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### The development and validation of a new technology, based upon 1.5% arginine, an insoluble calcium compound and fluoride, for everyday use in the prevention and treatment of dental caries

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#### ABSTRACT

*Objective*: This paper briefly discusses caries prevalence, the multi-factorial nature of caries etiology, caries risk and the role and efficacy of fluoride. The paper also highlights research on bacterial metabolism which provided understanding of the mouth's natural defenses against caries and the basis for the development of a new technology for the everyday prevention and treatment of caries. Finally, evidence that the technology complements and enhances the anti-caries efficacy of fluoride toothpaste is summarized.

*Conclusions*: Global data show that dental caries is a prevalent disease, despite the successful introduction of fluoride. Caries experience depends on the balance between consumption of sugars and oral hygiene and the use of fluoride.

Three scientific concepts are fundamental to new measures to detect, treat and monitor caries: (1) dental caries is a dynamic process, (2) dental caries is a continuum of stages from reversible, pre-clinical to irreversible, clinically detectable lesions, and (3) the caries process is a balance of pathological and protective factors that can be modulated to manage caries. Fluoride functions as a protective factor by arresting and reversing the caries process, but fluoride does not prevent pathological factors that initiate the process. A novel technology, based upon arginine and an insoluble calcium compound, has been identified which targets dental plaque to prevent initiation of the caries process by reducing pathological factors. As the mechanisms of action of arginine and fluoride are highly complementary, a new dentifice, which combines arginine with fluoride, has been developed and clinically proven to provide superior caries prevention.

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Dentistry

### 1. Dental caries is a global oral health issue and a public health concern

Despite the highly successful introduction of fluoride, dental caries is a globally prevalent oral health issue and a continuing public health concern.<sup>1–3</sup> Our understanding of global dental caries patterns has grown over the past several decades. The

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published data demonstrate that caries experience varies widely among populations, both within individual highly developed countries, such as the United States, and from country-to-country in developing and emerging economies.<sup>4</sup>

Detailed analysis of the changing global patterns of caries experience over several decades has led investigators to broadly categorize caries experience in three groups.<sup>5,6</sup> The first comprises populations with low caries experience (mean

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number of decayed, missing and filled teeth [DMFT] <1-2 in 12year olds), typically constant over time, found largely in rural populations in, for example, China, Africa, and remote areas of South America. The second comprises populations with moderate to high caries experience (mean DMFT = 3-4 and higher in 12-year olds), typically increasing over time, found largely in newly industrialized populations in developing and emerging economies, including Latin America, Central and Eastern Europe, and Asia. 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. The third comprises populations with low caries experience (mean DMFT  $\sim$ 1 in 12-year olds), as a result of a dramatic decline over time, found largely in established market economies, such as North America, Western Europe, Australia, and Japan. This decline in caries experience, which primarily results from the introduction of fluoride, has been accompanied by an increase in tooth retention in older adults. Interestingly, restorative dentistry in these populations has changed from a heavy focus on primary cavities in children and adolescents in the 1970s to a focus on "recurrent" caries in adults in 2000, reflecting the pattern of caries experience across life stages.<sup>6</sup>

Whilst the data from the developing and emerging countries clearly show that dental caries is a significant oral health and public health issue, at face value, the data from the industrialized countries suggest that caries is effectively managed and is no longer a major health problem. However, this is not the reality. The NHANES III study showed that dental caries remains an oral health problem in the USA.<sup>7</sup> Both the caries prevalence and severity data increased with age, showing that dental caries is not only a childhood disease, but also a prevalent adult problem. The data also showed that 80% of caries experience was found in 25% of the population, a "high risk" subset comprising groups of low social economic status.<sup>8</sup> The data from Europe and Australia show similar patterns, with dramatic declines in overall caries experience, yet widespread disparities in "high risk" groups, attributed to poor oral hygiene, diets rich in refined sugars and a number of factors stemming from low economic status.4

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 socio-economic inequality.<sup>9</sup> 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.<sup>10</sup>

Prevalence data also clearly demonstrate that dental caries is a disease which may affect individuals during each life stage, from birth through adolescence and adulthood to old age.<sup>11</sup> It appears that increasing caries experience with age is a broad global phenomenon resulting in an increasing need for caries diagnosis and management, both preventive and restorative, across life stages. In addition, gingival recession increasingly occurs in older adults, exposing the tooth root and posing root caries as a potential additional problem.<sup>11</sup> Thus, whilst dental caries has declined on a global basis over the past several decades, dental caries is an ongoing global oral health and public health problem throughout life.<sup>12</sup> Both the prevalence and severity of dental caries has been driven by two key behavioural factors: increasing caries has been driven by increasing consumption of refined sugars, whilst decreasing caries has been driven by improved oral hygiene and the effective use of fluoride. In essence, caries incidence and severity reflect the relative importance of these two factors to the individual and in the population.<sup>4</sup>

The fact that dental caries is prevalent globally does not, by itself, merit it as a major issue. However, the significant cost to treat the disease, as well as its impact on individuals and populations who cannot afford or do not have access to dental care, elevate dental caries to an oral health and public health issue. The most tangible costs are, of course, for dental restorations. A less tangible cost is the pain and suffering associated with caries; it can diminish the quality of life and may lead to malnutrition and other health problems, whilst the cosmetic consequences of dental caries can impact selfesteem and self-confidence.

### 2. Current concepts of the etiology of dental caries and the caries continuum

Dental caries is a multi-factorial disease with a complex etiology. The root cause of dental caries is bacterial production of acids, from dietary sugars and carbohydrates, at the interface of residual dental plaque and a susceptible tooth surface.<sup>1,13</sup> Time is a critical factor in determining caries severity, and factors, such as saliva function, behaviour, education and socioeconomic status, are also important.<sup>14,15</sup>

Dental plaque is a complex, highly diverse oral biofilm which develops on tooth surfaces over time and may remain in hard-to-brush areas, after brushing, for extended periods.<sup>1,13</sup> The properties of dental plaque 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. Specific species within the community are codependent and the community as a whole enjoys increased metabolic efficiency, greater resistance to stress, and enhanced virulence compared to its planktonic counterparts.<sup>16,17</sup> The composition and metabolism of plaque biofilm varies from site-to-site, for example on the teeth versus on the tongue, and deep in pits and fissures versus at gingival margin, yet at a particular site can remain relatively stable over time, in microbial homeostasis. Significant changes in the environment, however, can trigger changes from a "healthy" plaque biofilm to a "pathogenic" plaque biofilm, thereby predisposing a site to disease.<sup>18</sup>

The production of acid from fermentable dietary sugar can cause a significant change in biofilm environment that triggers a change from a "healthy" to a "cariogenic" plaque, predisposing the site to dental caries. Adaptive mechanisms play a critical role in the presence and persistence of specific bacterial species in the plaque biofilm. The aciduric/acidogenic bacteria implicated in the pathogenesis of dental caries, i.e., the mutans streptococci, would be unable to survive under the acid conditions they create at the biofilm-tooth interface, if they did not possess a mechanism of acid tolerance. Many of the bacterial species associated with oral health have difficulty

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surviving at the biofilm-tooth interface as they do not possess this acid tolerance mechanism.<sup>1,13</sup> However, several of these bacterial species possess an alternative mechanism, known as the arginine deiminase pathway, which breaks down the arginine present in saliva, produces ammonia and neutralizes plaque acids, and enables them to survive.<sup>1,13</sup> Thus, this mechanism protects the biofilm and prevents a change from a "healthy" to a "cariogenic" plaque.

When acid is formed at the tooth-biofilm interface in sufficient amounts and for sufficient time periods, the local pH drops below the critical pH (the pH below which calcium and phosphate from the tooth mineral are solubilized), the local environment at the interface becomes under-saturated in calcium and phosphate, and this drives dissolution of calcium and phosphate from the tooth enamel into the surrounding local environment, resulting in de-mineralization and loss of tooth mineral.<sup>1,13,19</sup> When fluoride is present, the critical pH for solubilization of calcium and phosphate ions is lowered, with the result that a lower plaque pH can be tolerated before solubilization occurs (Fig. 1).<sup>19</sup>

One of the most important concepts is that dental caries is a dynamic and reversible process.<sup>20,21</sup> When the caries process affects the crown of the tooth, it initially leads to a "subsurface" de-mineralized zone below the surface of the intact enamel, known as an "early caries" lesion.<sup>22</sup> Early lesions can be arrested and reversed by re-mineralization. Re-mineralization occurs following de-mineralization, when the acid challenge is removed, saliva becomes saturated in calcium and phosphate, once again, and these calcium and phosphate ions are driven back into the de-mineralized tooth tissue resulting in net mineral gain and "repair" of the enamel's hydroxyapatite structure.<sup>19,20</sup> However, if an early lesion is left untreated and continues to de-mineralize, it progresses beyond the point where it can be effectively reversed and re-mineralized, reaching the clinical end point of cavitation, which needs professional intervention, i.e., restoration.

A second important concept is that the caries process is a balance between pathological factors and protective factors. Fig. 2a 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 de-mineralization, and



Fig. 1 – Stephan curve showing the effects of a sugar challenge on plaque pH. Fluoride lowers the "Critical pH" below which solubilization of calcium and phosphate from the tooth occurs. Typical time to return to resting pH is 45–60 min (adapted from Kleinberg<sup>19</sup>).



a) In health, protective factors dominate over pathological



b) In disease, pathological factors outweigh protective factors

Fig. 2 – Schematic illustration of the caries balance in (a) health and (b) disease (adapted from Featherstone<sup>20</sup>).

the formation or progression of a caries lesion. If, on the other hand, the protective factors dominate, then the caries process results in net re-mineralization and existing caries lesions are arrested and reversed.<sup>21</sup> Dental professionals have found this concept helpful in assessing the pathological and protective factors in their patients and in recommending specific preventive and treatment steps to reduce pathological factors and to increase protective factors, to help manage the caries process.<sup>21</sup>

A third important concept is that dental caries is a continuum of disease states, ranging from sub-clinical early lesions to advanced, clinically detectable lesions. Clinical researchers have classified the various stages of primary coronal caries lesions and have defined diagnostic criteria for the detection and monitoring of intact and cavitated lesions in caries clinical trials and in clinical practice.<sup>23</sup> Early caries lesions, however, require more advanced and discerning methods of detection and monitoring, and this has important consequences for caries management and for the development and validation of new technologies to arrest and reverse early caries lesions.<sup>24</sup>

The preceding discussion has implicitly focused upon coronal (enamel) caries, but is also relevant to root (dentin) caries. Dental plaque biofilm, host tissue, and sugar are also critical factors in the development of root caries. An additional factor is the presence of gingival recession and exposure of the tooth root.<sup>25</sup> The critical pH for dentin de-mineralization is higher than the critical pH for enamel, which has suggested that more efficacious measures may be required to reduce demineralization and enhance re-mineralization of dentin.<sup>26</sup> In practice, root caries lesions can also be arrested and reversed and are, thus, amenable to preventive measures.

### 3. Caries risk factors and the concept of reducing risk factors to prevent and treat dental caries

Understanding of the factors driving the changing patterns of caries experience at the population level has sparked interest in the assessment and management of caries risk.<sup>27</sup> There is general acceptance that the true risk factors in dental caries are the presence of susceptible tooth surfaces, acid-producing bacteria, including *Streptococcus mutans*, frequent sugar intake; impaired salivary function, poor oral hygiene, past caries experience; inadequate fluoride exposure, limited access to dental care; and low socioeconomic status.<sup>12,21,28–30</sup> Understanding risk factors enables caries risk management and targeted prevention at both the population and the individual level. At its simplest, caries risk can be reduced by reducing caries risk factors.

The risk factors that can be readily addressed with new and improved oral care products are the two key biological factors: (1) the susceptible host tissue and (2) the cariogenic bacteria in the plaque biofilm, and as a consequence of the latter, the resulting production of bacterial acid.<sup>4</sup>

Historically, much of caries research and its translation into practical prevention and treatment measures have been focused on the host tissue. Specifically, attempts to reduce caries risk have focused on reducing the tooth's susceptibility to acid attack by rendering the tooth's surface less vulnerable to de-mineralization. In addition, by enhancing the uptake of calcium and phosphate ions into de-mineralized tissue, remineralization can be increased. Such measures to reduce the risk of caries can increase protective factors to restore the caries balance.<sup>4</sup>

In contrast, there has been limited research on the plaque biofilm, and its harmful consequences, that has translated into practical prevention and treatment measures. In principle, routes to reduce risk by targeting the plaque biofilm to reduce cariogenic challenge include: (1) reduction of total biofilm biomass, (2) inhibition of bacterial acid production and (3) promotion of microbial homeostasis and a dynamic balance favouring organisms associated with "health" within plaque biofilm. Such measures can reduce the risk of caries by reducing pathological factors to restore the caries balance.<sup>4</sup>

### 4. The role and efficacy of fluoride in reducing and preventing caries

The primary route to control the harmful effects of the plaque biofilm in initiating and perpetuating the caries process is thorough and effective removal of dental plaque from all tooth surfaces on a routine basis.<sup>31</sup> However, many individuals lack the knowledge, skills or motivation to achieve the necessary level of plaque control to be caries free, which is why therapeutic agents play an important role in supplementing mechanical oral hygiene.<sup>32,33</sup>

The dramatic decline in caries in developed countries over the past several decades has been attributed to the introduction of fluoride as a caries preventive agent. The widespread use of fluoride toothpaste has been widely acknowledged to be the single most important factor contributing to this decline in caries.<sup>34,35</sup> Indeed, fluoride is the only anti-caries agent that is routinely used as part of everyday oral hygiene by all segments of the population and this is likely because the benefits of fluoride are clinically proven in a spectrum of populations from young children to older adults.<sup>36</sup> Several comprehensive clinical reviews, which include a Cochrane systematic review, have shown that regular brushing with fluoride toothpaste reduces the development of coronal cavities by approximately 25% compared to brushing with non-fluoride toothpaste.<sup>37-42</sup>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.<sup>43</sup> The fluoride source itself is not critical to its effectiveness in cavity prevention. The Cochrane systematic review, which analysed the clinical 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.40 Fluoride toothpastes are also clinically proven to reduce the development and progression of root caries.26,44

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 mouth rinse, 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 sufficient time periods (>12 h) to exert sustained biological activity.  $^{45\text{--}47}$  Specifically, these low, sustained levels of fluoride reduce the critical pH value below which calcium and phosphate ions are solubilized from tooth enamel and dentin, thus, reducing de-mineralization.48-50 They also enhance re-mineralization;48,51 formation of calcium fluoride on the tooth surface acts as a reservoir of fluoride and significant drops in pH trigger fluoride release which drives calcium and phosphate ions back into the de-mineralized hydroxyapatite structure within the caries lesion.<sup>52</sup>

In summary, fluoride dentifrices, and other topical fluoride products, reduce dental caries by targeting the tooth surface and reducing its susceptibility to acid attack. Fluoride arrests the caries process, reducing de-mineralization and increasing remineralization of de-mineralized tissues. However, conventional fluoride toothpastes and mouth rinses have their limitations for high risk individuals, especially those with high plaque levels and frequent sugar intake. Fluoride does not act upon the plaque biofilm, an important modifiable factor in dental caries, to reduce cariogenic challenge. Specifically, fluoride does not primarily: (1) reduce total biofilm biomass, (2) inhibit bacterial acid production or (3) promote microbial homeostasis within the plaque biofilm and a dynamic balance favouring organisms associated with "health". Thus, topical fluoride products help to control, but cannot completely prevent dental caries.<sup>14</sup>.

### 5. Oral bacterial metabolism, the mouth's natural defenses against caries, and the basis of a new technology for caries management

More than a century ago, the emerging field of microbiology provided important new insights into the cause of many

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infectious diseases in humans. Miller applied this new science to understanding dental disease and the role of bacteria in the oral cavity. The results of his pioneering studies led Miller to formulate his acid decalcification theory and to describe a discrete two-step process for the disease, now known as dental caries. He described how, in the first step, oral bacteria metabolize fermentable carbohydrates, primarily sugars but also starches, to produce acid, which in a second step is able to de-mineralize the tooth mineral to initiate and sustain the caries process.<sup>53</sup> Whilst Miller's work has been superseded by many decades of research into virtually every aspect of dental caries, this early understanding remains a cornerstone of new approaches to prevent and treat dental caries.

The complexity of the oral flora and the role of specific species in the development and progression of caries have provoked interesting debate for many years. Two schools of opposing thought developed as investigators addressed the questions: "Is caries caused by a specific pathogenic species or dental plaque as a whole?", and "Is caries a classical infectious disease or does it result from an ecological overgrowth?"54,55 Proponents of the specific plaque hypothesis and caries as a classic infection have supported this theory with observations of frequent association of the mutans streptococci, especially S. mutans, and lactobacilli species with caries.<sup>54</sup> On the other hand, proponents of the non-specific hypothesis have advocated that dental caries can occur in the absence of mutans streptococci and lactobacilli species and in the presence of other bacteria that can produce acid.<sup>55</sup> It appears that aspects of each of these two opposing hypotheses have merit in respect of the true role of the oral flora in caries causation. Research has shown that a spectrum of resident bacterial species exists that are both acid-producing and acid tolerant, and are sufficiently numerous in the oral cavity to contribute to the development and progression of caries.<sup>56</sup> Further, research has shown that caries can be triggered by an increase in acidproducing species in the plaque biofilm and/or a decrease in resident species that produce counteracting alkali.55

Several decades of research in the detailed mechanisms of oral bacterial metabolism and the mouth's natural defenses against dental caries has provided a sound scientific basis for the development of a new technology for caries management based upon arginine, an insoluble calcium compound, and fluoride. Specifically, Kleinberg and coworkers conducted studies of the metabolic pathways involved in carbohydrate metabolism, and the factors driving acid formation, which provided a rational basis for the cause-and-effect relationship between glucose availability and plaque pH fall.<sup>57,58</sup> Kleinberg and coworkers also studied the effects of the nitrogenous substrate, urea, on plaque pH behaviour and showed the mirror image profile of the Stephan curve, i.e., a rapid rise in pH followed by a slow decrease back to baseline.<sup>59</sup> Importantly, the results with glucose and urea together demonstrated that both acid and base formation can contribute to plaque pH and serve as counteracting metabolic forces in the caries process.<sup>59</sup> These studies also led to the recognition that pH response to substrate challenges, with substrates such as glucose and urea, is an easy way to measure the dynamics of acid-base metabolism of plaque biofilm in real time.<sup>58</sup>

Kleinberg's more recent research has examined the role of saliva on the Stephan pH curve. In essence, saliva plays

multiple roles: facilitating fermentable carbohydrate and acid clearance,<sup>60</sup> providing bicarbonate for acid buffering<sup>61,62</sup> and nitrogenous substrates for base formation.<sup>59,63</sup> Of particular importance was the identification of arginine, mainly from small arginine-containing peptides, as a critical pH rise factor.63 Arginine metabolism was shown to involve the previously mentioned arginine deiminase pathway, with conversion to glutamate and alpha-ketoglutarate and, ultimately, to ammonia, carbon dioxide and acetate.<sup>64,65</sup> By studying the effects of glucose and arginine on numerous acidogenic oral bacteria in vitro and ex vivo, Kleinberg was able to establish the central role and characteristic pH behaviours of arginolytic and non-arginolytic bacteria.64,66 Experiments with mixed bacteria showed that a higher ratio of arginolytic to non-arginolytic bacteria resulted in a less cariogenic Stephan pH curve, and visa versa.<sup>67</sup> Importantly, these studies demonstrated that single bacterial species do not reflect plaque pH profiles observed in vivo, whereas mixed species with their integrated metabolic pathways could reproduce the different profiles of pH behaviour observed in vivo for individuals with different levels of caries activity. Kleinberg concluded that a deficiency in base formation can be as important in caries development as can excessive acid formation from fermentable carbohydrate.55

Another beneficial effect of saliva is that it provides a constant source of calcium and phosphate that helps maintains super-saturation with respect to tooth mineral. Adequate calcium and phosphate levels are critical to inhibiting mineral loss during periods of low pH and promoting mineral gain when pH returns to neutral. Many studies have focused on the direct role of these ions in step 2 of the Miller process. Saliva may have an additional indirect, and possibly more effective, way of providing calcium and phosphate. When saliva secretion is stimulated, a rapid rise in pH can occur, reaching as high as 8.3. Several salivary components come together in progressively greater amounts as the pH rises above neutrality. As a result, a complex of glycoprotein and calcium phosphate forms, named by Kleinberg-Salivary Precipitin, which is readily incorporated into dental plaque. Importantly, the calcium phosphate in Salivary Precipitin is 8-10 times more soluble than the calcium phosphate in tooth mineral and so it can serve as a sacrificial mineral that will dissolve preferentially before dissolution of tooth mineral, whilst providing a source of ions for re-mineralization of decalcified tooth tissue. In support of this role, Kleinberg has observed that levels of calcium phosphate carried by Salivary Precipitin are higher in non-cariogenic than in cariogenic plaque, and are effective in these buffering and re-mineralization processes.67

These discoveries have provided important understanding of bacterial metabolism and the mouth's natural defenses against dental caries, as well as knowledge of how to manipulate this metabolism. Together, they provided a basis for Kleinberg's design of a therapeutic that behaves like saliva and is able to change local environmental conditions at the plaque biofilm-tooth surface interface from a carious to a noncarious condition, enhancing intra-oral base-forming capability with an increase in mineralizing ability.<sup>67</sup> The composition described by Kleinberg comprises arginine, calcium, and preferably one or more cariostatic anion carriers. Kleinberg's

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hypothesis for the role of each of these components is as follows: firstly, the arginine favours the formation of base by the plaque biofilm to counterattack the first stage of the Miller process and the formation of cariogenic acids. At the same time, the arginine favours the presence of arginolytic over non-arginolytic bacteria, helping to maintain a less cariogenic biofilm flora. Secondly, the insoluble calcium compound suppresses the solubilization of tooth enamel by mass action and reduces the release of calcium from tooth mineral when attacked by plaque acids. Calcium precedes the release of phosphate from enamel, dentin, cementum, and other calcium phosphate-containing tissues during acid solubilization. This means that it is better to use calcium, rather than phosphate, as a suppressant because the calcium will attack the second stage of the Miller caries process at its initiation. Thirdly, the cariostatic anions are capable of enhancing or supplementing the anti-caries activities of calcium and arginine. For example, such anions may provide buffering, which may enhance the base forming and, thereby, the acid neutralizing activity of the arginine. Together, the individual components of the composition are coordinated to simultaneously counter both steps of the Miller process.<sup>67</sup>

The basic research that led to this new technology was followed by clinical research demonstrating that products containing it can actually deliver clinically meaningful benefits in cavity prevention. Only by conducting appropriately designed clinical studies, could the hypotheses underlying the development of the technology be proven to be well founded. In the first of two caries clinical trials, a fluoride-free calcium-based toothpaste containing arginine bicarbonate was evaluated as an alternative to regular fluoride toothpaste. The results of the study showed that, after two years use of the products, the fluoride-free arginine bicarbonate toothpaste reduced the formation of cavities as effectively as 1100 ppm fluoride toothpaste, demonstrating that the approach is valid and applicable to toothpaste.68 Review of the scientific literature indicates that the results for this new fluoride-free arginine-containing toothpaste are noteworthy, as it is the first fluoride-free toothpaste to demonstrate comparable clinical efficacy in preventing cavities to the efficacy of 1100 ppm sodium fluoride toothpaste.<sup>4</sup> In the second of the two clinical trials, the effects of chewing two sugarless mints containing the technology, twice daily after brushing with regular fluoride toothpaste, were compared to those of a placebo mint. The results of the study show that, after oneyear 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, once again demonstrating that the approach is valid.<sup>69</sup>

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.<sup>70,71</sup> Several studies have shown that loss of alkaligenerating potential in dental plaque through loss of urease activity has a positive relationship with dental caries experience.<sup>72,73</sup> 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<sub>24</sub> 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.<sup>74</sup> Similar observations have also been reported in an independent study.<sup>75</sup> 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 4weeks 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.76

### 6. The development and validation of a new dentifrice containing 1.5% arginine, an insoluble calcium compound and 1450 ppm fluoride as sodium monofluorophosphate

In recent years, there has been a conspicuous trend towards conservative dentistry and minimal invasive therapy.<sup>77–79</sup> Both academic researchers and the practicing dental professionals recognize that new approaches to intervene earlier in the caries process are an important step in taking conservative dentistry and minimal invasive therapy to the next stage. In fact, the profession are actively looking for new technologies that are proven to be effective in "high risk" children and adults.<sup>78–81</sup>

It is also acknowledged that detection and monitoring of lesions, in their earliest stages, is critical to changing the paradigm in caries prevention.<sup>1,13,14,20,21,78,79,82-84</sup> Indeed, traditional caries clinical methods that only detect caries at the cavitation level have been noted to be an impediment to the introduction of innovative new technologies. In particular, it was suggested that enhanced methods of detecting and monitoring pre-clinical enamel loss would accelerate the conduct of clinical trials and would, almost certainly, give more meaningful clinical results. Traditional caries clinical methods do not allow investigators to evaluate the effects of an intervention on the disease process, but solely allow evaluation on the clinical end point, cavities.<sup>1</sup> For this reason, a number of 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 monitoring of pre-cavitated lesions over time.<sup>85,86</sup> These advanced techniques are gaining wide acceptance as state-of-the-art caries detection methods.<sup>24</sup>

Given the success of fluoride, it would be prudent that any new caries management strategy recognize and complement the effects of fluoride, if the goal is to significantly impact the prevalence and severity of dental caries. As fluoride's benefits are focused on the host tissue as a means of damage control, once the caries process has been initiated and is in progress, combining fluoride with an agent that prevents the caries process at an earlier stage, by targeting the residual plaque biofilm, would have potential to deliver a step change improvement in caries prevention.

Based upon its proposed mechanism of action, which is complimentary to the well-known mechanism of action of fluoride, arginine with an insoluble calcium compound has the potential to significantly enhance the caries preventive benefits of traditional fluoride dentifrices. For this reason, a new and innovative dentifrice based upon 1.5% arginine, 1450 ppm fluoride, as sodium monofluorophosphate, in an insoluble calcium base has been developed and clinically validated.<sup>4</sup>

Three coronal caries studies, using Quantitative Lightinduced Fluorescence (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.<sup>87-89</sup> 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 product use, improvements from baseline in the representative parameter  $\Delta Q$  (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.<sup>87</sup> 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 product use, improvements from baseline in the parameter  $\Delta Q$  (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.  $^{\mbox{\tiny 88}}$  In a third study, the new dentifrice was compared to a matched positive control dentifrice containing 1450 ppm fluoride alone. After six months product use, improvements from baseline in the parameter  $\Delta Q$  (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).<sup>89</sup>

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.<sup>90,91</sup> 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 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% lesions showed improvement for the new argininecontaining 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.<sup>90</sup> In the second study, the new dentifrice was compared to a matched positive control containing 1450 ppm. After six months 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).<sup>91</sup>

Finally, a 2-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.<sup>92</sup>

This Special Issue of the Journal of Dentistry reports two of the coronal and one of the root caries clinical studies.<sup>88,89,91</sup> A Special Issue of the Journal of Clinical Dentistry reports the additional coronal and root caries studies,<sup>87,90</sup> together with a series of *in vivo* and *in situ* mechanism of action studies which demonstrate that this new technology works by targeting dental plaque to modulate bacterial metabolism and reduce fluctuations in plaque pH, which reduces de-mineralization and enhances re-mineralization of early caries lesions.<sup>93,94</sup>

The following papers in this journal discuss the topics: (1) dental caries as a dynamic process resulting in a continuum of lesions from early mineral loss through to cavitation, and the opportunities that this knowledge presents in terms of methods available to detect and monitor early reversible

lesions and the effects of state-of-the art technologies and products that prevent and treat them, (2) the results of two clinical studies, one conducted in China and the other conducted in Thailand, that demonstrate 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 and (3) results of a clinical study conducted in Brazil that demonstrates that this new dentifrice is significantly more effective in arresting and reversing the root caries process in adults than regular fluoride only dentifrice.

#### 7. Summary and conclusions

Global caries prevalence and severity data show that dental caries is a prevalent condition from childhood through to old age, despite the decline in caries experience in many countries. Caries experience for individuals, and populations, depends upon the balance between increasing consumption of sugars, which increases caries, and improved oral hygiene and increasingly effective use of fluoride, which decreases caries.

Three concepts are fundamental to caries research and the development of new preventive measures in the future. First, dental caries is a process, which may be arrested and reversed, in its early stages. Second, dental caries is a continuum of states ranging from the early pre-clinical to the advanced clinically detectable lesion. If left untreated, the early reversible lesion can progress to an irreversible endpoint that then needs to be restored by a dental professional. Third, the caries process is a dynamic balance between pathological and protective factors. Preventive and treatment steps, which reduce pathological factors and/or increase protective factors can help in effective management of the caries process.

Two of the key caries risk factors, the susceptible host tissue and the cariogenic bacteria in the plaque biofilm, are identified as targets for preventive therapy. The global decline in caries experience has been largely attributed to the introduction of fluoride toothpaste. The efficacy of fluoride toothpaste is well documented in caries clinical trials. Fluoride toothpaste works by delivering low levels of fluoride to the mouth, which are sustained between brushing occasions, reducing de-mineralization and enhancing re-mineralization. Thus, fluoride is a protective factor that helps to arrest and reverse the caries process. However, fluoride does not prevent the production of acid by cariogenic bacteria, which is the first step in the caries process. Thus, there is an opportunity for a step change improvement in caries prevention using a dual approach of combining an agent, which targets dental plaque, to control the plaque biofilm and its harmful effects, with fluoride to target the host tissue.

As the next step in conservative therapy and minimum intervention, academic researchers and dental professionals are seeking new technologies to promote re-mineralization of early caries lesions and reverse the caries process at the earliest possible stage. They recognize that conventional clinical methods are inadequate to detect and assess early caries lesions and the caries process itself. More advanced methods, such as QLF and ECM, which have been developed, refined and optimized, offer opportunities to detect, treat and monitor pre-cavitated lesions. These advanced techniques are gaining wide acceptance as state-of-the-art caries detection methods.

A new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride has been developed and validated. Its mode of action is based upon the arginine deiminase pathway of non-pathogenic arginolytic bacteria. These bacteria break down arginine to ammonia to neutralize plaque acids and to preserve undisturbed plaque biofilm on the tooth surface in a healthy state. As the mechanisms of action of arginine and fluoride are highly complementary, the addition of the arginine to a calciumbased 1450 ppm fluoride toothpaste enhances the anti-caries efficacy compared to the efficacy of fluoride dentifrices with the same level of fluoride alone. This has been clinically proven, using advanced and discerning new detection methods, in three coronal caries studies in children and two root caries studies in adults. Additionally, this new dentifrice has been evaluated in a conventional 2-year caries clinical trial and has been shown to be statistically significantly more effective than a dentifrice with fluoride alone in reducing the progression of caries to cavitation. Importantly, this new technology offers the potential to meet the unmet needs for early intervention.

#### **Conflict of interest statement**

Dr Cummins is an employee of the Colgate-Palmolive Company.

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