce caries risk by increasing ADS activity.[41] Most importantly, the clinical study by Acevedo, *et al.* has demonstrated that the delivery of exogenous arginine, to modulate bacterial metabolism, translates into a significant and clinically meaningful cavity prevention benefit. The same two dentifrices were evaluated in a two-year caries clinical trial among 11- to 12-year-old Venezuelan children. After two years, the fluoride-free, arginine-containing toothpaste demonstrated equivalent efficacy to the 1100 ppm NaF/silica positive control toothpaste. This clearly indicates that the effect of the arginine-containing toothpaste on plaque metabolism translates into a significant and clinically meaningful cavity prevention benefit.[22]

The dentifrice developed by Colgate, combining 1.5% arginine, an insoluble calcium compound, and fluoride, is an anticaries system capable of affecting the caries process in two ways: it builds upon fluoride's proven ability to protect the tooth surface from acids by adding a technology that enhances the natural protective mechanisms used by arginolytic bacteria to fight and survive against cariogenic bacteria. The development and validation of this new dentifrice represents a paradigm shift in caries protection. Much of the focus in caries research has centered on the physiochemical component of caries by trying to deliver fluoride more effectively, or by devising new remineralizing technologies. These approaches do not address the biological component that initiates and perpetuates the caries process. While some researchers have attempted to address the biological component of caries through use of broad spectrum antibacterial agents such as chlorhexidine, this approach appears to have limited potential.[42-44] The new dentifrice containing 1.5% arginine, an insoluble calcium compound, and fluoride is unique

and represents a simple solution to a very complex problem. It effectively utilizes natural biological protective mechanisms to tip the metabolic balance in favor of conditions that promote the maintenance of strong and healthy teeth.

In summary, the dentifrice containing 1.5% arginine, an insoluble calcium compound, and fluoride is an exciting new development in the treatment of dental caries. Its ability to provide enhanced anticaries efficacy compared to fluoride alone is well documented.[23-28,33] From a mode of action standpoint, there is still much to learn. But this study has provided insight by establishing that the new dentifrice has a powerful effect on plaque's metabolic activity with respect to alkali production, acid neutralization, and subsequent effects on pH. With respect to the drivers of the observed metabolic changes, there are several possibilities. The metabolic change could result from a general ecological shift favoring a higher proportion of arginolytic bacteria, increased expression of the genes controlling the arginine deiminase system in arginolytic bacteria, suppression of the genes controlling the virulence factors of the acid-producing bacteria, or a combination of any of the three. Further research is needed to address these questions.

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