THE CUTTING EDGE OF DENTISTRY:

Utilizing Nanotechnology for preventing Dental Caries & Oral Disease

A LITERATURE REVIEW
Novel Remineralization Strategies
Utilizing Nanotechnology

elementa
Revolutionary Oral Care

Abstract:

The science behind the prevention of dental caries has been embarrassingly stagnant for the past 100 years. This is due to the fact that current oral care product manufacturers have been approaching the problem from an antiquated knowledge base of what really causes caries. True prevention begins with a core understanding of pH-based concepts, which drastically affect the oral biofilms capacity to dissolve tooth structure, and the ability to direct the oral microenvironment back towards overall health. While many attempts have been made to alter the oral microenvironment, challenges have existed when addressing the fundamental issues pertaining to cariogenic biofilms and subsequent oral disease. While these problems are well known to the scientific community, there has not yet been a hygiene system capable of both penetrating and successfully introducing neutralizing agents into the biofilm. This is no longer the case, with recent advances in nanotechnology, it is now possible to bypass and suppress biofilm mechanisms that were previously known to prevent the uptake of neutralizing agents, and disturb the chain of cariogenic bacterial mechanisms and shifts towards oral disease.

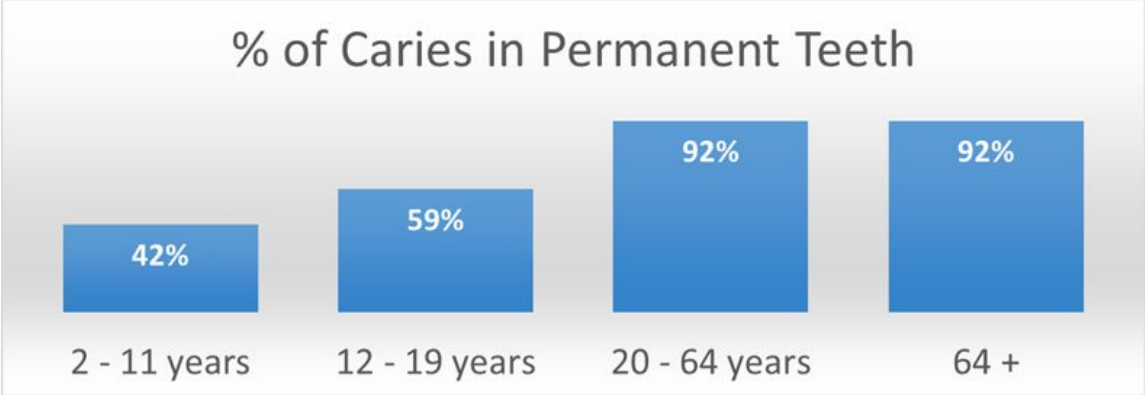
Keywords:

Oral Biofilm (OB), Plaque fluid, Exopolysaccharide (EPS) matrix, pH, Point of Zero Charge (PZC), Solubility product (Ksp), Ionic product (IP), Hydroxyapatite (HA), Silver Nanoparticles (AgNPs), Degree of Saturation (DS), Cation Exchange Capacity (CEC), Anion Exchange Capacity (AEC), Buffering Capacity, Alkalinity, Ion Substitution, Ion Competition, Structural Charge, Surface Charge, Adsorption, Absorption, Stern Layer, Diffuse layer, Substrate, Ecological Plaque Hypothesis, Remineralization, Demineralization.

Introduction - The Epidemic of Oral Disease:

For nearly one hundred years, dentistry has followed the same hygiene regimen to prevent oral disease. While fluoride treatments and sealants have had a significant positive impact on oral health, there still exists a large unaddressed gap in the prevention of oral disease. This is shown by our current epidemic-level rate of decay, which approaches 50% in children and increases towards 92% by adulthood[1]. Additionally, more than 80% of adults suffer from gingivitis and over 50% suffer from periodontal disease[1]. This extreme level of disease not only places a strain on the time and financial status of individuals, but can also lead to a concerning level of tooth loss. These disease paths are biofilm-based, and largely preventable.

Figure 1: Caries Prevalence Rates Based on Age[1]:



Current Modes of Prevention are Outdated:

You may be curious as to why our current methods of oral disease prevention are apparently failing. While oral disease is multifactorial, one core problem remains. Ultimately, the determining factor in the progression of oral disease is oral acid. Environment, sugar intake and socio-economical differences all play important roles in determining the presence of oral acid. These factors can culminate towards a low pH level in the oral biofilm that promotes certain disease paths. Not only does the pH of the oral microenvironment have the ability to promote or inhibit remineralization, it also induces changes in the types of bacteria present. Various bacterial strains may either facilitate or prevent disease processes. While these facts are well-studied, little has been done to innovate new agents and combinations to combat the underlying source of oral disease.

Cariogenic Disease Processes:

Caries is a term used to describe a physiological breakdown in the biological tissues of the oral environment[2,3,4,5]. The oral environment is coated with a biofilm that, when mechanically removed through brushing or flossing, is re-established within minutes. Upon reformation, the oral biofilm will regrow, mature, and disperse until disrupted again[4]. Cariogenic biofilms sequester and harbor oral acids as this maturation process occurs[6]. When the pH of the oral microenvironment is less than a critical value of 5.5, cariogenic disease processes occur[3]. Additionally, for individuals with low levels of calcium in their plaque fluid and saliva, the pH at which decay can occur may be as high as 6.5[3]. This effect can be further exacerbated when more aggressive strains of bacteria continue to grow and colonize, resulting in mineral dissolution[5,6]. This enamel dissolution re-buffers the plaque at the cost of additional tooth

structure[3]. This process creates an environment suitable for cariogenic organisms to grow, resulting in even more decay[5].

Natural Defense Mechanisms:

The human body's natural defence mechanism, saliva, works to restore balance by releasing enzymes that break through the plaque biofilm. When saliva floods into the plaque, calcium and phosphate salts are released, and the pH balance in the plaque fluid is restored[3,7,80]. Unfortunately, aggressive biofilms can interrupt this process and prevent timely salivary clearance of bacterial acids[6,7]. When substrate introduction is frequent, acid attacks occur more often, delaying the entry of saliva into the biofilm, leading to oral disease[6]. This constant attack on our oral environment happens every time substrates are consumed[80]. This is visualized by the Stephan Curve, whereby after substrate introduction, the plaque fluid pH level decreases[3,8,9]. Unfortunately, depending on the thickness of the plaque mass and the subsequent salivary clearance rate, it will occasionally take up to an hour for the plaque pH to resettle at a neutral level[6,9]. This is seen clinically as individuals struggle with new decay and recurrent caries around existing restorations. It is during these low pH events when demineralization and decay processes occur. If these issues are not addressed within a 6 month period, dental caries may develop.

Oral Disease Processes are Preventable:

While oral disease is not 100% preventable, the oral biofilm and its pH can be controlled and modified, preventing disease onset. Almost all current dental products are acidic, and contain preservatives and unnecessary supplemental ingredients, such as alcohol, SLS, and parabens. Not only are these compounds toxic to the oral environment, but they are added to products to primarily support an acid-base model thereby enhancing fluoride uptake and prolonging the shelf life of the product. Although fluoride has been shown to be taken up under acidic conditions, this subject remains controversial, as the specific level of acidity required has yet to be established[3]. This acidic model comes with many problems, ultimately promoting a low-pH environment preferred by cariogenic organisms[6]. Additionally, most dental products are unable to effectively penetrate the exopolysaccharide (EPS) structure of the biofilm, having little to no effect on the plaque fluid, which can lead to oral acid accumulation as the biofilm grows[6,10]. Furthermore, antibacterial agents, such as chlorhexidine (CHX), have been shown to penetrate oral biofilms, and may do so at the cost remineralization[13]. According to the Point of Zero Charge (PZC) concept, calcium exchange with hydroxyapatite (HA) is pH-dependant.[11] As the pH decreases, calcium's attraction to HA is severely reduced, and instead favors hydronium ions (H^+). This is especially true when the pH level sits below 5.5[3,8,11]. Calcium's attraction to HA is only restated when there exists a stable, non-acidic microenvironment. When considering the inner workings of the oral biofilm, a neutral pH promotes the growth of healthy bacterial

strains[6,8,12]. When these commensal bacteria are thriving at a neutral pH, homeostasis is promoted. By preventing more aggressive strains of bacteria from accumulating and acidifying the oral microenvironment, oral disease and tooth loss can be prevented[12].

The Challenge to Oral Homeostasis:

In order to promote homeostasis, the following goals and endpoints must be considered:

1. Increasing salivary flow
2. Increasing the pH value of plaque fluid and saliva in a short period of time.
3. Increasing calcium ion activity near the tooth surface (maintaining a degree of saturation)
4. Disrupting biofilm shifts towards pathogenesis
5. Producing a starved state for cariogenic bacteria (breaking apart, penetrating, and inducing biofilm dissolution of the EPS layer)
6. Utilizing a mechanism to deliver remineralizing agents into the biofilm and tooth structure

It has proven to be challenging to achieve these objectives, since the substances capable of stabilizing pH have difficulty bypassing and penetrating the oral biofilm[10]. Many studies have confirmed the sequestering nature of biofilms from host environmental conditions[6,10]. Many of the substances capable of penetrating the biofilm, such as CHX, may interfere with the remineralization process, thereby limiting their ability to be used with other agents[13]. In addition, fluoride and calcium have been shown to have very poor penetrance into the oral biofilm, especially at a low pH[44,45,91]. These factors have further challenged the remineralization pathway to allow for optimal calcium ion activity near the tooth.

Nanotechnology - Untapped Potential for Prevention:

With the development of engineered nanotechnology, we can now penetrate deep into the biofilm while simultaneously stabilizing pH[14]. Silver, in its many forms, has been used for thousands of years as a natural antimicrobial, antibacterial, antifungal, and antiviral agent[15]. However, previous attempts to utilize silver compounds have been sullied by the lack of a delivery mechanism for use in the oral microenvironment. Silver, in its ionic form, is not effective, as the silver ions interact with various salts, enzymes, proteins, and saliva in the oral microenvironment. These interactions form byproducts which cannot efficiently penetrate or alter the oral biofilm[16]. In order to combat this problem, nanoparticles can be used. Nanoparticles, up until recently, were not stable enough to be used in the oral microenvironment. Now, nanoparticles can be made using a capping agent which coats their surfaces and protects them from dissolving into ions[16,17]. It is this protection mechanism that

allows nanoparticles to be effective over long periods of time while remaining stable at a small size[17]. Nanoparticles can be used to penetrate through biofilms and retain their properties before releasing ions, allowing them to be used with other combination agents and under multiple conditions[10]. Ultimately, nanoparticles must not only be alkaline, but also be stable in the oral environment. However, this method is not challenge-free, as many properties of nanotechnology have yet to be elucidated; salt stability remains an obstacle for most nanotechnology applications, limiting their uses[17]. Nanoparticles must also have a targeted mechanism of delivery to allow them to penetrate the biofilm alongside a neutralizing agent, which is typically a calcium salt. In the oral microenvironment, this is equivalent to penetrating the biofilm and stabilizing pH. Most nanoparticles cannot remain stable in the presence of other ions to achieve this, nor can they target the biofilm selectively. Engineered nanoparticles can now be designed with a coating that matches the EPS bacterial coating of the biofilm. This provides a mechanism for selection while preventing the nanoparticles from being disrupted by other ion activity in the oral microenvironment[17]. This not only provides a strong buffering capacity against acid attacks, but also offers antibacterial and antimicrobial properties at a concentration of 1/80th that of CHX[91]. These particles can also be made completely non-cytotoxic to any oral tissue, thanks to their superior coating and slow release of silver ions over time[17]. This antibacterial, biofilm penetrating, alkalizing agent stabilizes the PZC exchange complex around the tooth. Furthermore, it provides optimal calcium and phosphate delivery, subsequently stabilizing pH.

Combination Therapy - Enhancing Protective Factors Against Oral Disease:

It has been discussed that the best method for disease prevention usually involves combination therapy, with multiple agents and modes of action working simultaneously. Thus, targeting bacterial mechanisms of action using several different agents may be more effective in controlling biofilm-based disease paths. Moreover, by combining nanoparticles and other agents which are proven to be highly effective, such as xylitol and calcium salts, the likelihood for biofilm resistance, acid release, and cariogenic processes can be severely reduced[91]. Xylitol is akalkine and non-fermentable, and is able to reduce the proliferation of cariogenic strains of organisms below critical threshold levels[18]. Calcium also acts as a buffering agent to prevent demineralization, and is a key player in the remineralization process[19]. As noted previously, nanoparticles were unable to be combined with other agents due to their lack of stability. Hence, they have not been used in combination therapy for oral care until recently[17]. By using advanced engineering methods, these agents can now be combined with xylitol and neutralizing agents, such as calcium salts, to promote remineralization and stabilize oral pH levels. This has opened up a new realm for exploratory prevention of oral disease.

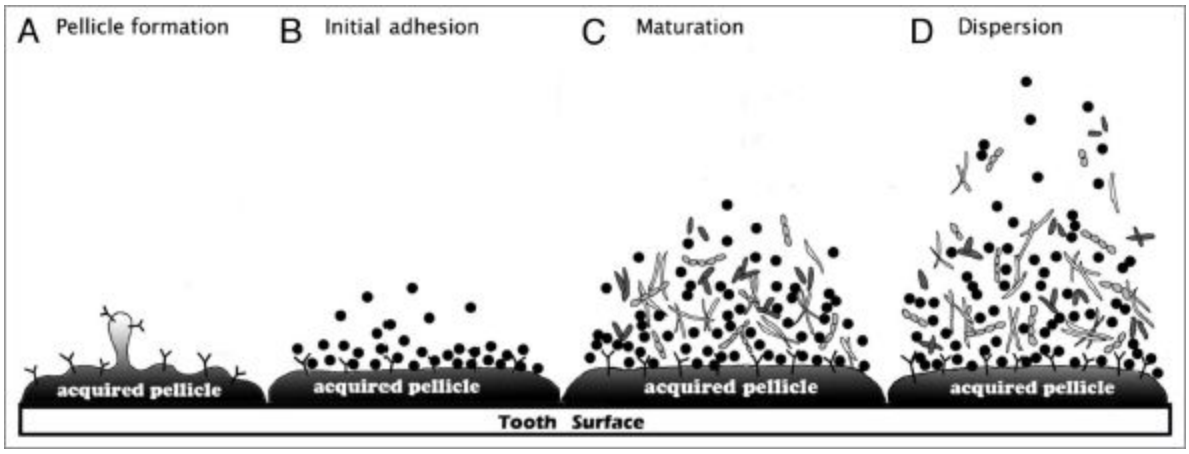
Table of Contents:

I: Biofilms, Bacteria, and Associated Challenges:

The Composition of a Biofilm:

The oral biofilm is a self-produced matrix of extracellular polymeric substance (EPS) that hosts a complex myriad of microorganisms[20,21]. Microbial cells that make up the oral biofilm adhere to each other on a living or non-living surface[20,21]. Oral biofilms are infectious in nature, and can initiate disease processes, resulting in dire consequences such as a loss of tooth structure[5,6]. Figure 1-1 depicts the growth and maturation of the oral biofilm[21]:

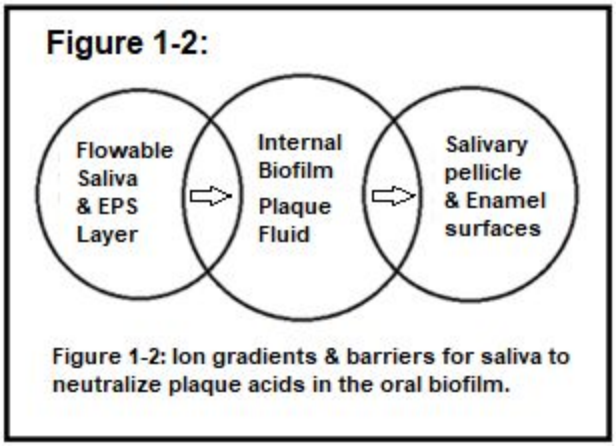
FIGURE 1-1: Biofilm Growth and Maturation Phases[21]:



The first step in the development of the oral biofilm is the formation of acquired salivary pellicle. The acquired salivary pellicle is a thin layer composed primarily of salivary proteins and bacterial enzymes, such as glycosyltransferases (Gtfs)[6]. Gtfs are able to bind directly to some oral bacteria, synthesizing glucan. The presence of glucan in the salivary pellicle further increases the adherence of bacteria to the oral biofilm. After the formation of the salivary pellicle, initial adhesion, maturation, and dispersion phases occur[6]. During the dispersion phase, newly developed biofilms migrate to new sites, promoting further colonization[6].

Oral biofilms efficiently self-regulate their uptake of nutrients and charged compounds. It has been noted that oral biofilms can form isolated pH microgradients in these regions. It is difficult for neutralizing agents alone to diffuse into and alter these gradients[5,6,32]. This mechanism allows biofilms to contribute to the development of dental caries, and even worse, tooth loss. The outer EPS matrix has been shown to limit the inward diffusion of charged ion buffers, while uncharged solutes such as sucrose can pass through the EPS matrix, and be readily metabolized into acids by bacteria embedded in the biofilm[10,22,32]. This process is visualized in figure 1-2[91]:

Figure 1-2: The Challenge of Ion Gradients:

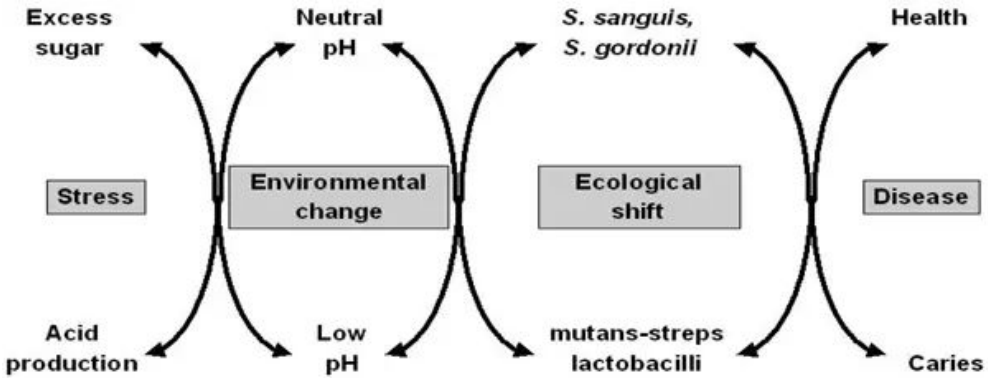


Furthermore, extracellular glucans appear to directly trap hydronium (H^+) ions to help maintain and further acidify the pH of the biofilm[6,32]. Dental caries can develop when acidic microenvironments are formed, maintained, and protected within the biofilm[6]. Without the buffering capacity of saliva to neutralize oral acids in plaque fluid, oral acids can remain in contact with tooth enamel for longer periods of time, contributing to decay[2,3,8].

Bacterial Growth and Shifting: The Ecological Plaque Hypothesis:

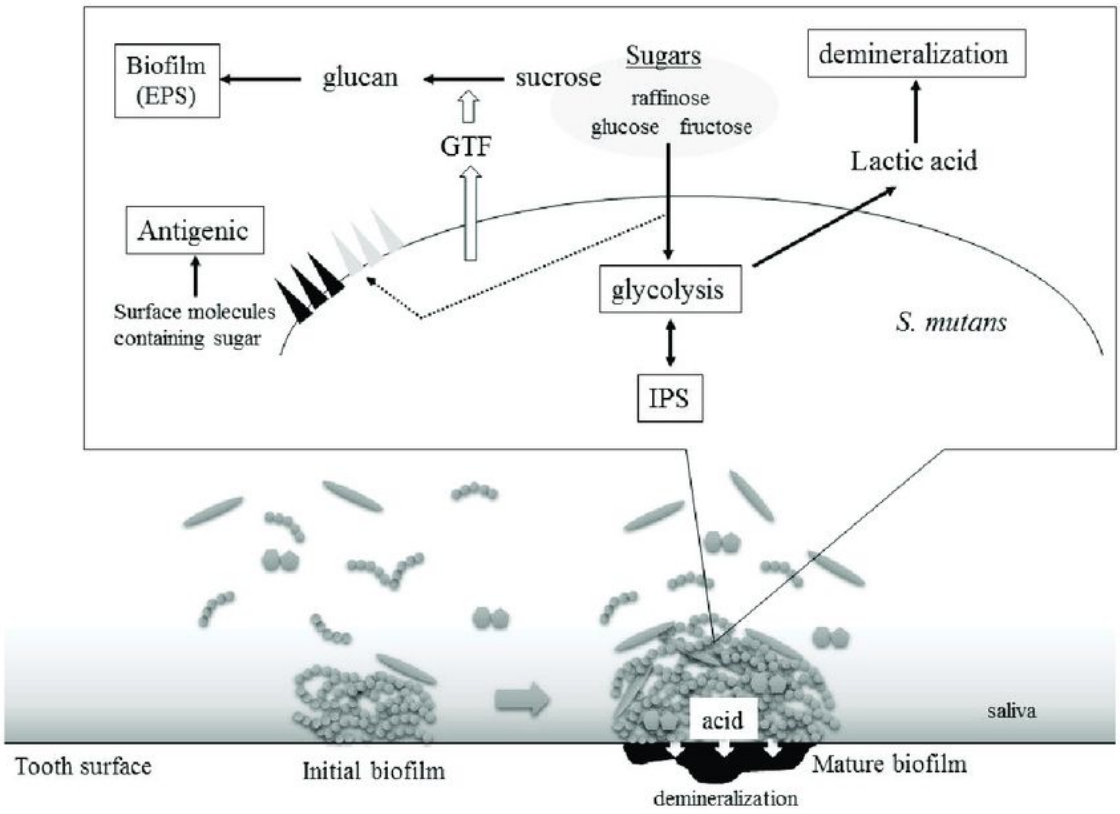
It has long been understood that oral biofilms are well-controlled and undergo shifting over time[5,6]. The ecological plaque hypothesis suggests that as a biofilm matures without disruption, pathogenic organisms will be favored due to shifts away from pro-commensal conditions[5,6]. This is due to shifts in pH, and oxygen levels from cariogenic organisms which tend to be anaerobic and thrive under acidic conditions[87]. This is illustrated in Figure 1-3:

Figure 1-3: Ecological Plaque Hypothesis: Shifting Towards Disease[12]:



Biofilm growth and attachment mechanisms should also be considered for cariogenic challenges. As the level of the substrate, or nutrient, increases, the ability of the biofilm to secrete glucan, dextran, and other glycoproteins increases; this allows them to attach more readily to the salivary pellicle[5,6,9,12]. By interrupting this process, demineralization can be inhibited or delayed, as the process of acid release from the biofilm will not occur as readily. Therefore, it is important to consider this in the maturation process of a biofilm. This attachment process is illustrated in Figure 1-4 below:

Figure 1-4: Glucan Bonding (*S. mutans*) and Subsequent Demineralization[23]:

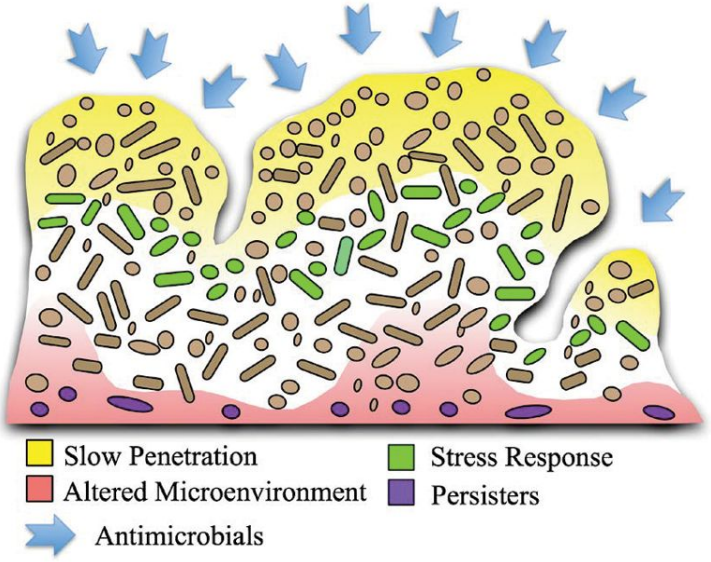


Caries occur as a result of changes in the environment of the biofilm due to acid production from the fermentation of dietary carbohydrates[6,23]. This selects for acidogenic and acid-tolerating species such as *S. mutans* and lactobacilli[6,23]. Disease can be prevented not only by targeting the putative pathogens directly, but also by interfering with the key environmental factors driving these deleterious ecological shifts in the composition of the oral biofilm[5,6,9,12,23]. Therefore, the ability to interrupt, reset or prevent these shifts plays an important role in dental caries prevention[6,91].

Challenges Associated with Oral Biofilm Homeostasis:

As mentioned previously, oral biofilms are known to suppress the uptake of charged species, especially those with the ability to neutralize acid[22]. This tendency must be taken into account when assessing remineralization strategies. As a biofilm grows, its tendency to shift towards a pathogenic nature increases. Bacteria such as *S. mutans,* which thrives in crowded, anaerobic conditions, can flourish by creating environments which are too acidic for host commensal organisms, thereby gaining a competitive advantage[5,6,12,23]. When these biofilms form, they secrete glucan, dextran and other glycoproteins, which allow for attachment and isolation from saliva[5,6,23]. Biofilms use their EPS layer to slow the diffusion of charged species, and can readily limit the amount of antibacterial and neutralizing agents which enter[24]. This is illustrated in Figure 1-5:

Figure 1-5: Slow Penetration and Diffusion of Antibacterial Agents (Oral Biofilm)[24]:



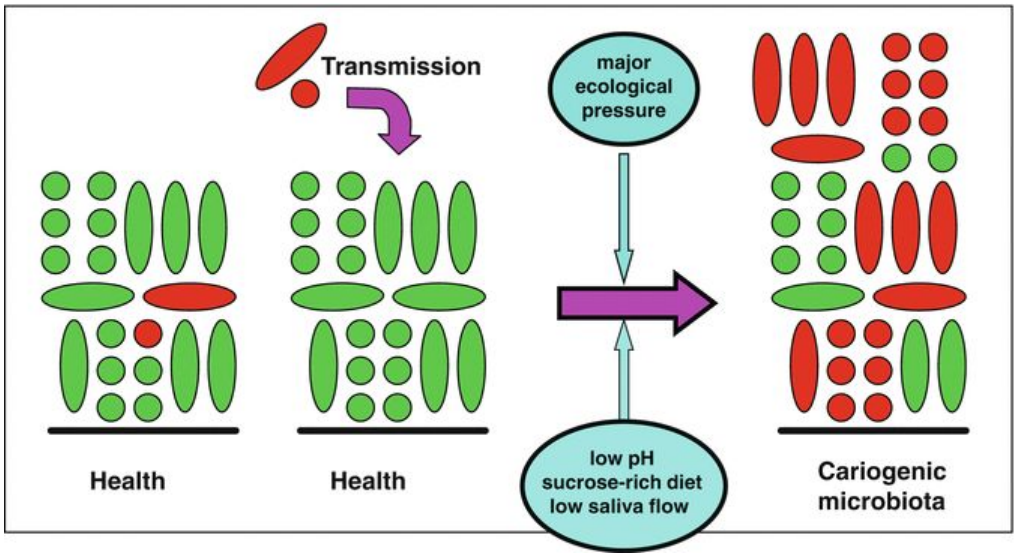
The EPS layer is able to slow the entry of saliva, salivary proteins and other beneficial complexes, rendering remineralization attempts difficult[22,24,32]. The bacterial composition of the biofilm reacts to changes in the local microenvironment, leading to an imbalance in compositional changes of the biofilm[5,6]. These changes to the overall metabolic activity of the biofilm can subsequently lead to disease, unless they are interrupted[6].

II: Importance of pH in Oral Microenvironments:

Importance of Homeostasis: A pH Problem:

Most commensal processes from oral biofilms occur at a neutral to alkaline pH level[5,6]. Opposing commensal organisms, cariogenic organisms are constantly attempting to render the ecosystem more suitable for their own growth[5,6,8,12]. A retentive site is colonized by these organisms present in saliva. *S. mutans*, although scarce in the initial inoculum (fewer than 0.1% of the initial colonizers), is selected for if the average pH value in the site is not well buffered by saliva[26]. Frequent ingestion of sucrose-containing products predisposes the biofilm to lower pH values, selecting for *S. mutans*[28,87]. When the pH remains in the vicinity of 5.0–5.5, tooth mineral is solubilized, thereby buffering the plaque and maintaining an environment suitable for the growth of *S. mutans*[26]. Once enough mineral is lost, a cavitation occurs in the enamel. If the cavitation enlarges and extends into the dentin, a semi-closed system will be formed in which the pH drops below 5.0[26]. Under these acidic conditions, growth of lactobacilli is favored, and these organisms succeed as the predominant flora in the carious lesion[5,6,26]. This progression towards disease is illustrated in Figure 1-6:

Figure 1-6: Effect of Cariogenic Organisms and Low pH on Disease State[25]:



Since pH is such a critical component in the success of cariogenic organisms, their release of lactic acid has been shown to give them a competitive advantage, while other organisms must

adapt to the acidic conditions or die[5,6,26]. It has been shown in multiple studies that *S. mutans* is excellent at recycling lactic acid through a carrier-mediated process. Therefore, *S. mutans* can use very little energy to maintain homeostasis, making better use of compounds with little to no toxicity, as opposed to other bacterial strains[27]. These highly acidic microenvironments can readily dissolve tooth structure. Thus, by neutralizing these microenvironments, cariogenic organisms lose a critical piece of their competitive advantage: pH control[27]. Ultimately, these organisms thrive in a low-pH environment, away from saliva and neutralizing agents.

III: pH-Dependence of Equilibrium and Charge-Based Concepts:

The Importance of Solubility Product (Ksp), Ionic Product (IP), and Equilibrium States:
As the scientific community's collective understanding of chemical equilibria has progressed, it has become obvious that chemical balances must be satisfied. This is especially true for tooth structure and HA, which is the building block of enamel. When looking at the demineralization-remineralization cycle, HA undergoes frequent changes within a central equilibrium[3]. Le-Chatelier's principle states that if there is an imbalance between products and reactants, the reaction must be driven back to a state of equilibrium. The equilibrium solubility product, known as Ksp, is the exact amount of mineral that can dissolve into a liquid. This value is temperature and pH-dependent[3]. The Ksp equation is depicted below 1-1[3]:

Ksp (Solubility product) = Fixed value of $[Ca]_3[PO4]_5[OH]_2$

Thus, at a neutral pH of 7, HA is hardly soluble and will only dissolve at a rate of 30mg/L[3]. However, under acidic conditions, this can reach up to 30g/L due to the logarithmic nature of pH[3]. With each drop in pH level, the acidity level jumps by a factor of 10. This means that a pH of 4 is one thousand times more acidic than a pH of 7. The ionic product is the non-equilibrium amount of mineral species that is dissolved in a liquid at any given time, and must follow Le-Chatelier's principle. Therefore, when there is a lack of calcium, phosphate and hydroxyl ions, the ionic product is negatively affected, placing pressure on tooth structure to dissolve[3]. Due to the dynamic nature of these ions, the dissolution of tooth structure can occur at a pH level as high as 6.5 in patients with calcium-deficient saliva and/or plaque fluid[3]. In Figure 1-7, it is demonstrated how the changing concentrations of various ions can still satisfy Ksp, even though these ions may have different activity levels. Since phosphate is pH-dependent in its ionic state, it is easy to understand how there are different burdens on different ions at various pH levels. For example, many different states of ion combinations can satisfy this equilibrium:

Figure 1-7: Satisfying Ionic Product (Calcium and Phosphate) Dependence:

$[Ca^{2+}]$ x $[P_i]$ = Ionic Product: (Where P_i= all complex ions of Phosphate species):		
mM $[Ca^{2+}]$	mM $[P_i]$	mM^2 (Ionic Product=Ksp)
0.84	0.84	0.7
0.7	1	0.7
0.07	10	0.7
0.1	7	0.7

$[Ca^{2+}]$ x $[P_i]$ = Ionic Product: (Where P_i= all complex ions of phosphate species):
mM $[Ca^{2+}]$ mM $[P_i]$ mM^2 (Ionic
Product=Ksp)
0.84 0.84 0.7
0.7 1 0.7
0.07 10 0.7
0.1 7 0.7

In order to visualize the importance of pH on HA dissolution, it is important to see how phosphate acidification is affected by pH in its many ionic forms, as noted above by (P_i). Carbonate is another common ion to consider in plaque fluid and saliva. When carbonate is present, a pH dependency also exists, demonstrating how carbonate substitution for phosphate is also pH- and ion composition dependent. This is shown in Figures 1-8 and 1-9:

Figure 1-8: Phosphate Complex Ion Equilibria pH-Dependence[3]:

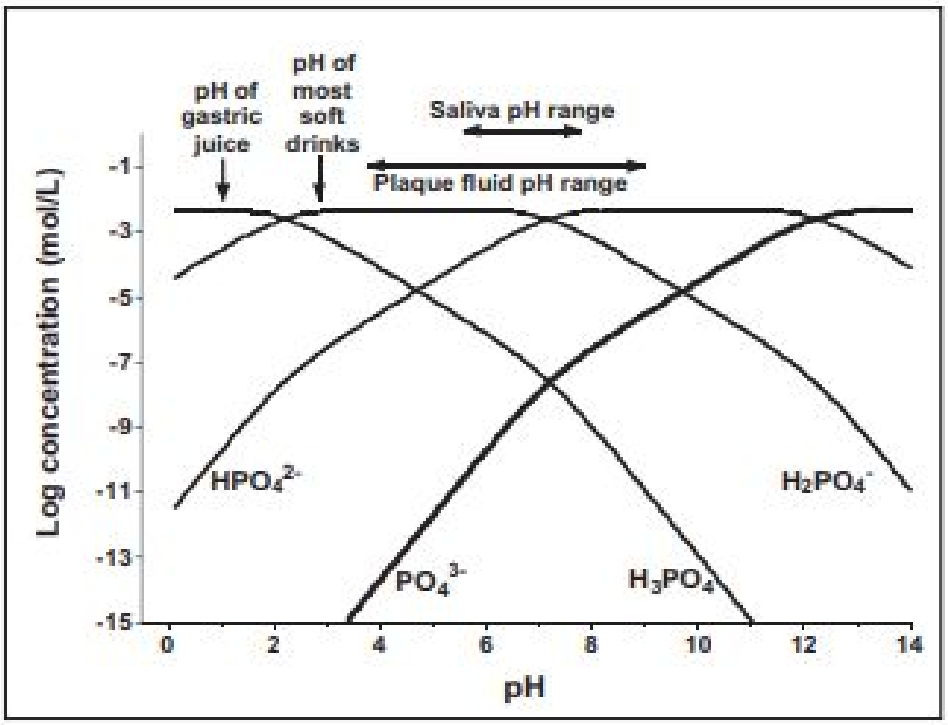
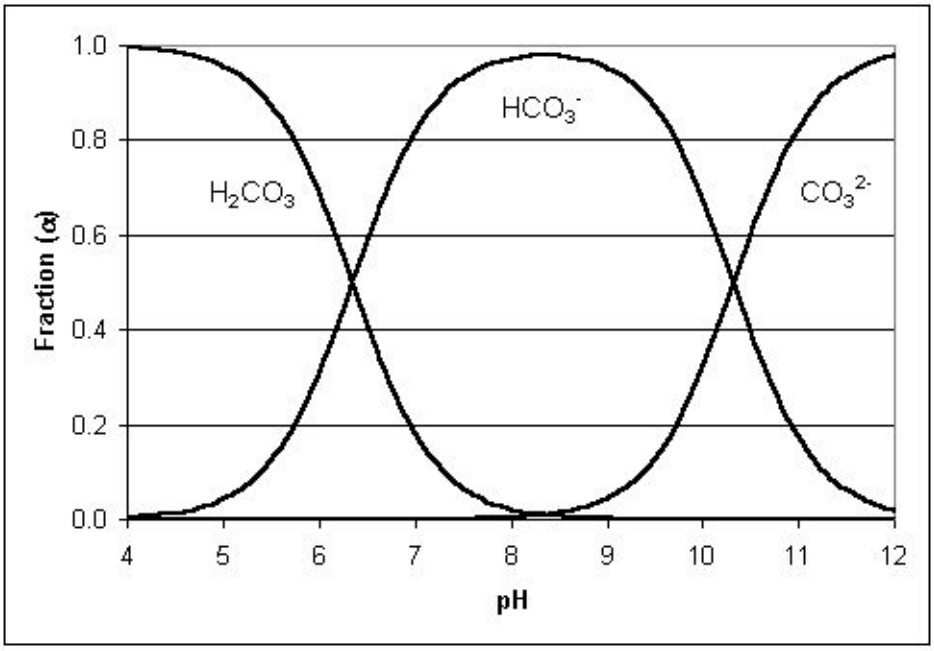


Figure 1-9: Carbonate Complex Ion Equilibria pH-Dependence[28]:



As depicted in Figures 1-8 and 1-9, each graph has a retracement between a pH of 7.5 and 8, whereby the preferred forms of these minerals exist for remineralization processes. This suggests that these complex ion equilibria can ultimately determine how remineralization processes occur. Based on this knowledge, it is critical that hygiene products have a pH level in the range of 7-8, in order to obtain superior results. White spot lesions on teeth are the result of lost calcium as well as substitution of phosphate with carbonate ions. Thus, carbonate substitution can be avoided at a neutral or alkaline pH. Furthermore, as shown in Figure 1-7, dependence on phosphate can also be reduced by increasing calcium levels, to maintain in a state of equilibrium while enhancing remineralization.

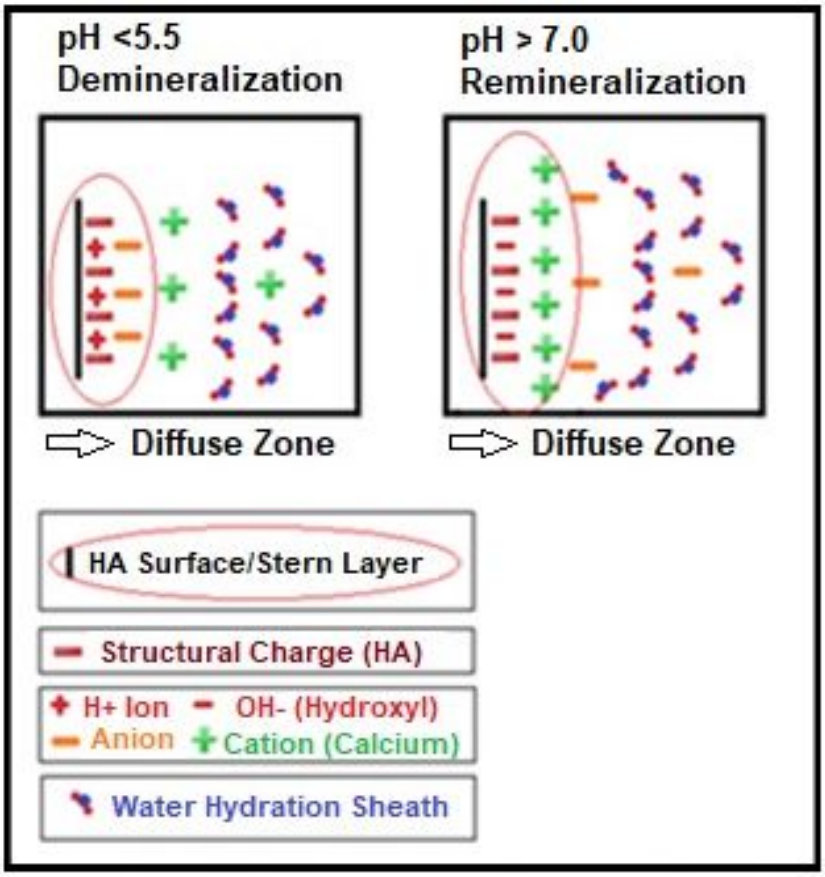
It should also be mentioned that the solubility coefficients, especially for precipitation (supersaturated), can be influenced by proteins, biofilm components, and other agents that can enhance or detract from the solubility of calcium phosphate salts[3,29,30].
This data contributes to a lack of understanding of the complexity of ion equilibria. However, it does not detract from the idea that in undersaturated conditions, demineralization occurs to satisfy the lack of minerals and buffering capacity present from oral acid buildup[5,6,12]. It is the continuum of oral acid buildup which places a constant strain on ion equilibria, forcing dissolution of tooth structure, subsequently leading to the formation of caries[3]. Therefore, to alleviate the stress on HA and satisfy undersaturated conditions presented by chronic bacterial insult, it is important to introduce remineralizing agents from an external source, such as saliva or dentifrice[3].

Point of Zero Charge (PZC) - A pH-Dependent Function for Remineralization:

One of the most critical concepts in understanding remineralization is the PZC[11,31]. This concept has been cited several times in dental journals, but is often forgotten in the schema of the remineralization hierarchy, despite its significance. The PZC concept determines the pH at which hydroxyl anions (OH^-) and hydronium ions (H^+) are in equal proportions on HA surfaces[11,31]. It is known that HA is negatively charged on account of its phosphate backbone. Thus, HA emits a negative charge into solution[11]. However, the total charge of any mineral is dictated by considering the structural charge and pH of the solution simultaneously[31]. This provides what is known as a net charge, and allows us to assess how the mineral will act in various situations. The total charge of HA can be affected in two ways. First, by incorporating foreign ions like fluoride, silver, stannous, or others into the lattice, ion substitution (absorption) occurs, affecting the structural charge of HA permanently[11,31]. Secondly, the surface charge, which is dictated by the pH and the ions present in plaque fluid and saliva, also affects the compound's overall charge[11,31]. The surface charge of HA is mainly determined by the pH of the solution in which it exists, as the structural charge is relatively fixed. When HA dissolves, it may undergo unfavorable substitutions or become saturated with acid, becoming more net positive over time. On the contrary, if the pH of the solution exceeds the PZC, more hydroxyl anions will

be present at mineral surfaces, rendering a more net negative surface charge. These conditions, which are favorable for remineralization[11,31], are depicted in Figure 1-10:

Figure 1-10: The pH-Dependence of HA Surfaces[91]:



As the enamel (HA's) total surface charge becomes more negative, calcium ions become increasingly attracted to these surfaces, as shown by Coulomb's Law in Equation 1-2 below[37,38,91]:

$$F\,(attraction) \;=\; \frac{Z_{Ca^{(2+)}}\, Z_{HA^{(-)}}}{\varepsilon\,(R_h^2)}$$

The PZC of HA compared to other minerals is listed Figure 1-11:

Figure 1-11: Various PZC Listings for Minerals[38]:

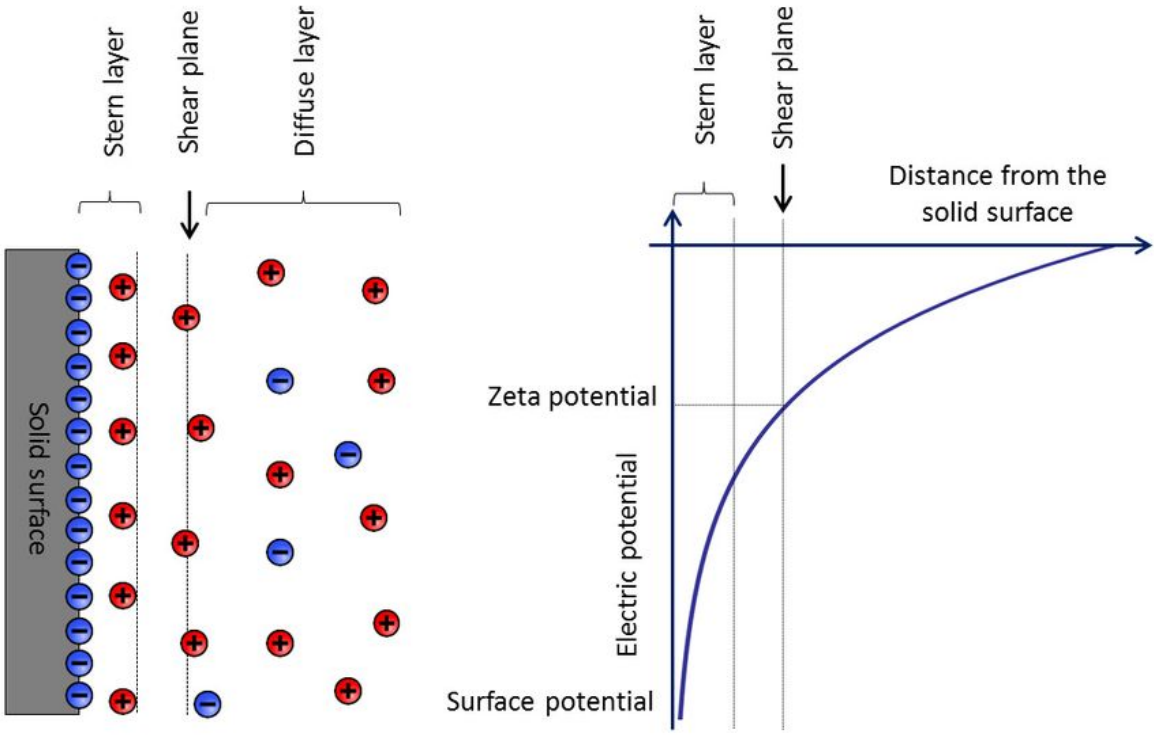
Material	pH_{pznpc}	Material	pH_{pznpc}
α-Al_2O_3 Corondum	9.1	δ-MnO_2 Birnessite	2.8
α-$Al(OH)_3$	5.0	β-MnO_2	7.2
γ-AlOOH	8.2	SiO_2	2.0
CuO	9.5	$ZrSiO_4$	5.0
Magnetite	6.5	Feldspars	2-2.4
α-FeOOH Goethite	7.8	Kaolinite	4.6
α-Fe_2O_3 Hematite	8.5	Montmorillonite	2.5
		Hydroxyapatite	7.6
$Fe(OH)_3$am	8.5	Albite	2.0
MgO	12.4	Chrysotile	>10
Calcite	9.5	Rutile TiO_2	5.8

This process of pH-dependent attraction is what elicits remineralization. Multiple literature sources cite the PZC for HA and enamel as being equal to 7.6[11]. This means that solutions with pH levels above 7.6 are ideal for the purpose of remineralization. This also agrees with data on saliva, which matches this value closely under stimulated conditions[33]. It should also be noted that as the surface charge of enamel becomes more negative, negatively charged bacterial species, especially cariogenic ones, are repelled from the HA surfaces[34]. On the contrary, when HA is acidified and the pH drops to a level where the net charge is more positive, bacteria such as *S. mutans* can more readily attach, grow, and produce acid[34,35].

Cation Exchange Capacity - A pH Proven Model:

Another important factor to consider is cation exchange capacity (CEC), which measures the capacity of a mineral to exchange cations[31]. A mineral's CEC is determined by the concentration of unfixed cations in the diffuse layer of the plaque fluid surrounding HA. This concentration is dependent on the magnitude of the total mineral charge determined by the pH[31]. Thus, CEC varies with pH. Since PCZ determines the pH at which cation or anion exchange occurs, CEC is simply an extension of this concept[11,31]. Since mineral surfaces have a finite charge (unless the pH is at the PCZ), minerals with opposite surface charges will attract one another, and minerals with similar surface charges will repel one another. It should be noted be noted that the exchange capacity is also drastically affected by the mineral species present. Therefore, ionic, competition can affect favorable uptake of critical ions if they become outcompeted despite pH being ideal. This concept is illustrated in Figure 1-12:

Figure 1-12: Stern Layer Model (Adapted for HA)[36]:



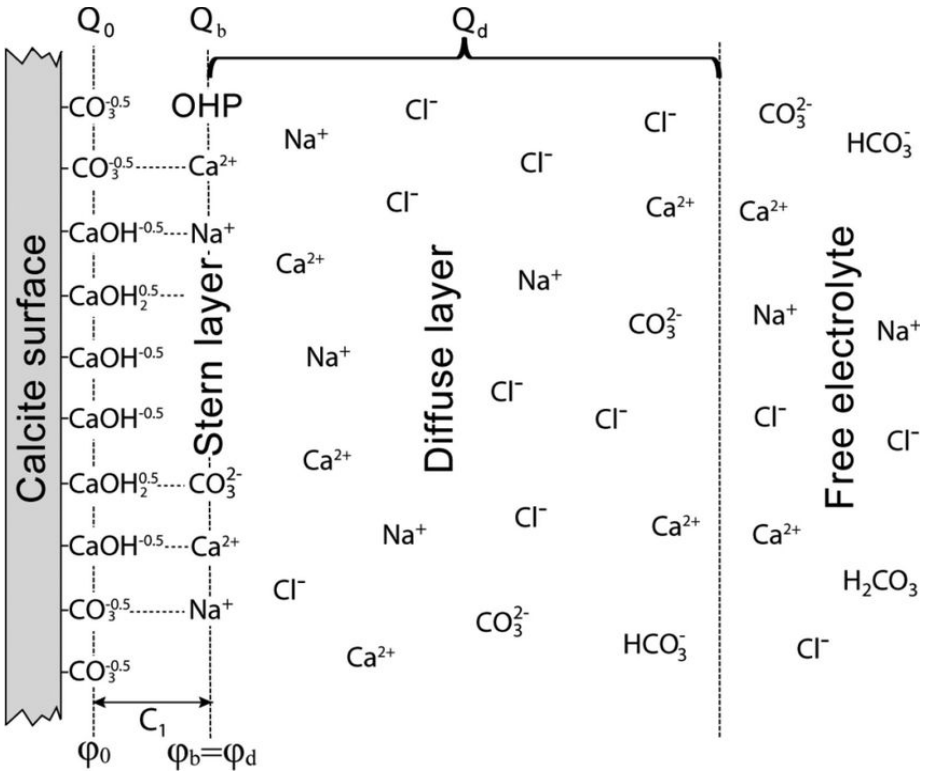
This explains why in certain conditions, despite a tendency towards supersaturation at a high pH, calculus buildup may not occur if a biofilm is not present to facilitate mineral deposition[34,35]. This is because calcium phosphate precipitates are negatively charged, and at a higher pH level, the surface charge of HA is also negative. These similar charges repell one another, reducing the attraction for precipitates. Additionally, remineralization can only occur when the PZC of enamel is exceeded by the pH[11]. Therefore the attraction of calcium to HA increases due to Coulomb's Law, which is pH-dependent[37]. As a result, when CEC increases with increasing pH, so does the potential for remineralization. Fluoride, calcium, and phosphate can also occupy space on the surface of HA, thereby decreasing the PCZ to 6.8[11] or lower. This means a pH of 7 or higher, for most individuals, could be satisfactory for cation exchange and subsequent remineralization to occur. However, this number varies on an individual basis.

Ion Substitution and Ion Competition:

Ion substitution is the process by which certain anions or cations are exchanged at mineral surfaces and incorporated into the tooth structure. On the other hand, ion competition is the process whereby certain ions have a higher tendency than other ions to occupy surface area on the outer surfaces of HA, often called the "charged fixed" or stern layer of tooth structure. These

ions can either promote or deter other ions or substances from occupying the tooth structure. Ion competition can also control how much ion substitution occurs, especially in cases where one type of ion dominates over another. As discussed previously, the overall charge of HA can be determined by summing the structural charge and the pH-based charge[31]. When cations with a charge of +2 are present, they tend to outcompete cations with a charge of +1 when ionic strength is low to moderate. However, if there is an overabundance of a cation with a charge of +1, the amount of ion substitution or surface competition of that cation is favored[38]. It should be noted that as the degree of charge increases, ion substitution of these highly charged species is lower. Figure 1-13 shows a calcite mineral complex, and how various ions and solutions can contribute to ion adsorption and absorption into the lattice:

Figure 1-13: Stern Layer Diagram of Calcite[88]:



All ionic species have the ability to compete for surface sites, and potentially deter the uptake of other charged species[38]. When considering certain cations or anions for incorporation into tooth structure, it is critical to limit competition between critical and non-critical ions. For example, sodium ions should be limited in dentifrices to prevent them from outcompeting calcium ions for surface sites. Likewise, chloride ions may interfere with fluoride ions for uptake into surface sites. Therefore, although compounds like sodium bicarbonate can neutralize oral pH, they will undoubtedly interfere with calcium and phosphate uptake onto into tooth structure. This concept is illustrated in figures 1-14 and 1-15:

Figures 1-14[39] and 1-15[38]: Sodium Interference with Calcium for HA Surfaces

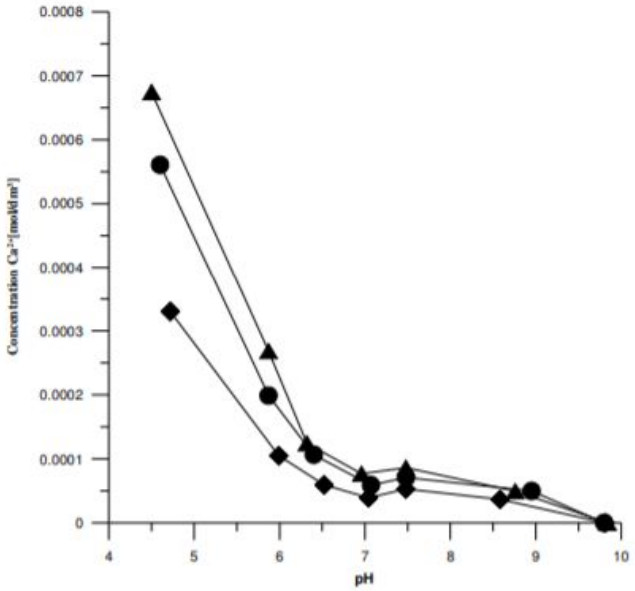


Fig. 6. Ca^{2+} concentration at the hydroxyapatite /NaCl solution interface as a function of pH.

Selectivity of ions relative to Na, using equivalent fractions.

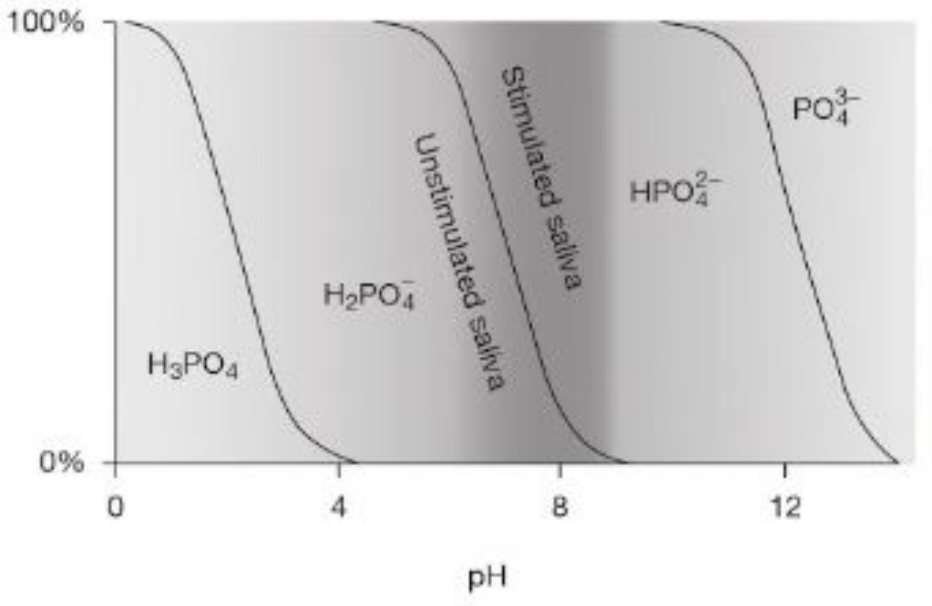
Ion I^{+}	**$K_{Na/I}$**	**Ion I^{2+}**	**$K_{Na/I}$**	**Ion I^{3+}**	**$K_{Na/I}$**
Li^{+}	1.2	Mg^{2+}	0.5	Al^{3+}	0.6
K^{+}	0.2	Ca^{2+}	0.4	Fe^{3+}	
NH_4^{+}	0.25	Sr^{2+}	0.35		
Rb^{+}	0.1	Ba^{2+}	0.35		
Cs^{+}	0.08	Mn^{2+}	0.55		
		Fe^{2+}	0.6		
		Co^{2+}	0.6		
		Ni^{2+}	0.5		
		Cu^{2+}	0.5		

In addition, compounds that utilize acid may prevent calcium uptake, and may occupy key sites in the sorbed charge-fixed stern layer[11,31,38]. It is important to consider that if you have a specific beneficial ion of interest, competition from other ions for surface sites may ultimately inhibit the frequency and degree of uptake of these critical ions[31,38]. This effect can also be observed with the quaternary ammonium salts such as chlorhexidine (CHX), which has shown to interfere with calcium and fluoride uptake. It is suspected that CHX becomes charge-fixed and can outcompete other ions (e.g. F, OH, PO4, Ca) for surface sites, inhibiting remineralization[13].

Degree of Saturation: A pH-Dependent Function

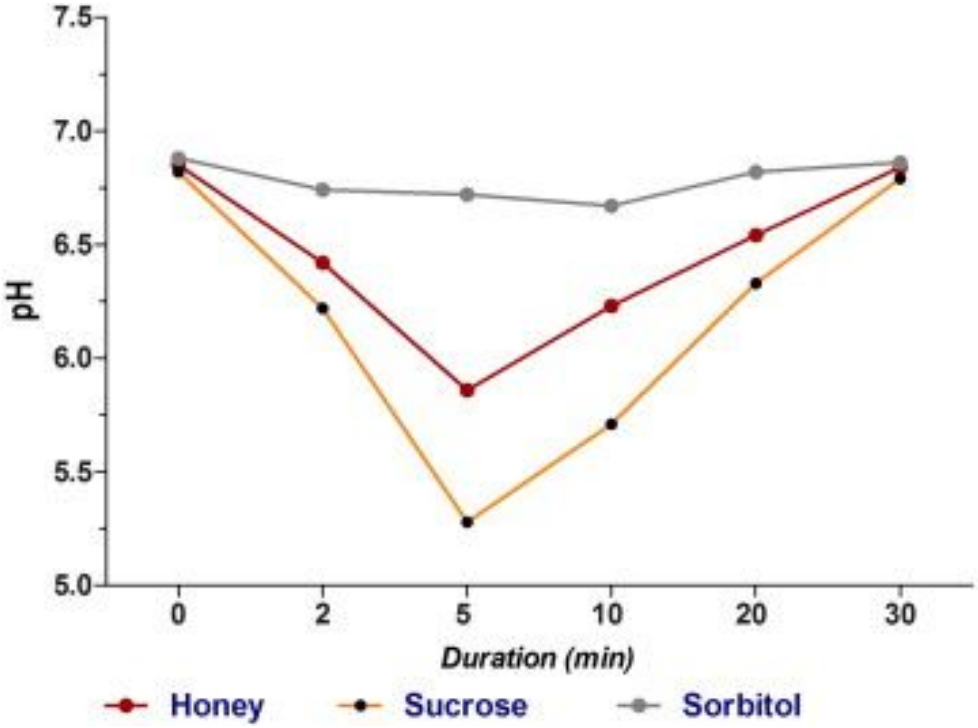
It has already been established that degree of saturation depends fundamentally on pH[3]. This is because two of the primary components of HA are directly affected by pH. For instance, phosphate has multiple ionic forms, depending on the acidity of the environment[3]. Once the pH level exceeds 7.5, the primary form of phosphate is HPO42-, which is one of the most basic forms, and can act as a buffer for acid attacks. The hydroxyl anion (OH^-) is directly affected by pH, since the definition of pH is the negative log of hydronium ions (H^+). Additionally, as hydronium ions increase, the attraction for hydroxyl groups decreases[11,31]. Therefore, a degree of saturation ensures demineralization does not occur. This agrees with a salivary pH range close to 8. This is depicted by Figure 1-16:

Figure 1-16: Ideal pH Range for Saliva (Degree of Saturation):



The surface attraction of calcium changes along with pH, as the concentration of hydroxyl ions increases[37]. To summarize, the overall degree of saturation of an ionic product is crucial for remineralization[3]. It can be concluded that each and every aspect of this ionic product dictates how tooth enamel dissolves, remineralizes, and functions. This is evident when looking at stimulated saliva, of which the pH is typically 7.5 or higher[33]. Stimulated saliva also contains an abundance of these minerals alongside antibacterial enzymes and proteins that enhance the stability of these minerals, promoting remineralization. It is always best to mimic or enhance the existing system, which, in this case, is saliva. This is evident when looking at how plaque pH is affected by a sucrose challenge, as illustrated in Figure 1-17:

Figure 1-17: Salivary pH with a sucrose challenge[40]:

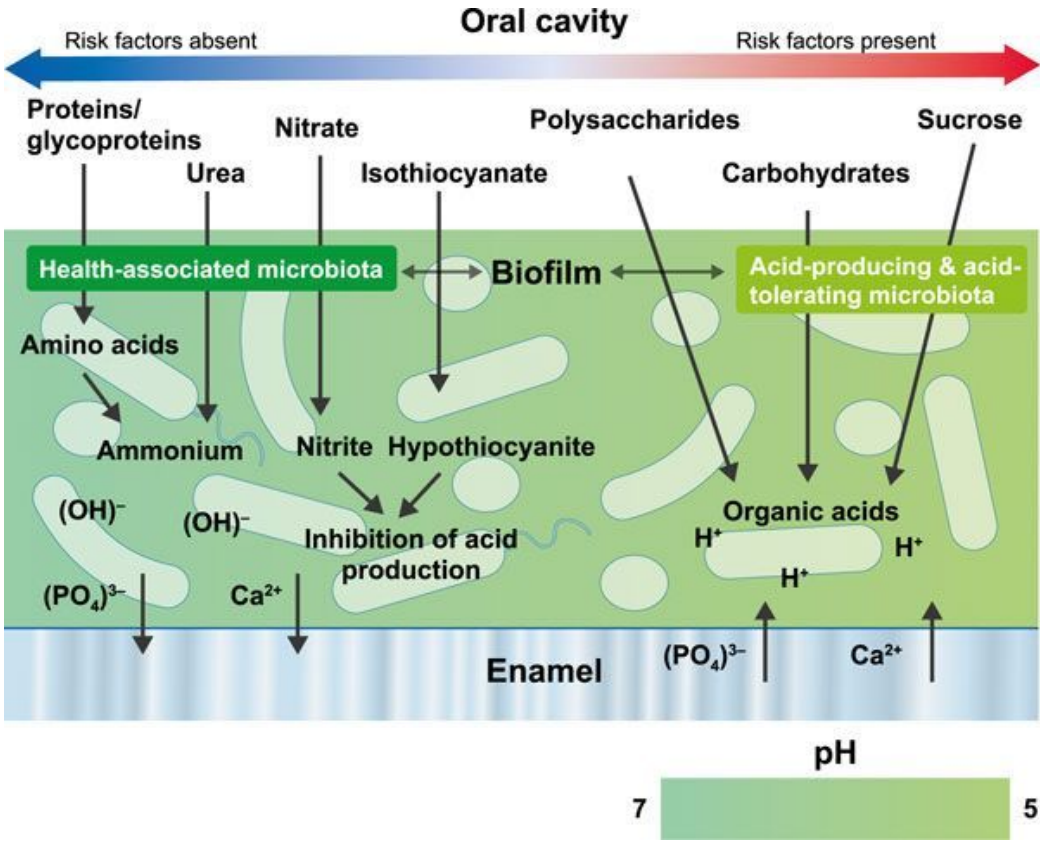


IV: Remineralization and Demineralization Concepts:

Saliva: The Natural Medium for Remineralization:

Saliva is a natural medium that removes bacteria and clears substrates from the teeth and gums[7,41]. It also buffers against acid attacks by breaking down the biofilm and neutralizing oral acids in plaque fluid[7]. Saliva has many other functions, among which are lubrication and protection of oral tissue, maintenance of tooth integrity, digestion and taste[7,41]. Cariogenic bacteria are directly impacted by salivary processes, which alter their ability to release acid and ultimately survive. Saliva is made up of many components, both organic and inorganic, including electrolytes, proteins, immunoglobulins, enzymes, mucins, urea, and ammonia[7,41]. This is outlined in Figure 1-18:

Figure 1-18: Oral Biofilm pH and Caries Risk Factors[41]:

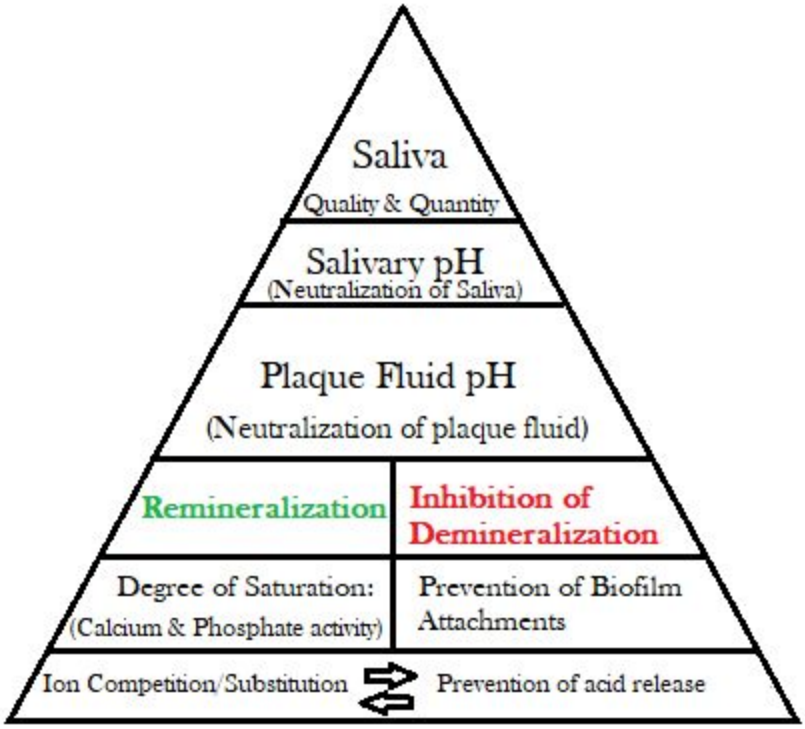


The various components of saliva help to modulate the the attachment of the oral biofilm as well as the pH and buffering capacity of plaque fluid[3]. Saliva is directly coordinated to the demineralization/remineralization axis, as the quality and quantity of saliva largely impacts this process[7]. This is why it is crucial for high-risk individuals to increase their intake of substances that increase salivary mineral content, antibacterial capacity, and overall salivary flow[7].

Remineralization Concepts Reviewed:

When considering remineralization, it is critical to consider all relevant concepts. These include two core points: promotion of remineralization and inhibition of demineralization. Both are highly valuable in predicting success, but have different relative importances according to the remineralization hierarchy. This is displayed in Figure 1-19:

Figure 1-19: Callister et. al Remineralization Hierarchy:



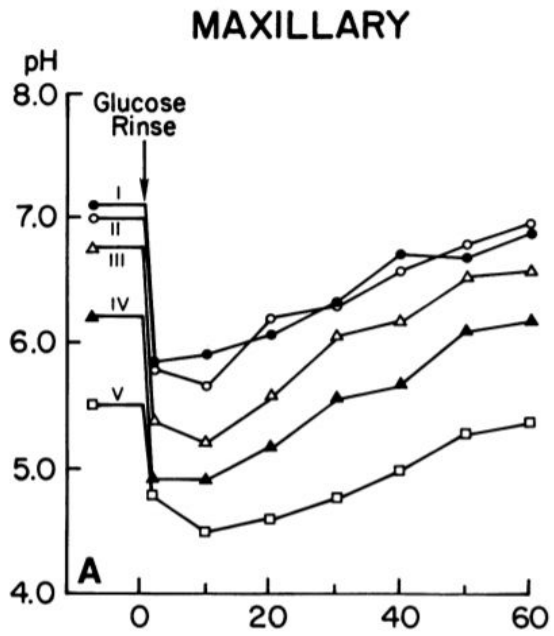
1. Promotion of Remineralization:
1.1. Salivary quality and quantity and subsequent clearance capacity 1.2. pH buffering capacity of saliva and plaque
1.2.1. → Degree of Saturation 1.2.2. → PZC and CEC 1.2.3. → Enamel (HA) surface charge 1.3. Ion competition and substitution

2. Inhibition of Demineralization:
2.1. Prevention of biofilm shifting (Ecological Plaque Hypothesis)
2.1.1. → Removal of biofilm attachments 2.2. Prevention of oral acid release 2.3. Ion competition/substitution

It is fundamental to understand that remineralization follows a hierarchy based upon the relative importance of factors that produce a state of remineralization and subsequently prevent demineralization. Although both concepts are important, the hierarchy primarily revolves around the quality and quantity of saliva[7]. Dawes and colleagues have extensively reviewed how salivary flow affects the body's ability to clear bacterial buildup[7]. Mechanisms that increase salivary flow are the first stage of the hierarchy. After this, the most important concept is pH and buffering capacity[3,25]. If an individual has acidic saliva or plaque fluid with minimal buffering

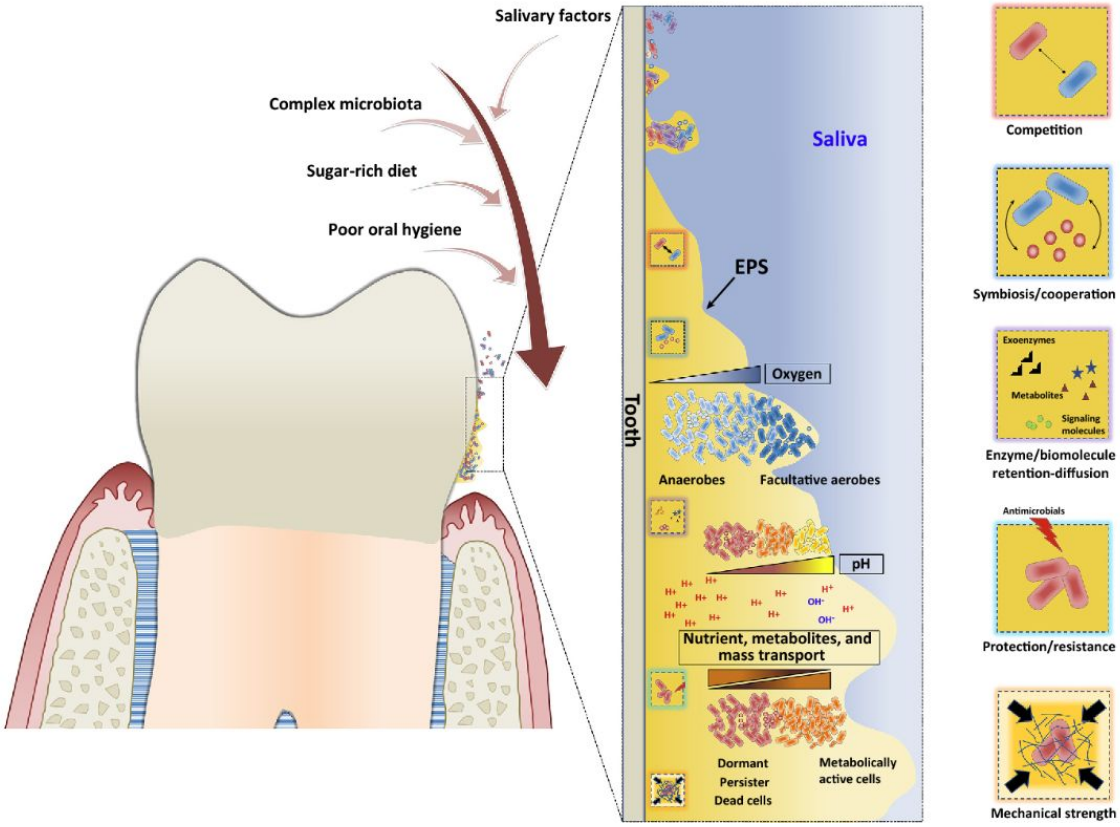
capacity, they will be at a high risk for caries[3]. This is not just because the saliva is acidic, but because it is unable to increase the alkalinity of plaque fluid inside the oral biofilm[3]. This is one of saliva's greatest roles, as it helps to dismantle biofilms and raise their respective pH levels[7]. This is shown by Figure 1-20:

Figure 1-20: Plaque Fluid pH Over Time After Sucrose Challenge[9]:



In addition, pH directly impacts other core concepts involved in remineralization, many of which are of equal importance. The third component under this hierarchy is the pH of plaque fluid. This is directly impacted by the pathogenicity of the formed oral biofilm. Despite having excellent saliva and an alkaline microenvironment, many cariogenic organisms focus their attention on isolating themselves from their external environment to form acidic 3D pockets[5,6]. Without mechanical plaque removal or chemical alteration, oral biofilms undergo continuous shifting to become increasingly more pathogenic. This is why the enzymes in saliva, in addition to mechanical plaque removal and combination therapy, are key to keeping this shifting under control[5,6,7]. When the oral biofilm becomes thicker and is left intact, it makes it increasingly more difficult for saliva to enter and break down bacterial attachments and buffer against bacterial acids[24,25,41]. It has also been noted that certain bacteria, which are non-pathogenic at higher pH levels, may have stress response mechanisms that allow them to produce acid, promoting survival in cariogenic conditions[10].
This is why it is critical to have multiple modes of action that enhance salivary action, preventing these shifts towards pathogenesis. This is reviewed in Figure 1-21:

Figure 1-21: An Overview of Biofilm Risk Factors[6]:



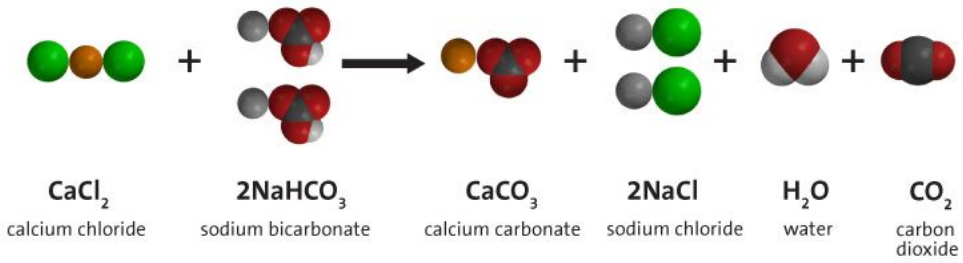
V: Problems with the current prevention model:

Lack of Bioavailability of Calcium Salts

One of the long-standing problems with calcium salts is that many are not bioavailable. This is because they have low solubility, and, as a result, are prone to precipitate or become unstable in the gastrointestinal tract[3]. Additionally, there are very few alkaline calcium salts that are highly soluble. What determines the ultimate success of a calcium salt is its counterion. This is because the counterion directly reacts with the solution to form either hydronium or hydroxyl ions. This counterion can also play a large role in chelation, or subsequently influence how calcium ions interact with enamel surfaces. As a result, calcium phosphate and calcium carbonate dentifrices lack the bioavailability to participate in traditional remineralization reactions[81,82,83]. This is because they can become unstable and form precipitates prior to reaching tooth structure. Historically, many have tried different forms of “saturated” calcium phosphate solutions[43]. The generation of these solutions involves combining different salts to form calcium phosphate, which relies on the patient to rinse before precipitation occurs. This becomes counterproductive and creates an additional compliance issue for patients as well. At

a high level, foreign ion competitors, such as sodium bicarbonate, may seem like a good idea, but this agent can cause calcium to precipitate in an exchange reaction as illustrated in Figure 1-22. This can also create increased competition from sodium for enamel surface sites instead of calcium[44,45].

Figure 1-22: Substitution and Precipitation of Calcium Salts with Sodium Bicarbonate:



This is because single charged ions become more attracted to enamel surface sites as the ionic strength (level of salt) increases[31,38,44,45]. This can make it even more difficult for remineralization to occur, even though sodium bicarbonate increases pH. Fundamentally, the largest crux for calcium salts is their lack of solubility in the alkaline range combined with their associated increased risk of precipitation. However, it should be noted that when possible, the stabilization of calcium ions in alkaline conditions can provide a landscape of opportunity for reducing caries challenge while keeping a degree of saturation possible. The balance and choice of calcium salt cannot be overlooked, as it is critical for success.

Poor Penetrance of Neutralizing Agents and Fluoride:

When considering remineralization, one of the largest obstacles to overcome is the oral biofilm. The oral biofilm has the ability to seed and mature quickly, allowing it to become denser. The oral biofilm also creates pockets of oral acids, and shields itself from saliva by utilizing its EPS coating[5,6]. Because of this tactic, when helpful agents are introduced prior to mechanical removal of plaque, these agents fail to incorporate into plaque fluid, and instead can get stuck in the EPS layer of the biofilm[22]. This limited entry has been observed clinically, especially with calcium and fluoride dentifrices which show minimal entry into deeper biofilm sites. It has even been seen that at lower pH levels (where most fluoride dentifrices are) there is less fluoride uptake into the biofilm[44]. This is in contrast to the acidulated in-vitro studies with no biofilm present. It should be noted that when a biofilm is present, few active remineralization agents from a dentrifice may enter at critical thresholds in plaque fluid. This places a damper on the success of these products which are tested almost entirely in vitro with no biofilm present. This is illustrated in the Figures 1-23 and 1-24:

Figure 1-23: Poor Penetrance of Fluoride Into Deeper Sections of Biofilm Mass[45]:

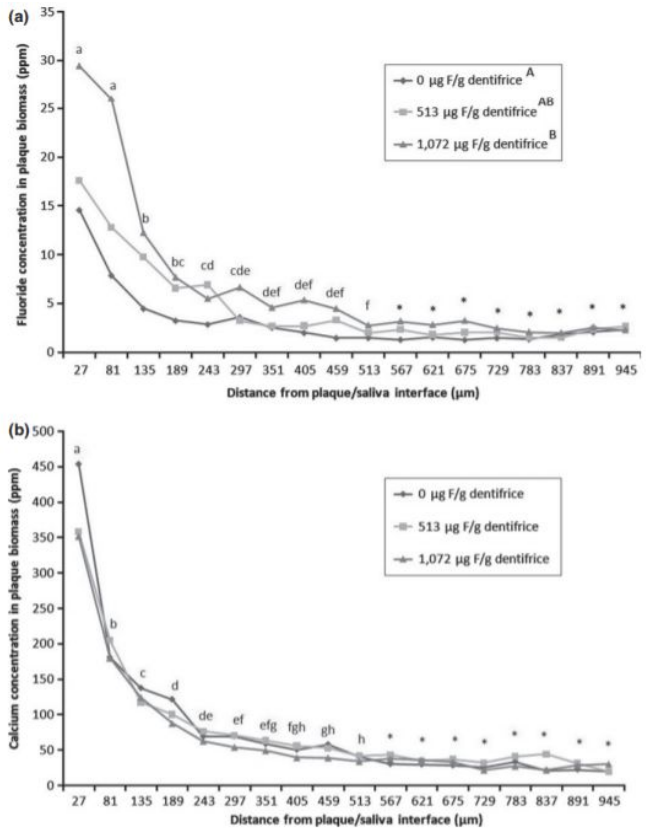


Figure 3. Mean biomass-associated fluoride (3a) and calcium (3b) concentration profiles (ppm) in serial sections of plaque biofilms generated *in situ*, after exposure to dentifrices containing 0, 513, and 1072 µg F/g. Each point refers to the mean of the 11 volunteers (values obtained for 1 and 12 h after brushing analysed together). Different lower case letters indicate significant differences among the sections for all dentifrices tested. Different upper case letters indicate significant differences among the dentifrices for all the sections. Comparison made by three-way, repeated-measures ANOVA on the natural log of the outcome and Tukey's *post hoc* test ($P < 0.05$). Samples obtained from sections 594 to 972 µm (asterisks) were not included in the statistical analysis.

Figure 1-24: Lower Fluoride Uptake in Deeper Biofilm at Low pH Compared to Neutral pH[44]:

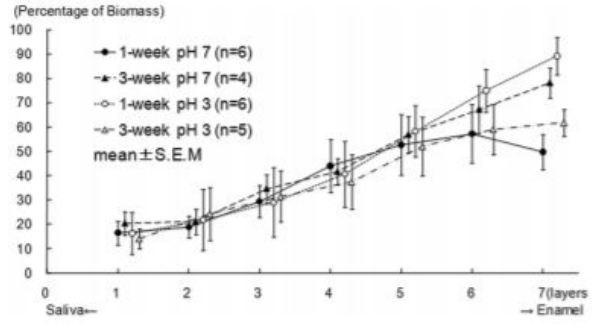


Fig. 1 Mean biomass throughout 1 week and 3 week plaque biofilms exposed to 1,000 ppm fluoride at pH 3 and pH 7

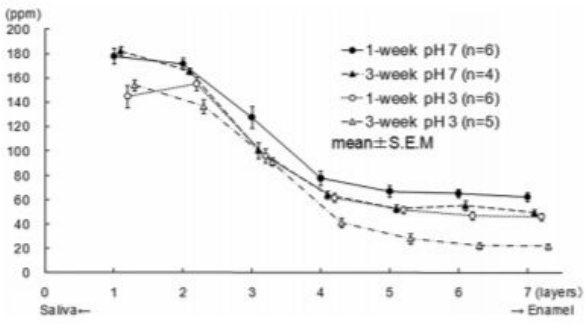


Fig. 2 Mean fluoride concentrations throughout 1 week and 3 week plaque biofilms exposed to 1,000 ppm fluoride at pH 3 and pH 7

The diffusion of saliva across the EPS layer is also slowed, and the gradient of entry for saliva, salivary proteins and buffering agents takes an extended period of time. This is consistent with the Stephan Curve[5,6]. This is also true for antibiotics and traditional antibacterial compounds, which may only diffuse into the outer EPS layer[10,24]. In contrast, silver nanoparticles are highly effective in penetrating biofilms as depicted in Figures 1-25 and 1-26:

Figure 1-25: Poor Diffusion of Antimicrobials into EPS Layer of Oral Biofilm[46]:

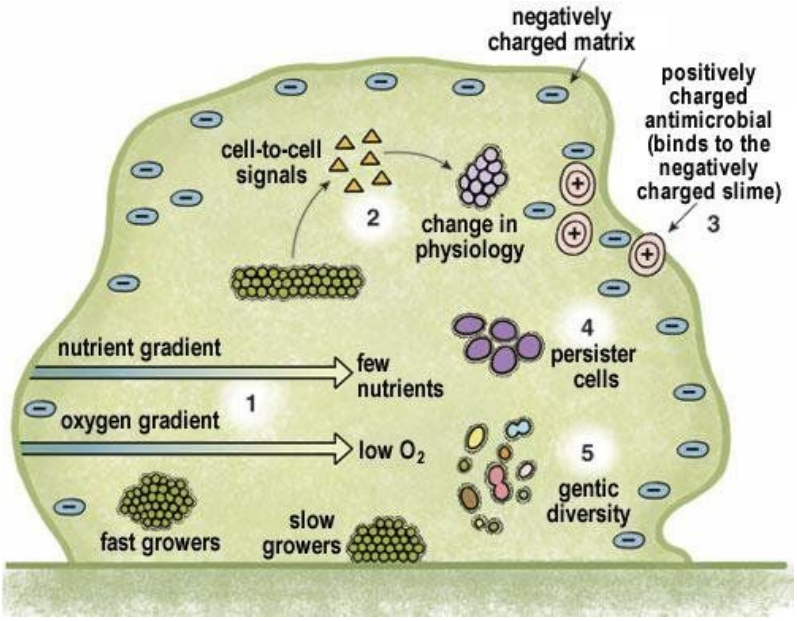
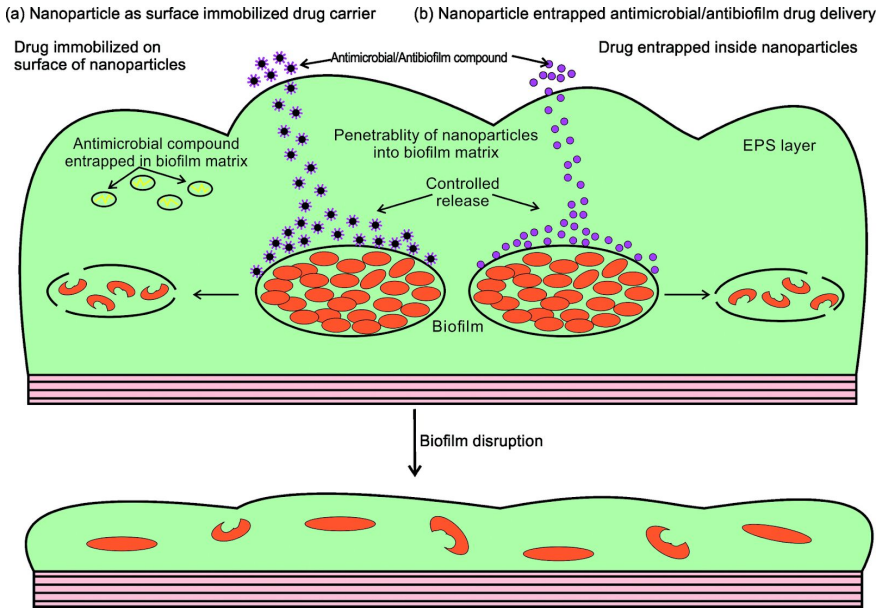
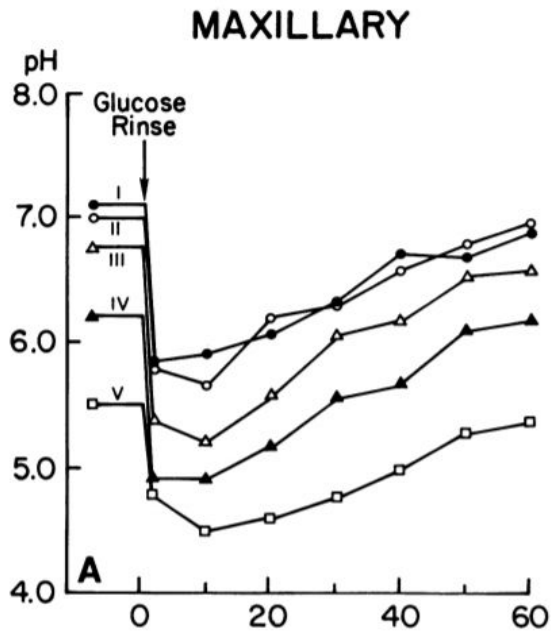


Figure 1-26: Silver Nanoparticles Increased Penetration into Oral Biofilm[10]:



The drop in plaque fluid pH from substrate challenge can be easily observed by using a Stephan Curve. The curve shows how after consumption of substrates, the pH of plaque fluid does not rebuffer upwards, sometimes, for hours. However, this depends on individual patient factors, such as salivary flow and clearance rate. This poses a problem, since the biofilm has channels which allow it to easily uptake nutrients, but at the same time delay entry of saliva into plaque fluid that directly contacts tooth surfaces[5,6]. This is illustrated by the reiterated figure 1-20[9]:



All cariogenic processes occur at the point of contact between plaque fluid and tooth structure[3]. Substrate availability results in extended periods of the pH level existing under 5.5, where leaching of HA occurs via continuous release of oral acid and subsequent buildup[5,6]. Furthermore, plaque fluid has been noted to have much more variation in pH level compared to saliva[3], making it a key indicator of the level of risk a patient has at any given time. Ionic content, pathogenic activity and pH are all contributing factors[5,6,8,80]. Due to difficulties that exist with delays in buffering capacity and pH drop after carbohydrate consumption, existing beneficial agents demonstrate poor entry into the plaque fluid[22,44,45]. It is because of these extended delays in neutralization that white spot lesions and subsequent caries develop[9]. This is a major issue that has yet to be addressed by existing hygiene agents.

Acidiculated Model is Counterintuitive:

It has long been known that in order to remineralize teeth effectively, acidic dissolution of tooth structure is necessary to incorporate fluoride and other agents into the tooth structure via substitution. Although fluoride does show an increased uptake at low pH levels with in vitro models, there is evidence to suggest that adequate fluoride uptake can also be achieved at neutral and alkaline pH levels[44,47]. The question becomes: how much benefit is achieved by acidification vs. non-acidification? This question can be answered by looking at ion equilibria coefficients. Essentially, if the amount of ions leaving exceeds the amount of ions entering, this becomes a net negative loss to the tooth structure[3]. This phenomenon is apparent when pH levels fall to 2-3, as phosphoric acid, dihydrogen phosphate, and monohydrogen phosphate are present, but mostly in the form of phosphoric acid[3]. This is reiterated in Figures 1-27 and 1-28:

Figure 1-27: Phosphate Complex Equilibria Based on pH:

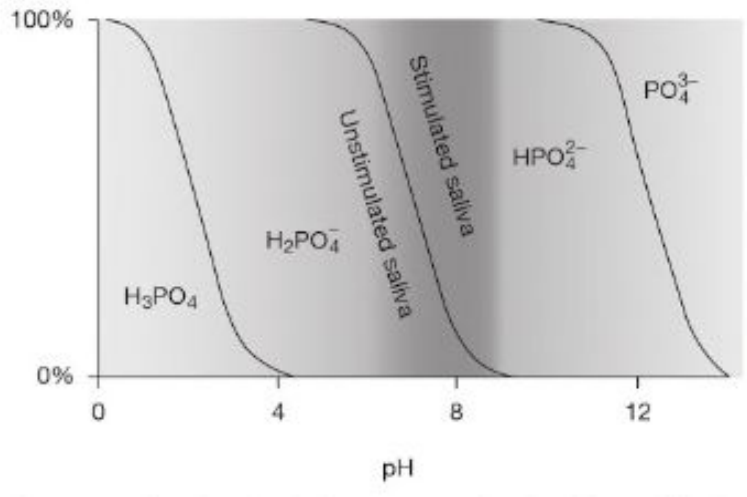
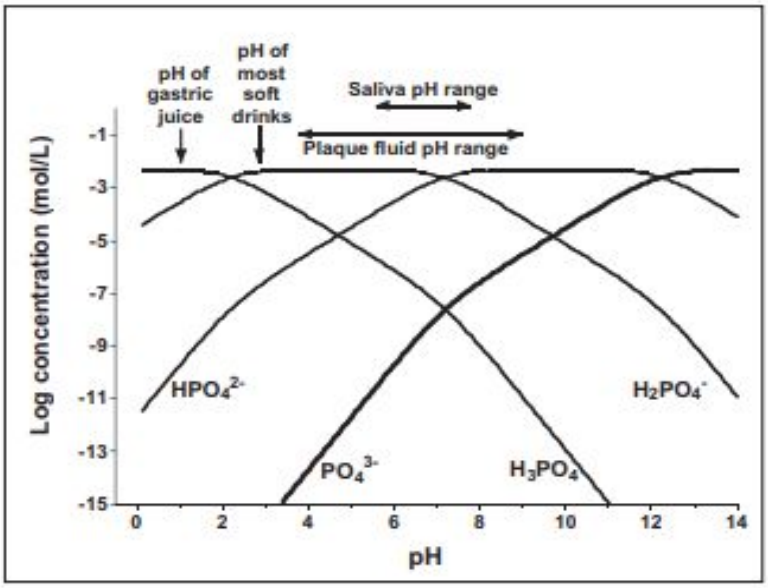


Figure 1-28: Phosphate Complex Equilibria Based on pH (Plaque Fluid and Saliva)[3]:



Below a pH of 2 is the critical zone whereby no buffering capacity exists for phosphate, and almost 100% of the species present is phosphoric acid[3]. Since phosphoric acid is a strong acid, it dissociates completely and can cause rapid dissolution of tooth structure. Inversely, a point of retracement occurs at a pH level between 7 and 8. This is the ideal point for remineralization, as buffering capacity is optimal. Furthermore, overlap occurs between multiple phosphate species, of which phosphoric acid is a minor component[3]. At the intervals of these overlaps, a much greater buffering capacity exists. This means that unless the pH is further increased, the constituent ions at these equilibrium points will be the dominant species observed[3]. This agrees with current knowledge surrounding remineralization concepts. As seen in the ionic product, as the pH approaches 7-8, phosphate dependence decreases for remineralization. This means that calcium is more relevant to remineralization paths in this range. This is not to say that phosphate will not incorporate at this pH, but rather the dependence is lessened for remineralization to occur based on the ionic product. Therefore, the use of fluoridated varnishes and gels which approach a pH of 2 - often 2.3 or below - are not necessary to remineralize teeth, and may cause considerable damage if individuals are exposed for extended periods of time[3]. In addition to the notion that fluoride uptake is very low[44,45] in oral biofilms, especially in the deeper sections, this uptake is also pH-dependent, showing less uptake at an acidic pH of 3[44]. This is reiterated in the figures below:

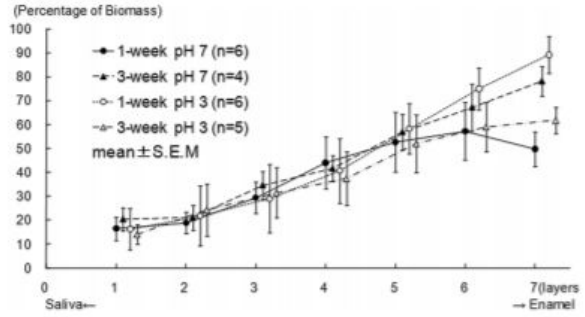


Fig. 1 Mean biomass throughout 1 week and 3 week plaque biofilms exposed to 1,000 ppm fluoride at pH 3 and pH 7

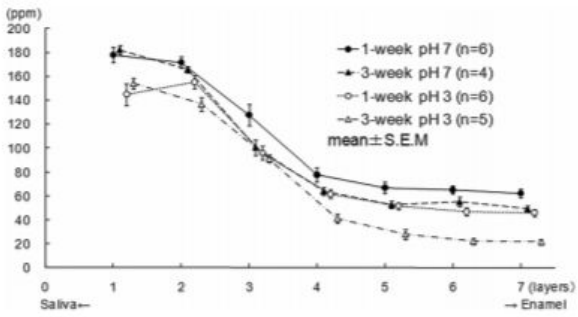


Fig. 2 Mean fluoride concentrations throughout 1 week and 3 week plaque biofilms exposed to 1,000 ppm fluoride at pH 3 and pH 7

A strong example to counter the existing acidulated model is silver diamine fluoride. A study was conducted showing that as the pH of the solution increased, fluoride incorporation also increased dramatically. The figures from the study are shown below:

Figures 1-29 and 1-30: Fluoride Incorporation as a Function of pH (SDF)[47]:

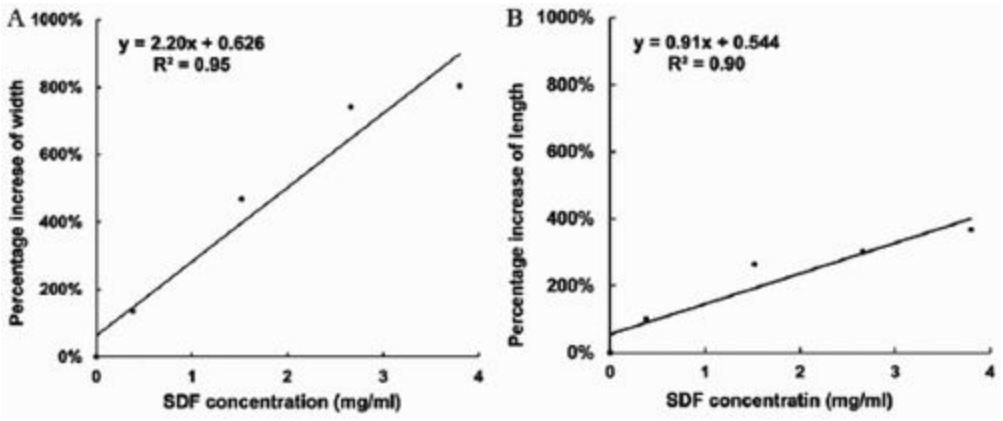


Table. Calculated Hexagonal Unit Cell Parameters *a* and *c* Axes, F/Ca, F/P, and Final pH in Experimental Groups.

	P-XRD, Å				
SDF,[a] mg/mL	*a*-axis	*c*-axis	F/Ca	F/P	Final pH
None[b]	9.577 ± 0.0012	6.833 ± 0.0010	N/A	N/A	7.07 ± 0.02
0.38	9.554 ± 0.0011	6.833 ± 0.0010	0.022 ± 0.002	0.043 ± 0.006	8.02 ± 0.01
1.52	9.552 ± 0.0036	6.833 ± 0.0010	0.037 ± 0.007	0.055 ± 0.006	8.14 ± 0.01
2.66	9.548 ± 0.0024	6.833 ± 0.0010	0.043 ± 0.004	0.070 ± 0.009	8.60 ± 0.02
3.80	9.542 ± 0.0047	6.833 ± 0.0010	0.072 ± 0.005	0.111 ± 0.011	8.95 ± 0.01

All the data are normally distributed. Values are presented as mean ± SD.
F/Ca, fluoride/calcium; F/P, fluoride/phosphorus; N/A, not applicable; P-XRD, powder X-ray diffraction; SDF, silver diamine fluoride.
[a]No crystal was detected in the SDF control group (no calcium phosphate).
[b]Calcium phosphate control.

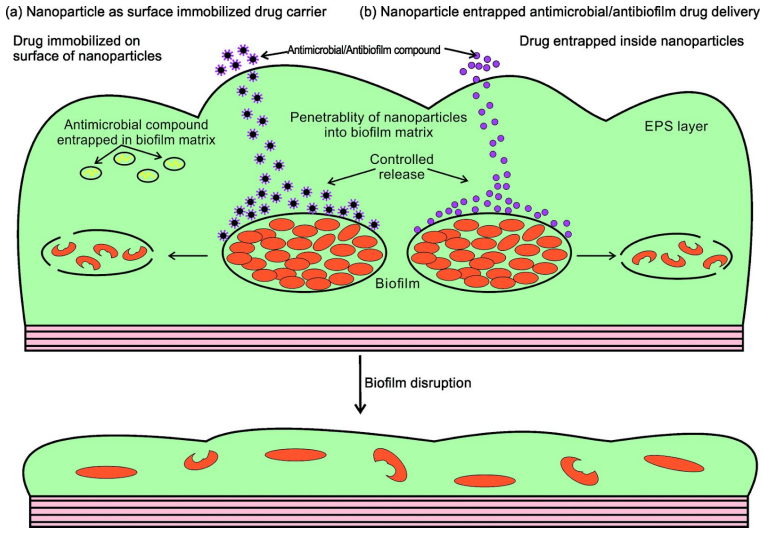
As demonstrated, by incorporating higher concentrations of silver while increasing the pH level, fluoride incorporation into HA increased drastically[47]. This increased the length and width of the HA crystals. This increase in size renders the crystals more resistant to acid attacks[47].

It should be noted that acidulation is not the only possible mechanism by which remineralization can occur, and that mimicking the natural mechanism that saliva uses with an alkaline pH should always be considered as a first option.

VI: Suggested Solutions to Address Issues with Current model:

Nanotechnology - A New Horizon:

There have been many improvements to nanotechnology in the last decade. Specifically, recent advancements have been made to make nanoparticles more size consistent, stable and biocompatible. The biocompatibility of nanoparticles is dependent upon not only their size, but primarily the capping agent with which the particles are coated[48]. Nanoparticles release ions over time, and the rate at which these ions are released is dependent on whether or not the nanoparticles break down due to instability[48,49]. This is the primary reason as to why toxicity may occur, besides the harsh reducing agents used in chemical methods of production[49]. This means that modulated high stability nanoparticles can be engineered with low toxicity, and outperform comparable compounds such as quaternary ammonium salts (e.g. CHX) which are considerably more toxic to human fibroblasts & other cell types[48,49,50]. Additionally, nanoparticles can be used at much lower concentrations, and can penetrate biofilms with ease[10,91]. This is not true of some charged compounds which can become stuck in the EPS layer and contribute to resistance over time[10]. This is reiterated below[10]:



Many authors have contributed to the pool of data surrounding nanoparticles and their effects. Contamination of nanoparticles with salt or other compounds directly impairs their ability to release ions over time[51]. This is the primary attribute that sustains their antimicrobial action. When nanoparticles are exposed to contaminants such as salt, a flood of ions is generated, leading to dissolution, aggregation and increased toxicity[48,49,51]. Older chemical methods also utilize harmful reducing and capping agents, such as DMSO, sodium borohydride or strong amine bases, which also lead to increased toxicity. Therefore, nanoparticles which are stable in the presence of salts will continue to display stability over long periods of time. Furthermore, if capped with plant extracts, nanoparticles can be rendered non-cytotoxic to human tissues[52]. This new bioengineering method supersedes older methods, and has shown to be superior in its ability to create nanoparticles that release ions slowly, even in highly contaminated media[51]. Considering the human body and oral cavity are filled with different salts, substrates, saliva and bacteria, nanoparticles that can hold up to these conditions can be highly useful in various oral care applications[51,52,91].

Engineered Nanosilver Particles (AgNPs) vs. Conventional Silver Products Silver has been used for thousands of years, especially for its antiviral, antifungal, and antimicrobial properties, which are widespread[15]. However, one of the largest problems with silver salts and colloidal silver products is their lacking ability to stay stable in various conditions.

Generation 0: Silver Salts (Ionic Silver):

The major difference between a silver particle and a silver ion is that silver ions tend to react immediately, and do not retain antimicrobial properties over time[56]. This is because an unfavorable reaction with chlorine, among other potential compounds, can transform them into less useful byproducts, which can increase toxicity[54]. For example, silver chloride does not have any marked capacity for extended antimicrobial action and is a precipitate with low solubility[55]. Due to the fact that the human body is high in salts, many of which contain chloride, this bodes poorly for distribution into wounds, biofilms, and other key sites for which this antimicrobial action would be valuable[54]. Additionally, it has been noted that because the biofilm EPS layer is negatively charged, it is common for positively charged antimicrobials to get stuck in this layer, rendering them ineffective[10]. Therefore the downsides can be summarized:

1. Unstable with chlorine and other compounds
2. Limited antimicrobial action over time compared to nanoparticles
3. More toxic than nanoparticles
4. Less effective for penetrating biofilms

Generation 1:

Traditional colloidal silver (The First Silver Particles): Many companies claim to have the best colloidal silver. Colloidal silver is made using the physical electric model. This process involves using silver wires, through which a current is run, producing cleavage of silver particles and ions. This method has many downsides.

1. Incomplete reactions to particles (mostly ions)
2. Size of particles is highly variable with current (inconsistent size profile)
3. Particles do not retain stability outside of water
4. Particles are not protected by capping agents
5. Cannot be used with other beneficial agents

When considering colloidal silver made by these methods, it is easy to see with a simple experiment that by adding anything to these compounds, besides water, yields the same unfavorable reaction as seen with silver salts[57]. This is because these colloidal particles have no engineered protective coating or capping agent. This is the most archaic, inefficient, and ineffective way to make silver particles, most of which will not be nano in size. Additionally, many who make this solution do not realize that without a secondary reaction involving UV light or heat, true silver particles are made in sparing proportions. It should be noted that multiple forms of energy can be used to produce these uncapped particles such as lasers, microwaves, and heat among other methods. Regardless, it is not uncommon to test these products only to find that they are nearly 100% ionic in form (comparable to silver salts). In addition, they suffer from a wide range of size profiles due to inconsistencies with the electric current or other energy means, which can be difficult to control. When exposed to human tissues, a flood of silver ions is generated from the particles as salts and other biomolecules tear apart the minimal chemical attraction between these atoms which form these particles with ease[54,56,57]. The clear indicator that particles are not present is by testing with a UV-VIS to show no reflection in the UV or typical range for silver nanoparticles (400-420nm). This is because silver nanoparticles absorb blue light and reflect orange to yellow in the visible range for humans.

Generation 2:

Chemically Engineered Nanoparticles: Some of the best research that has been done in the last two decades has utilized methods to make better silver nanoparticles. Unfortunately, many chemically modified silver nanoparticles still suffer from low stability in human tissues, because their coatings are still susceptible to decomposition[57]. The other issue with chemically modified nanoparticles is that some have been observed to be toxic, due to a fundamental instability when exposed to biomolecules and salt, as well as the use of very strong reducing agents in their production[58]. However, these particles have shown a great increase in consistent size profiles as well as extended antimicrobial action over longer periods[58]; electrochemically-made colloidal silvers were unable to achieve this outcome. Therefore, although these particles were a

step in the right direction, these methods still required improvement. The downsides can be summarized as follows:

1. Suffers from instability with salts and biomolecules
2. Cannot be used with other agents
3. Considerably toxic due to strong reducing agents

Generation 3:

Plant-Based Engineered Nanoparticles: Although extensive research has been done on plant compounds of various kinds, there has been little success in achieving the same outcomes as chemically modified silver nanoparticles. This is due to the poor reducing ability of many plant compounds. In turn, this yielded many types of nanoparticles with a wide range of sizes and incomplete reactions. However, these compounds are very biocompatible as opposed to their predecessor, since their coatings are made from plant compounds, which interact favorably with human tissues[58,59]. It was only recently that these compounds were first used to make a new generation of nanoparticles. By using advanced engineering methods, it was possible to make salt-stable, highly biocompatible, silver nanoparticles with size profiles similar to the chemical method without using toxic reducing agents[59]. In addition, it has been noted that the stability of these compounds in various materials has been high, allowing for long-term use and extended antimicrobial action[58,59]. This allows these engineered nanoparticles to be used alongside other agents that, historically, would have been impossible to use. These nanoparticles can be selectively modified to match the outer coating of the oral biofilm EPS layer, giving rise to a greater ability to latch onto and penetrate biofilms, subsequently releasing silver ions once inside the biofilm. In summary, these nanoparticles are:

1. Highly stable in multiple conditions
2. Highly biocompatible and non cytotoxic
3. Extended antimicrobial action
4. Modifiable for use in the oral environment (Biofilm selective)

Anti-Caries Effects of Silver Nanoparticles (AgNPs):

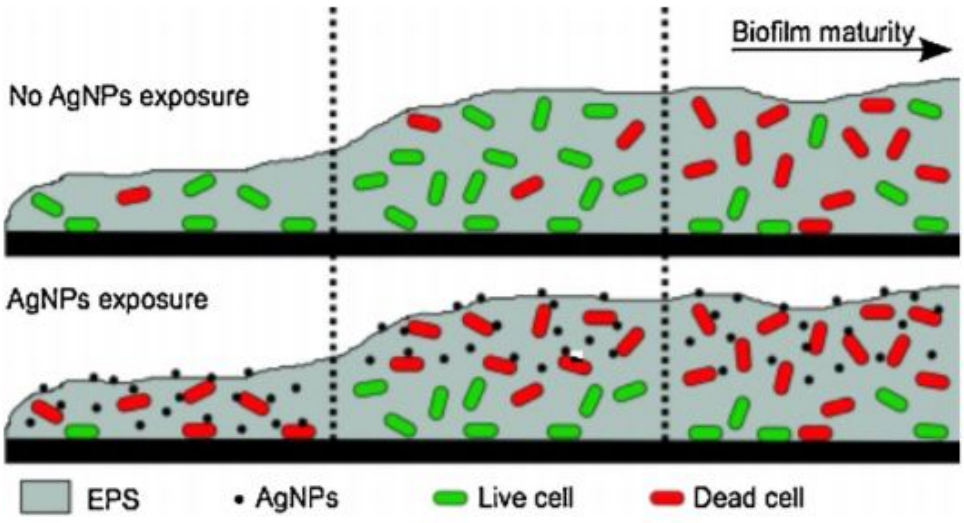
Nanotechnology has been extensively explored in the last decade, and has served as a promising outlet for combating the effects of various disease processes[10,60,61,91]. Nanosilver particles (AgNPs) have been used for a variety of functions, including against cariogenic bacteria[60], their acidic release[61], and as a mechanism to penetrate oral biofilms[10] to increase the overall bioavailability of neutralizing agents[91]. Silver nanoparticles provide multiple modes of action in order to prevent caries:

1. Penetrance of biofilms and disruption of biofilm attachments
2. Prevention of bacterial adhesion to enamel
3. A delivery system for remineralizing agents

4. As an alkaline neutralizing agent for oral acids
5. As an agent to reduce acid release from oral bacteria
6. Silver ion substitution into HA lattice (Remineralization)

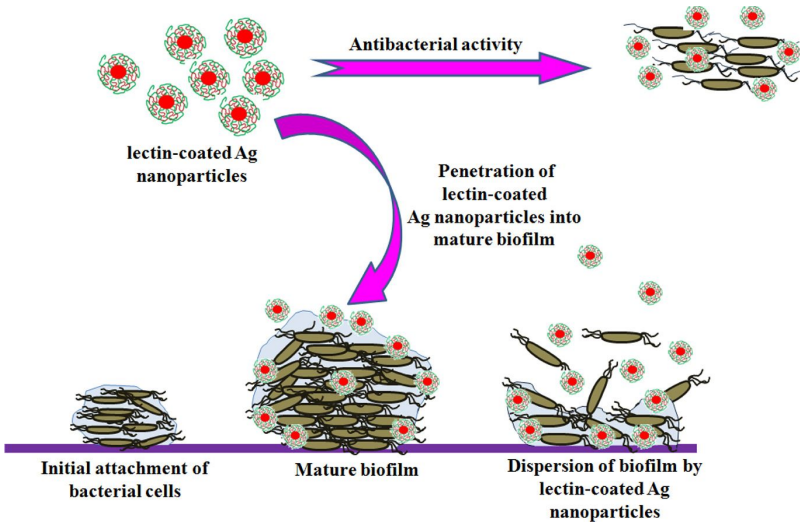
In the figure below, penetration of the biofilm is noted, causing a disruption even in the deeper parts of the biofilm[62]:

Figure 1-31: AgNP Penetration of Biofilm[62]:



In addition, biofilm attachment has been proven to be severely affected by silver nanoparticles, as shown in the figure below[63]:

Figure 1-32: AgNP Disruption of Biofilm Attachment[63]:



In a pilot clinical trial, it was noted that nanoparticles were able to deliver 4.6 times as much calcium into the plaque fluid of the oral biofilm[91]. CHX has also shown these properties, but may interfere with remineralization, as shown clinically by calculus buildup and staining[64]. However, the idea of using biofilm penetrants such as CHX confirms the findings from the clinical study by showing a nearly 5 times increase of calcium and phosphate uptake compared to the control treatment[64]:

Figure 1-34: Calcium Uptake into Oral Biofilm with AgNPs[91]:

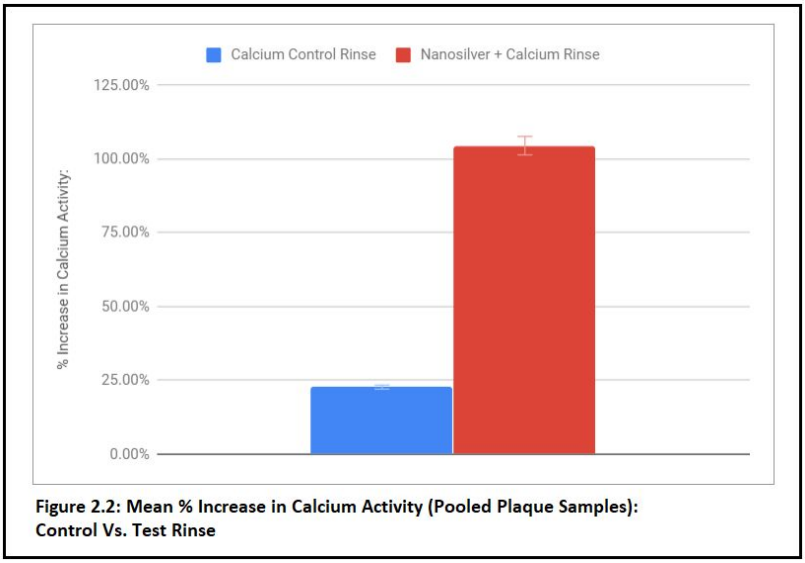
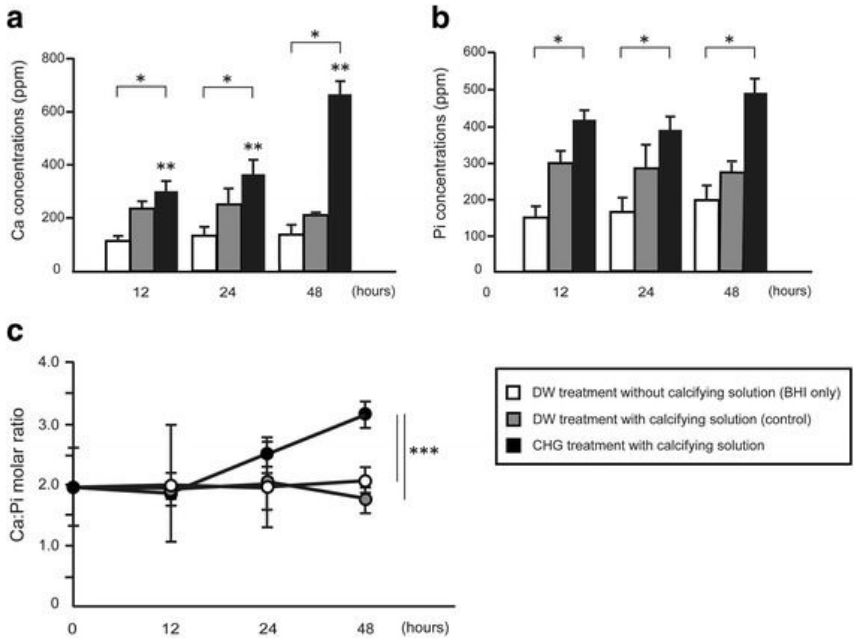


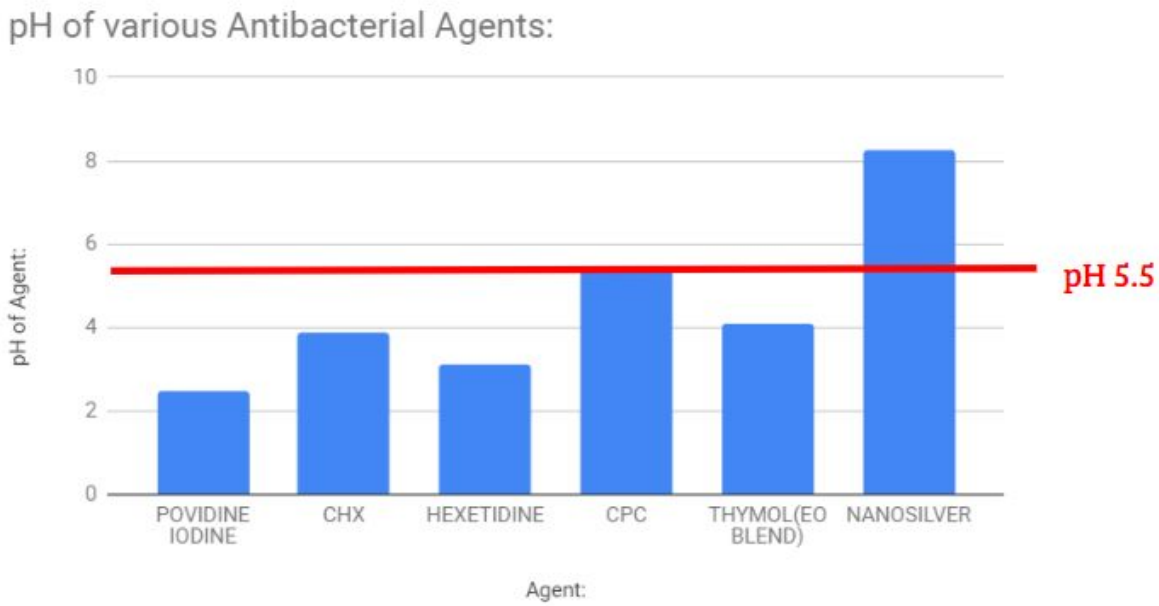
Figure 2.2: Mean % Increase in Calcium Activity (Pooled Plaque Samples): Control Vs. Test Rinse

Figure 1-35: Calcium and Phosphate Uptake into Biofilm Using CHX[64]:



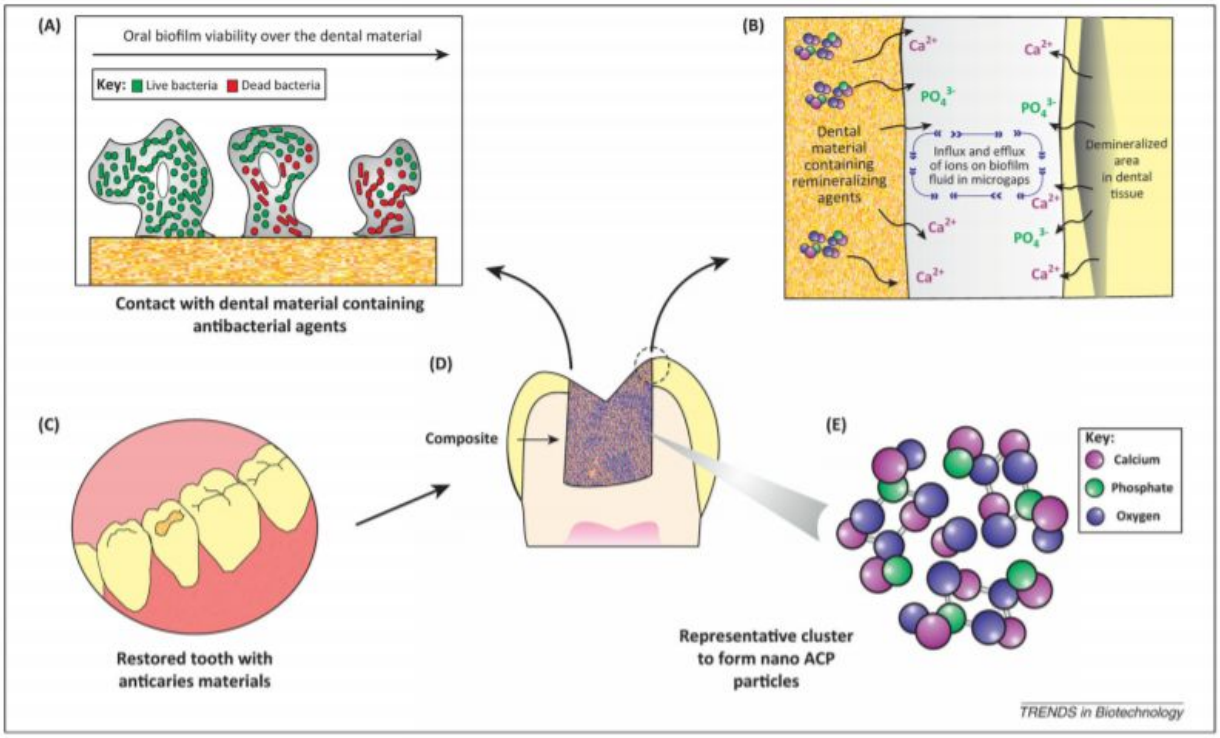
In addition, nanosilver falls into an alkaline range. It typically exists in a pH range of 7-9[91], offering additional neutralization for oral acid once entering and penetrating deep into the plaque biofilm. This is in contrast to other antimicrobial agents, which typically exist at a pH of lower than 5.5[92]:

Figure 1-36: A Comparison of pH of Antibacterial Agent[92]



In recent studies, the addition of silver nanoparticles has been mirrored in restorative materials to contribute to a reduction in oral acid release[65]:

Figure 1-37: Use of Nanomaterials in Composites to Reduce Acid Release[65]:



Furthermore, as observed with silver diamine fluoride, silver ions can incorporate into tooth structure at an alkaline pH. This in turn can increase the charge density of the mineral. As is known, the unhydrated radius of silver is far greater than calcium. This can add more charge density to HA, potentially improving its resistance to acid attacks. This is reiterated below[47]:

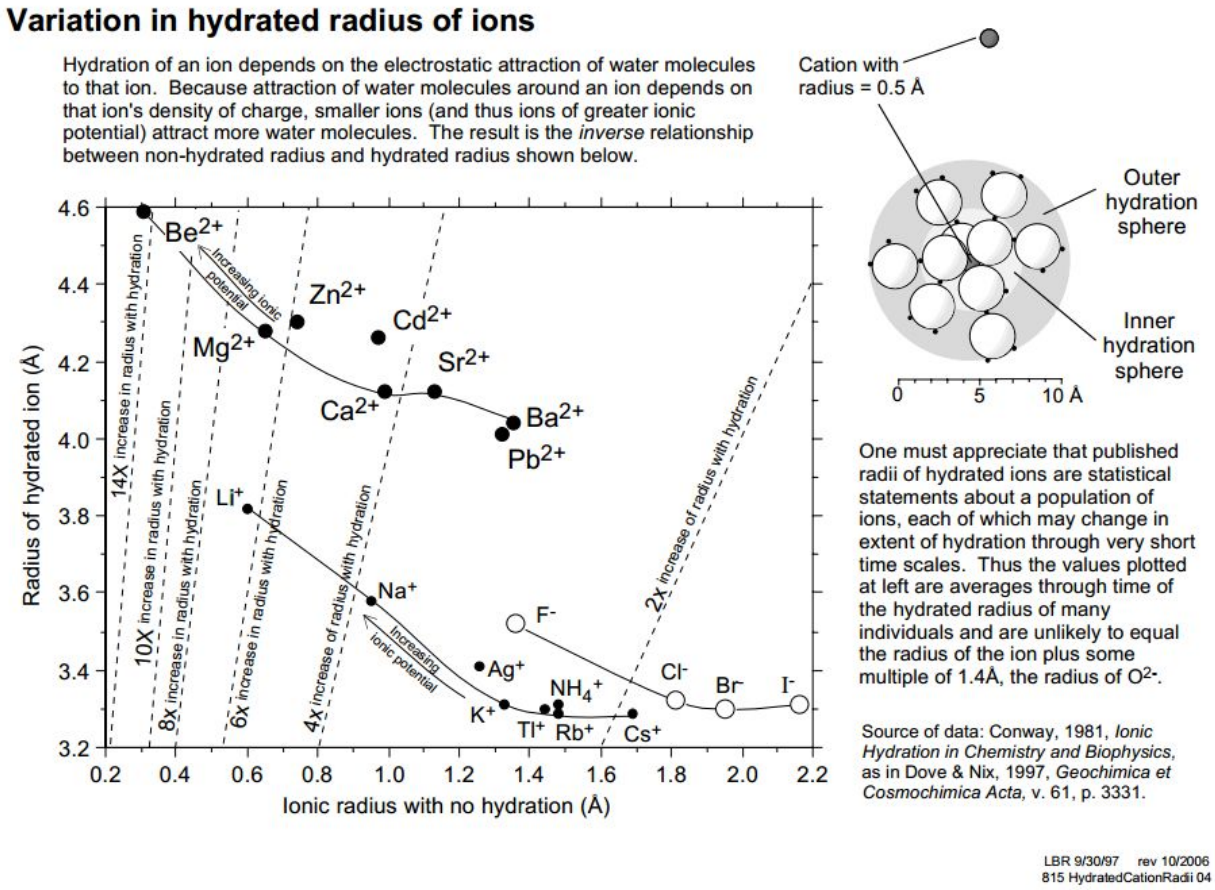
Table. Calculated Hexagonal Unit Cell Parameters *a* and *c* Axes, F/Ca, F/P, and Final pH in Experimental Groups.

	P-XRD, Å				
SDF,[a] mg/mL	*a*-axis	*c*-axis	F/Ca	F/P	Final pH
None[b]	9.577 ± 0.0012	6.833 ± 0.0010	N/A	N/A	7.07 ± 0.02
0.38	9.554 ± 0.0011	6.833 ± 0.0010	0.022 ± 0.002	0.043 ± 0.006	8.02 ± 0.01
1.52	9.552 ± 0.0036	6.833 ± 0.0010	0.037 ± 0.007	0.055 ± 0.006	8.14 ± 0.01
2.66	9.548 ± 0.0024	6.833 ± 0.0010	0.043 ± 0.004	0.070 ± 0.009	8.60 ± 0.02
3.80	9.542 ± 0.0047	6.833 ± 0.0010	0.072 ± 0.005	0.111 ± 0.011	8.95 ± 0.01

All the data are normally distributed. Values are presented as mean ± SD.
F/Ca, fluoride/calcium; F/P, fluoride/phosphorus; N/A, not applicable; P-XRD, powder X-ray diffraction; SDF, silver diamine fluoride.
[a]No crystal was detected in the SDF control group (no calcium phosphate).
[b]Calcium phosphate control.

Studies that focused on the anti-caries effects of sealant materials containing nanosilver reported a significant reduction in demineralization and increased remineralization compared to a conventional sealant[93]. It should also be noted that when nanosilver was applied to enamel, a 15% increase in remineralization occurred compared to when the control was used[94].

Figure 1-38: Unhydrated and Hydrated Radius of Various Ions[66]:



Silver Nanoparticle Summary:

The two most important factors when considering remineralization are rapid entry and preventing the oral biofilm from controlling its ionic content in plaque fluid[5,6]. Since the oral biofilm is able to sequester away pockets of oral acid using channels and the EPS matrix[5,6,8], penetrance into these isolated areas requires a sophisticated delivery system[10]. Due to the small particle size to surface area ratio of nanoparticles, nanotechnology can be very effective at penetrating biofilms, thus preventing them from properly controlling their internal ionic environments[63,91]. This, combined with penetration, allows ions in saliva and dentifrices to enter at a much higher rate. This has been observed clinically with nanosilver particles and CHX, which both showed a 5 times increase in calcium entry into the plaque fluid of the biofilm[64,91]. Just like fluoride ions, silver ions can also incorporate into HA through a mechanism called substitution. Many studies have been done on this substitution process, and have shown that silver ions do not change the crystal lattice parameters[47]. However, silver ions have a much larger unhydrated radius than the calcium they replace[66]. Additionally, a buildup of silver ions

can create an increase in the structural charge of HA. This is because more charge is encompassed in the same space resulting in an excess of electrons. This excess may allow fluoride to enter at a much faster rate, which has been shown with silver diamine fluoride[47]. Additionally, by creating an alkaline environment, silver ions are more attracted to tooth structure, further disrupting biofilm attachment[10,63,91]. Due to the fact that nanosilver is highly alkaline, it also serves as a neutralizing agent for oral acid. Studies have also shown that in dental materials, silver nanoparticles reduce acidic mechanisms of action in oral bacteria[65]. With various protective mechanisms, silver nanoparticles show promising potential for use in commercial dentifrices.

Xylitol - pH Neutralizing and Anti-Biofilm Polyol:

Xylitol is a sugar alcohol (polyol) naturally found in many fruits and vegetables[90]. Xylitol has been extensively studied for its anticariogenic properties, and fits the criteria for both promotion of remineralization and inhibition of demineralization[67]:

1. Breaks up biofilm attachment to tooth structure and prevents new biofilm attachments from occurring[70]
2. Displays strong antibacterial activity towards cariogenic organisms[71]
3. Reduces lactic acid output from cariogenic organisms[70,71]
4. Penetrates biofilm and tooth structure, acting as a delivery system for calcium and phosphate (stabilizes calcium and phosphate in solution-acts as a statherin mimic)[68]
5. Raises the pH and acts as a pH neutralizing agent[69]
6. Non metabolizable by oral bacteria[68]

Xylitol has been known for its ability to break up biofilm attachments[70]. This property is evident from its high water activity compared to other sugar alcohols. This allows xylitol to displace water surrounding tooth surfaces effectively cleaving biofilm attachments[72]. This can also be seen from the low plaque mass weight:

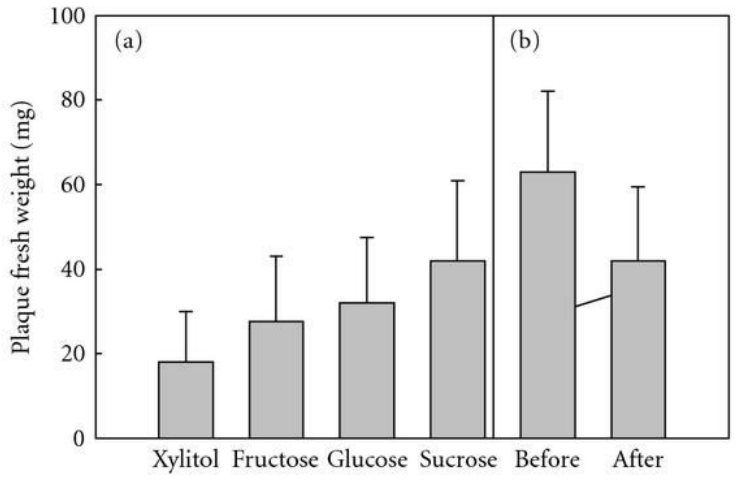
Figure 1-39: Sugar Alcohol Water Activity[72]:

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

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

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

Figure 1-40: Xylitol Plaque Mass Compared to Other Sweeteners[73]:



It has been evident in multiple clinical studies that xylitol is effective at reducing cariogenic CFU counts, even at low concentrations. This can be seen in the figures below:

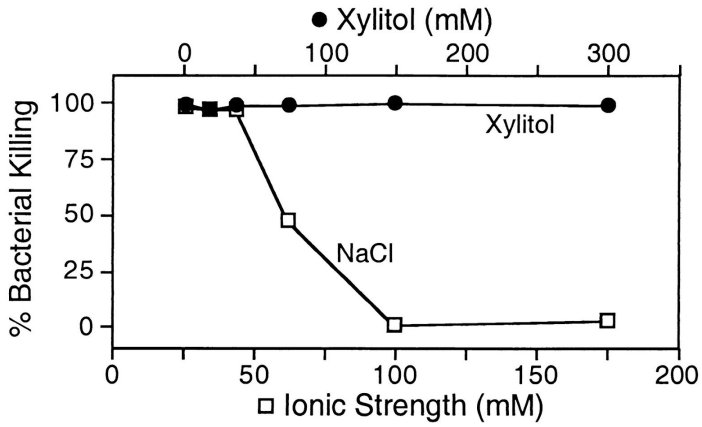
Figure 1-41: Cariogenic Bacterial Reduction from Xylitol[76]:

Table 1 Effect of xylitol (xyl) at two concentrations (1% and 3%) on biofilm bacteria. Results are presented as average CFU/ml subjected to logarithmic transformation

	Lactobacilli			Streptococci			Actinomyces			Anaerobes		
	Control	Xyl 1%	Xyl 3%	Control	Xyl 1%	Xyl 3%	Control	Xyl 1%	Xyl 3%	Control	Xyl 1%	Xyl 3%
Average	7.23	1.40	NR*	7.30	NR*	NR*	7.74	5.90	2.46	7.32	NR*	NR*
SD	0.22	1.68	0.00	0.07	0.00	0.00	0.05	0.15	1.23	0.03	0.00	0.00

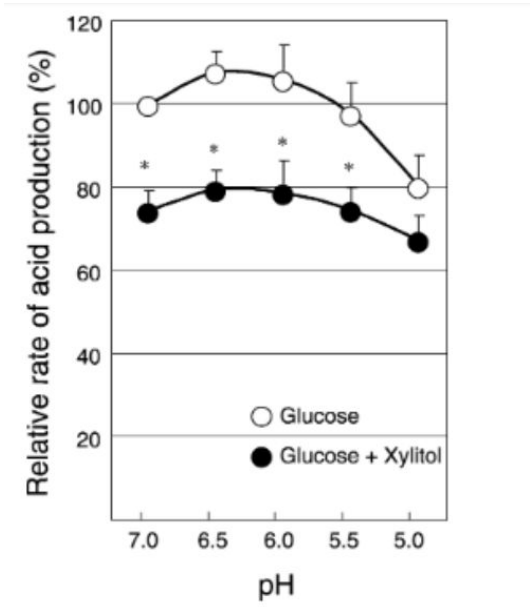
NR*: Not recorded.

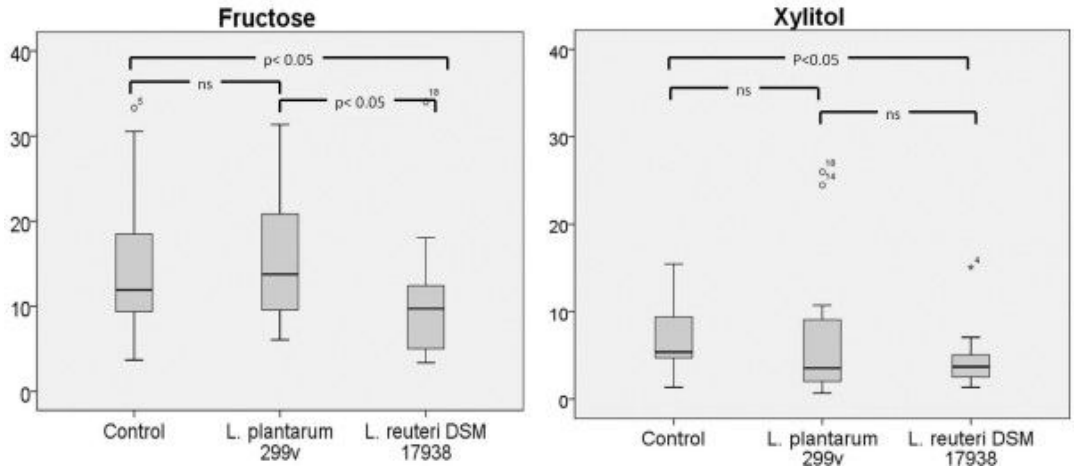
Figure 1-42: Bacterial Killing Effects of Xylitol[74]:



It has also been noted as an effective disruptor of lactic acid release from cariogenic organisms. This can be seen in the figure below:

Figure 1-43 and 1-44[75]: Lactic Acid Reduction of *S. mutans* (Xylitol):





It has also been noted that xylitol is an effective complexer of calcium and phosphate salts, especially at a neutral pH. This can be observed in the figure below:

Figure 1-45: Xylitol Complexation with Calcium Ions[47]:

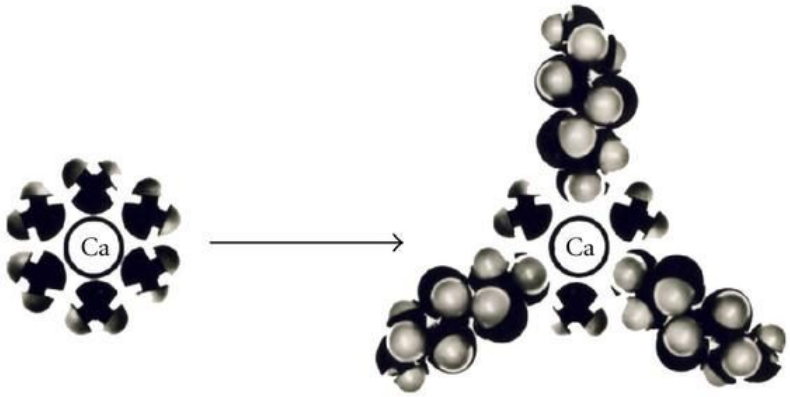


Figure 1-46: Increase in Calcium in Plaque Mass After Use of Xylitol Agents[47]:

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

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

As shown, xylitol is excellent at raising the pH of the biofilm. It has also been observed that individuals who frequently consume xylitol tend to have higher protein levels in plaque, which increases pH and generates compounds such as urea:

Figure 1-47: Effect of Xylitol on Plaque pH after 10% Sucrose Solution[77]:

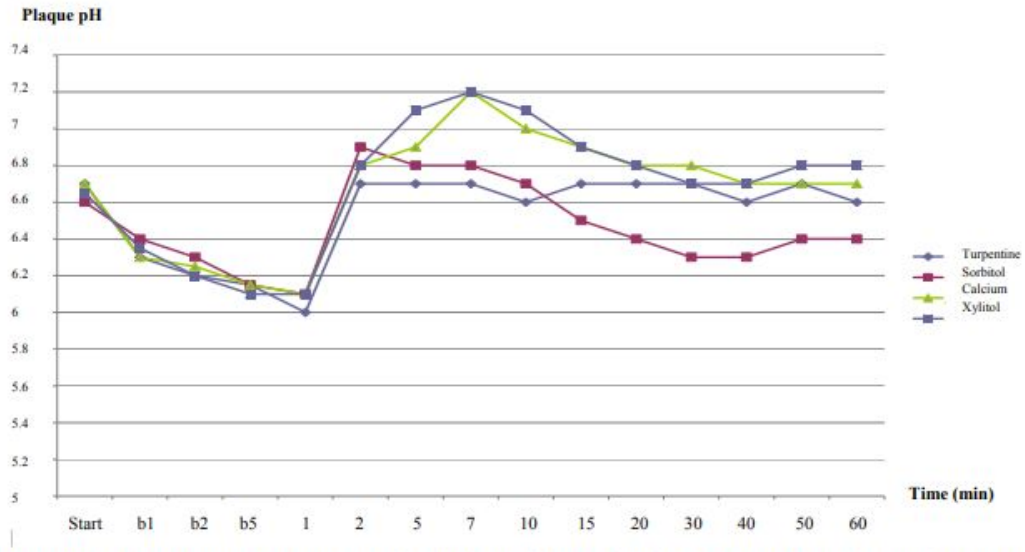
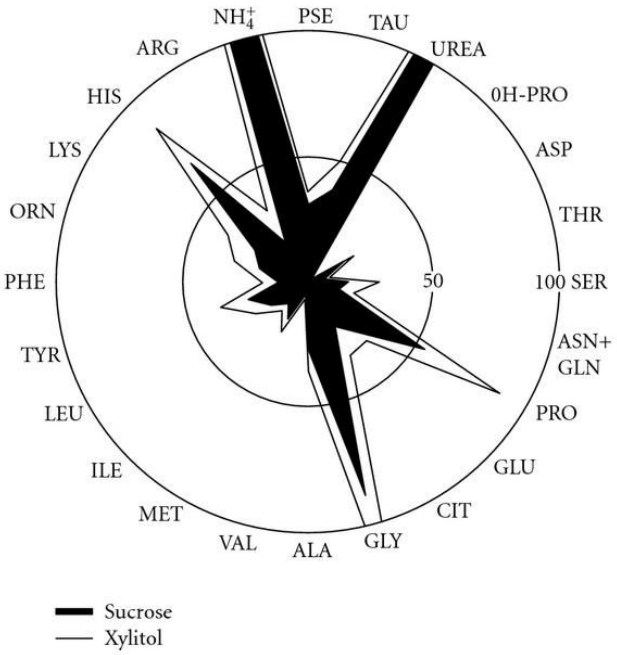


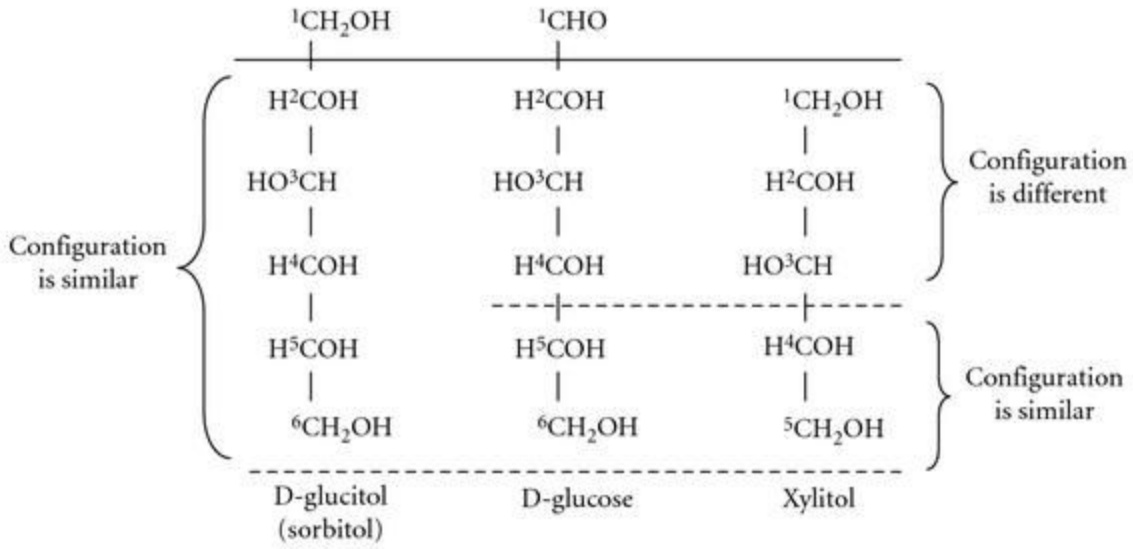
Diagram 1- Mean changes in plaque pH of subjects at different time intervals due to chewing studied gums after consumption of 10% sucrose solution.

Figure 1-48: Xylitol Exposure Affects Alkaline Protein Content in Plaque[47]:



It is well known that xylitol is not metabolized by oral bacteria. This is evident by its structure, which is unlike glucose or sorbitol. Unlike these compounds, xylitol cannot be converted into lactic acid as shown by the figure below:

Figure 1-49: Structure of Xylitol Compared to Sorbitol and Glucose[47]:



Xylitol Summary: Xylitol has been shown in many clinical studies to not only break up biofilms[68,70,74], but to reduce plaque buildup, increase the pH of plaque fluid[77], and act as a calcium delivery system into HA crystals[47]. When compared to fluoride, xylitol was shown in a study to remineralize from the deepest inner portion of the lesion outward, a complete surprise to researchers[78,79]. This initiated the notion that xylitol can penetrate deep into tooth structure and deliver calcium and phosphate to deeper portions of existing enamel lesions, even surpassing the effects of fluoride[47,78,79].

In order for xylitol to be the most effective, it cannot be part of an acidic solution. Substances such as citric acid can compete for xylitol surface sites (complexation), thereby reducing the effects of xylitol. This is because xylitol has a weak complexing effect with calcium salts[47]. Weak complexing via hydroxyl groups allows xylitol to complex onto calcium and phosphate[47]. This is due to xylitol's high water activity, which allows it to displace water from calcium and phosphate, stabilizing them but not chelating them[47,72]. However, if xylitol is in an acidic environment, ions such as citric acid will easily complex with xylitol and simultaneously chelate free calcium[47]. This reduces the bioavailability of calcium salts, in addition to rendering xylitol far less effective. As a result, xylitol attaches to acids almost exclusively over calcium salts, deterring its ability to deliver these salts into and onto tooth surfaces where they are most beneficial[47]. This acidic presence can also prevent the stabilizing nature of xylitol from occurring, rendering calcium salts less stable, leading to undesirable precipitation[47]. Therefore, the xylitol synergy with calcium salts is clearly a pH-dependent function. As a result, pH levels

can lead to varying effects and outcomes for xylitol. This means that in an acidic environment, the attachment of xylitol to calcium salts will be minimal[47]. This would explain why xylitol in non-pH-controlled conditions would show effects sometimes but not others. Xylitol can still break up biofilms in an acidic delivery system, but it will not stabilize calcium, and if it's not complexing with calcium, the chances of it delivering calcium and phosphate to demineralized sites is much lower[47]. Additionally, in acidic conditions, xylitol is less competitive for tooth structure due to its hydroxyl groups. Ultimately an alkaline pH allows xylitol to be much more effective at entering cariogenic organisms, disrupting biofilms and promoting remineralization[47]. Another benefit of xylitol besides its stabilizing effects on calcium salts, is that its weak complexation with calcium salts can also decrease precipitation of calcium phosphate. This process occurs without removing these ions from solution like a chelating agent would, thus, not affecting ionic product[47]. Interestingly enough, when xylitol is used, studies have shown a downregulation in enzymes which normally digest sugars (sucrase, dextranase, and amylase)[47]. This prompts bacteria in the oral microbiome to scramble for energy, turning to slow digesting proteins and glycoproteins instead[47]. This can increase nitrogen output in the oral biofilm, subsequently increasing pH[47]. When looking at other sugars and sugar alcohols like sorbitol (hexose), their yield from glycolysis produces one mole of lactic acid upon breakdown. In contrast, xylitol glycolysis yields one mole of acetic acid[47]. This is interesting, because lactic acid is far more active and damaging than acetic acid, which is considered a protective acid in the oral biofilm[8]. One of the very interesting properties of calcium phosphate derivatives such as HA, is that they are highly insoluble in acetic acid. Additionally studies have also shown no increase in acetic acid during caries challenge[80], suggesting organisms involved in the development of caries are not producing it. In conclusion, xylitol has many anti-caries benefits, including stabilizing calcium salts, reducing plaque mass, reducing oral acid output, interrupting biofilm attachment, and increasing the pH of saliva and plaque fluid[47,68,74,77].

Calcium Salt - pH Neutralization Agent and Acid Inhibitor:

Although many calcium agents have shown success in remineralization, very few products have made use of them, and instead tend to favor fluoride. This is because fluoride and calcium cannot be used together, due to the formation of a calcium fluoride precipitate. As a result, there has been very few products released which make use of this core component of HA. This is seen by the reaction below, which forms the insoluble precipitate, calcium fluoride[81].

$2NaF + CaCl_2 \rightarrow CaF_2 + 2NaCl$

Further, it should be noted that calcium fluoride is only soluble in highly acidic conditions, sometimes far below the threshold levels bacteria reach. This, combined with its transient contact time with dentifrices, can render this precipitate less valuable. This is observed in the figures below:

Figure 1-50: Low Solubility of Calcium Fluoride[81]:

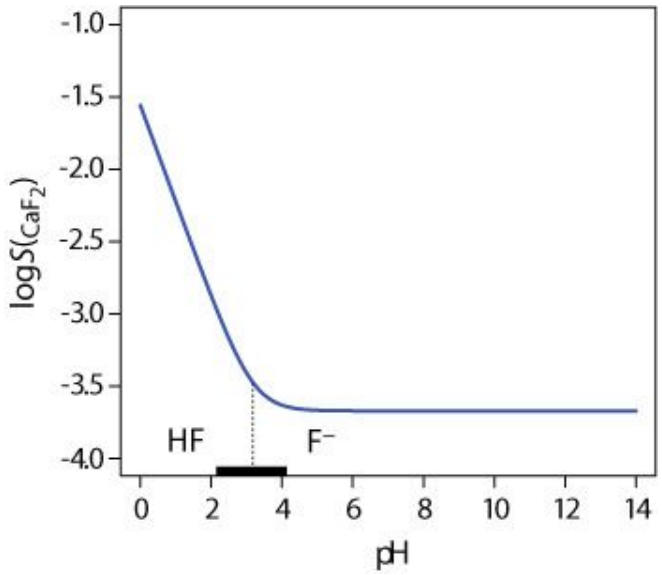
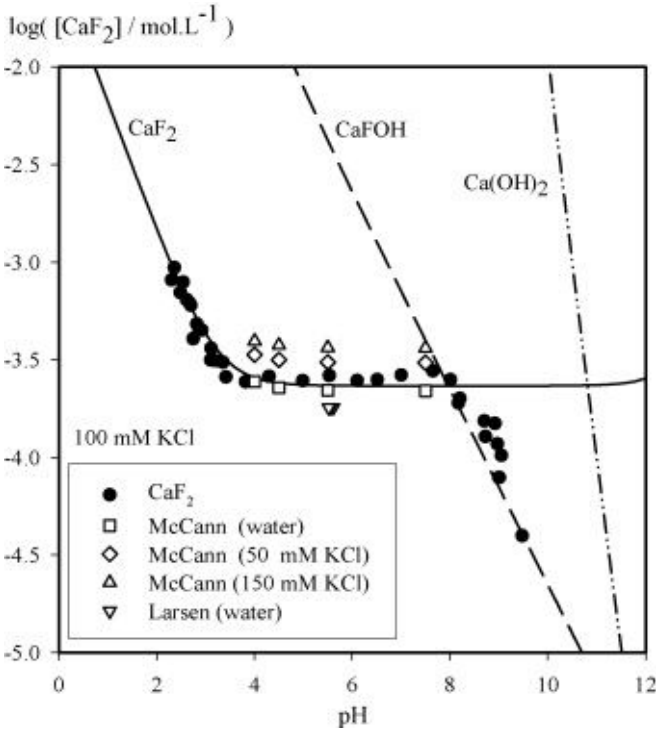


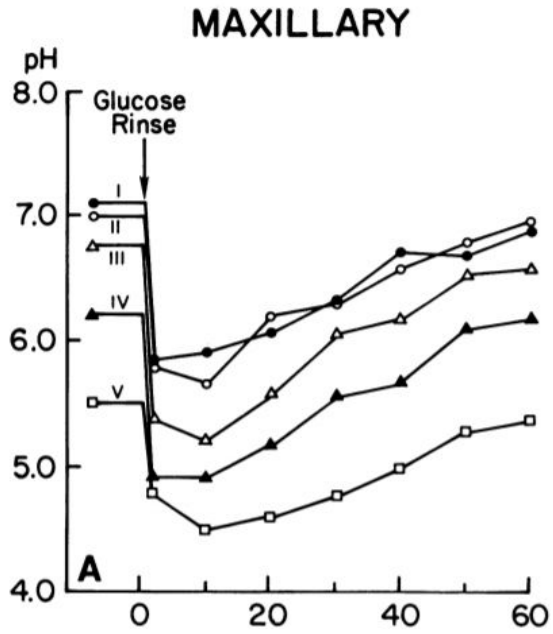
Figure 1-51: Solubility of Calcium Fluoride at Varying pH[82]:



When considering traditional models which only utilized fluoride, the logic was that the benefits of fluoride are proven beyond a doubt. However, the key issue that has gone unaddressed is that

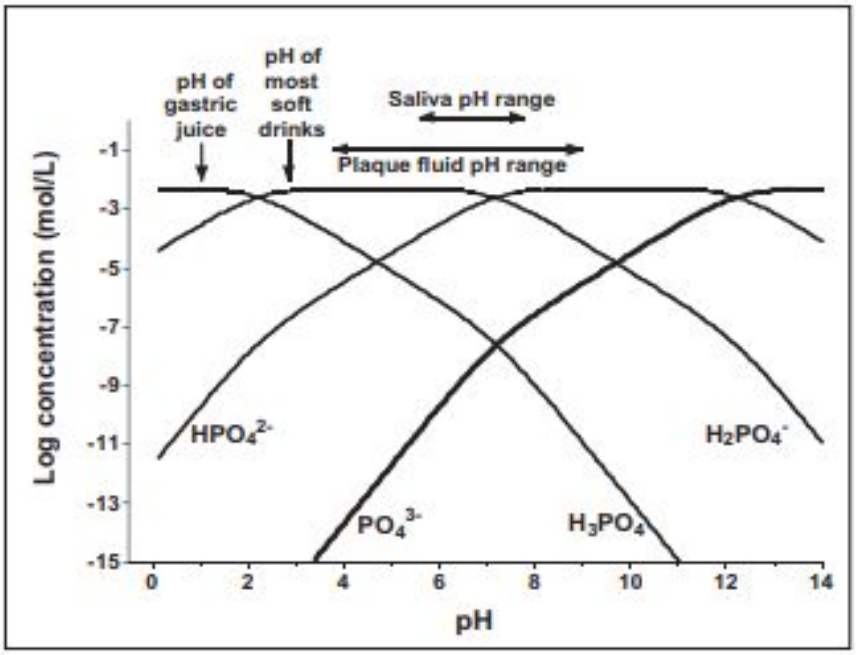
simply using fluoride alone does not remedy the situation of satisfying a need for calcium in tooth structure through the equilibrium known as the solubility product I.e. Ksp[3]. This is shown below:

Ksp (Solubility product) = Fixed value of $[Ca]_3[PO4]_5[OH]_2$



Since saliva naturally contains calcium and utilizes it for remineralization at a pH level between 7 and 8, it would make sense that the supply of saliva significantly impacts cariogenic processes[3]. This has been demonstrated with calcium lactate, among other calcium salts and compounds, which successfully remineralize teeth[3]. The issue for fluoride, as well as calcium, comes down to bioavailability in the plaque fluid of the oral biofilm. Saturation of Ksp in this area fluctuates drastically with increased substrate in the biofilm. Thus, plaque fluid is the ultimate determinant of cariogenic activity[3,5,6]. This is visualized by the Stephan Curve and reiterated here[9]:

Although there will always be a use for fluoride, once it has reached its maximal incorporation into the tooth structure, the lattice will no longer accept fluoride. At this point, additional use of fluoride may not show an increased benefit for tooth remineralization. This is shown in calculations determining that fluorapatite's Ksp is approximately 30% more resistant to acid attacks. Certain individuals may have plaque fluid or saliva that is chronically low in calcium. Thus, despite the use of fluoride, these individuals may still suffer from cariogenic attacks and subsequent decay[3]. The figure below depicts the pH dependence of phosphate[3]. This leads us to conclude that as pH increases, dependence on calcium increases for remineralization to occur:



It should also be noted that a fluoride dentifrice alone does not contribute to the ionic product, and can actually cause dissolution of HA[3]. Since many of these solutions are also acidified, it remains a question as to the degree of damage caused by extended contact[3]. Therefore, it is instrumental that we look for alternatives that can be used in addition to fluoride to help reduce the cariogenic activity and acid attacks responsible for demineralization and subsequent decay. Calcium offers some concrete benefits, and the type of calcium is very important in order to ensure the highest benefits can occur:

1. Satisfies Ksp and Degree of saturation (Remineralization)
2. Neutralizes acid upon contact
3. Interferes with acid production processes from cariogenic bacteria

One of the interesting components about calcium salts is actually that the counterion is of severe importance. Since calcium salts are usually insoluble due to their low bioavailability, this means that very few can be used clinically in a stable form. Even less so, very few calcium salts display high bioavailability in the alkaline range compared to solubility in acidic media. This is illustrated by calcium sulfate, calcium hydroxide, calcium phosphate among other calcium salts. This can be observed in the figure below:

Figure 1-52: Calcium Salt Solubility at Varying pH[83]:

Table 4. Percentage of 500 mg of Calcium Dissolved in 500 ml of Water at One Hour, According to the pH of the Water.

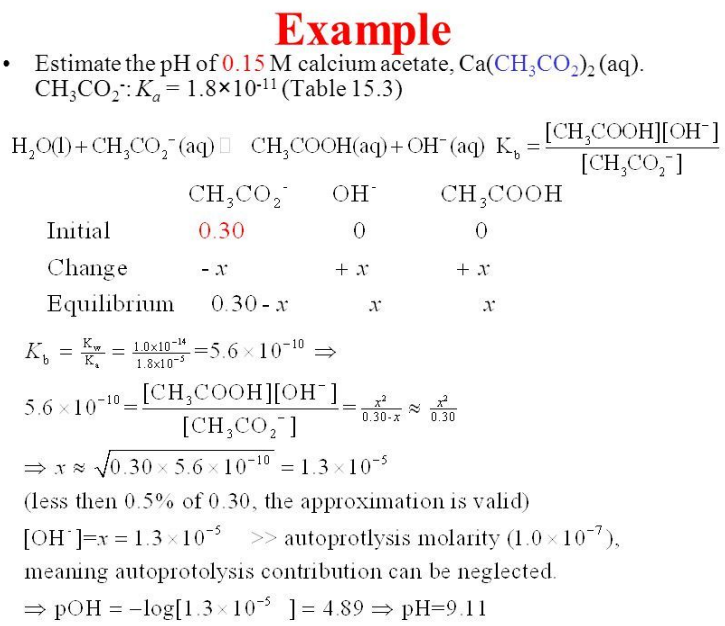
Calcium Salt	pH Not Adjusted	pH 5.0	pH 2.5
		percent	
Calcium carbonate	1	86*	100
Calcium citrate	17	23†	100
Calcium gluconate	100	100	100
Calcium lactate	100	100	100
Calcium acetate	100	100	100

*100 percent had dissolved at three hours.

†24 percent had dissolved at two, three, four, and five hours.

In contrast there are calcium salts that are soluble in the acidic range, but these do not serve to enhance the pH. Fortunately, a highly soluble, alkaline calcium salt exists: calcium acetate. Calcium acetate has a pH of approximately 7.5-9, and is highly soluble and bioavailable in water. The pH of calcium acetate salts can be observed in the figure below:

Figure 1-53: Calcium Acetate pH[84]:



Example

- Estimate the pH of 0.15 M calcium acetate, $Ca(CH_3CO_2)_2$ (aq).
  $CH_3CO_2^-$: $K_a = 1.8\times10^{-11}$ (Table 15.3)

$$H_2O(l) + CH_3CO_2^-(aq) \rightleftharpoons CH_3COOH(aq) + OH^-(aq) \quad K_b = \frac{[CH_3COOH][OH^-]}{[CH_3CO_2^-]}$$

	$CH_3CO_2^-$	OH^-	CH_3COOH
Initial	0.30	0	0
Change	$-x$	$+x$	$+x$
Equilibrium	$0.30-x$	x	x

$$K_b = \frac{K_w}{K_a} = \frac{1.0\times10^{-14}}{1.8\times10^{-5}} = 5.6\times10^{-10} \Rightarrow$$

$$5.6\times10^{-10} = \frac{[CH_3COOH][OH^-]}{[CH_3CO_2^-]} = \frac{x^2}{0.30-x} \approx \frac{x^2}{0.30}$$

$$\Rightarrow x \approx \sqrt{0.30\times5.6\times10^{-10}} = 1.3\times10^{-5}$$

(less then 0.5% of 0.30, the approximation is valid)

$[OH^-] = x = 1.3\times10^{-5}$ >> autoprotlysis molarity (1.0×10^{-7}), meaning autoprotolysis contribution can be neglected.

$$\Rightarrow pOH = -\log[1.3\times10^{-5}] = 4.89 \Rightarrow pH = 9.11$$

There are many studies on acetate, the counterion of calcium acetate, in the literature. Some of these studies concluded that *S. mutans,* among other cariogenic organisms, have no carrier-assisted mechanism to displace this counterion[27]. In contrast, salts such as calcium lactate, with a pH of approximately 6, may contribute lactate to cariogenic organisms. This lactate can be recycled for reuse. *S. mutans* is a particular strain that employs this mechanism[27]. Essentially, with very little energy, *S. mutans* can produce more lactic acid in the presence of lactate[27]. Upon investigation it was confirmed that acetate may drain the energy (ATP) stores of cariogenic organisms in order to maintain a pH gradient without rupturing[27]. In addition, acetate has been shown to reduce *S. mutans* glycolysis, whereas lactate has shown not to impede this process[27,85]. This is shown by the figures below:

Figure 1-54: Glycolytic Activity of *S. mutans* with Acetate vs. Lactate[85]:

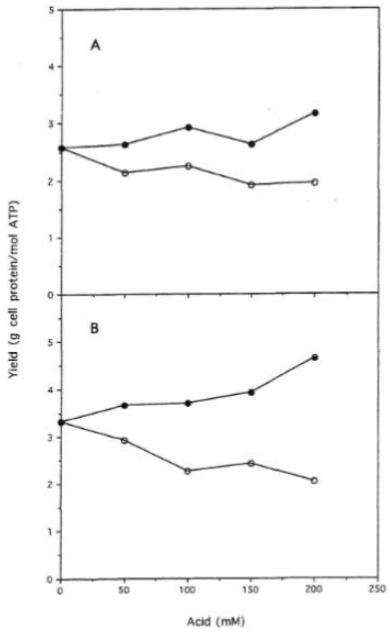
Table 3. Glycolytic Activity of Washed Cells of *S. mutans* and *S. sanguis* in the Presence of Organic Acid Anions at an Extracellular pH of 7.0

Additions[a]	Glycolytic Activity Concentration of Anion (mmol/L)			
	0	50	100	150
S. mutans				
Control	182 ± 8 (12)^{b,c}			
Acetate		158 ± 15 (3)^{d}	143 ± 5 (3)^{d,e}	130 ± 9 (3)^{e,f}
Lactate		193 ± 4 (3)^{c,f}	196 ± 3 (3)^{c,f}	182 ± 3 (3)^{c,g}
Formate		169 ± 4 (3)^{c,d}	151 ± 25 (3)^{d}	152 ± 2 (3)^{d}
KCl		185 ± 6 (3)^{c,f}	160 ± 6 (3)^{d}	132 ± 7 (3)^{e,f}
S. sanguis				
Control	170 ± 14 (12)^{c}			
Acetate		190 ± 6 (3)^{d,f}	185 ± 10 (3)^{c,d,f}	172 ± 13 (3)^{c,f,g}
Lactate		189 ± 7 (3)^{d,f}	188 ± 5 (3)^{c,d,f}	174 ± 1 (3)^{c,f}
Formate		176 ± 6 (3)^{c,d}	156 ± 2 (3)^{c}	159 ± 8 (3)^{c,g,h}
KCl		163 ± 18 (3)^{c}	159 ± 10 (3)^{c}	153 ± 7 (3)^{c,h}

Table 4. Glycolytic Activity of Washed Cells of *S. mutans* in the Presence of Organic Acid Anions at an Extracellular pH of 5.0

Additions[a]	Glycolytic Activity Concentration of Anion (mmol/L)			
	0	50	100	150
S. mutans				
Control	169 ± 6 (12)^{b,c}			
Acetate		135 ± 4 (3)^{d,f}	91 ± 11 (3)^{e,f}	53 ± 3 (3)^{f}
Lactate		101 ± 3 (3)^{d,g}	64 ± 3 (3)^{e,g}	28 ± 2 (3)^{g}
Formate		98 ± 10 (3)^{d,g}	71 ± 4 (3)^{e,g}	25 ± 2 (3)^{g}
KCl		184 ± 5 (3)^{d}	196 ± 6 (3)^{e}	184 ± 4 (3)^{d}
S. sanguis				
Control	104 ± 10 (12)^{c}			
Acetate		83 ± 5 (3)^{d,f}	50 ± 5 (3)^{e,f}	29 ± 2 (3)^{f}
Lactate		41 ± 6 (3)^{d}	24 ± 4 (3)^{e,g}	16 ± 1 (3)^{g}
Formate		60 ± 11 (3)^{d}	23 ± 8 (3)^{e,g}	7 ± 4 (3)^{h}
KCl		106 ± 5 (3)^{c,g}	78 ± 7 (3)^{d}	72 ± 5 (3)^{d}

Figure 1-55: Acetate as a Biomass Reducer of *S. mutans*[27]:



Furthermore, when acetate is turned into acetic acid, it is far less damaging to tooth structure than when lactate is turned into lactic acid[8]. This is because acetic acid has very limited ability to dissolve HA and has a higher pka[8].

Acetic Acid **Acetate Ion**

Compared to lactic acid with a pka of 3.86, acetic acid has a pka of 4.76 and is considered a protective acid[8]. This makes it a more ideal counterion, and thus serves as a better buffer in the case of acidification. This is confirmed by the work done by Morghalis and colleagues, whereby during cariogenic attacks, acetic acid does not fluctuate, indicating that cariogenic organisms do not favor acetic acid, and instead produce lactic acid[80]:

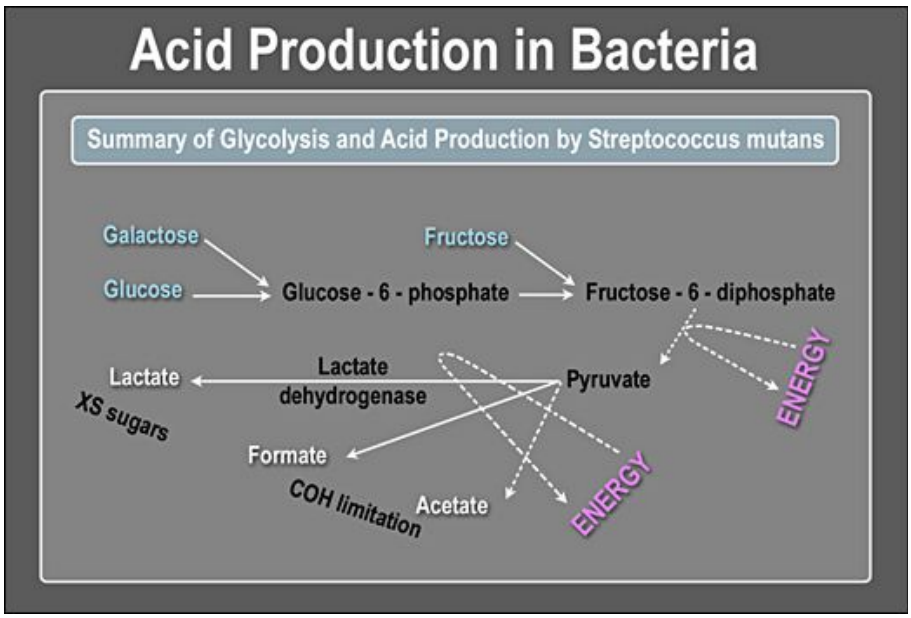
Figure 1-56: Acid Anions Present After 10% Sucrose Challenge in Plaque Fluid[80]:

TABLE 12
Mean Concentrations of Selected Ions in Pooled Plaque Fluid from Caries-Free (CF) and Caries-Positive (CP) Groups Before and at Selected Times Following a 1-min 10% Sucrose Rinse[a]

		Time (min)				
		0	7	15	30	60
pH	CF	7.02[b]	5.63[c]	5.80	6.02	6.19
	CP	6.79	5.38	5.58	5.73	5.88
NH_4^+	CF	26.3	18.5	18.5	19.6	21.2
	CP	22.6	16.5	18.7	22.1	20.7
Ca_{tot}	CF	2.8	9.6	5.9	4.2	3.5
	CP	2.7	8.2	5.8	4.3	3.5
P_i	CF	13.9	17.7	15.0	14.8	13.5
	CP	15.6	20.6	20.0	17.4	16.4
Succinic	CF	4.4	3.2	4.0	5.2	5.6
	CP	4.6	3.0	2.8	6.0	7.5
Lactic	CF	1.8	36.0[c]	21.9[c]	12.5[c]	6.8
	CP	2.6	51.1	39.9	19.1	13.2
Acetic	CF	19.9	17.6	18.7	20.7	19.8
	CP	20.3	19.8	24.1	29.9	25.3
Propionic	CF	5.8	8.0	7.4	8.9	7.6
	CP	9.2	11.7	13.7	17.5	14.3
DS(En)	CF	7.11[b]	1.76[d]	2.29	2.48	3.30
	CP	5.42	1.02	1.35	1.52	1.87

It has also been noted that in carbohydrate limiting conditions, acetate and formate are produced more favorably. Since carbohydrate limiting conditions are ideal for the host, it would seem that these are more favorable for host commensal conditions, as they contribute far less to dissolution and may cause disturbances in biofilm homeostasis. This is observed in the figure below:

Figure 1-57: Oral Acid Production in Starved vs. Substrate Rich Plaque[86]:



This is likely because at higher acetate concentrations, cariogenic cellular homeostasis is compromised[27,85]. Although multiple calcium salts could be used, calcium acetate presents a fascinating opportunity for use in a dentifrice, as it satisfies multiple needs as a neutralizing agent.

Combination Therapy - Utilizing Multiple Modes of Action to Achieve Superior Results:
Whenever a hygiene strategy is implemented, it is important to approach the problem with multiple modes of action. This greatly increases the chances of success by suppressing the ability of cariogenic organisms and their mechanisms to a minimum. This agrees with the CAMBRA philosophy of looking at risk factors in the development of disease. One of the biggest problems with current solutions is that they do not take into account how the oral biofilm operates. It has been observed time and time again that the oral biofilm can sequester and protect plaque fluid from being neutralized by saliva[5,6]. This is especially critical for high risk patients, who may have aggresive biofilms and poor salivary clearance, among other factors[3,6,7]. Therefore, delivery of neutralizing agents should be of primary importance. In order to increase delivery of neutralizing agents, there has to be a biofilm penetrant, which can enter the oral biofilm, and open up channels to equilibrate ion flow into the biofilm[64,91]. This allows for faster neutralization of plaque fluid, and decreases the time whereby plaque fluid pH is decreased, as has been observed in the Stephan Curve[9]. This curve shows how after eating, the pH of the plaque fluid dips dramatically, and can sometimes take hours before rising back to safe levels. Therefore, isolated mechanisms of action may not be enough to sever the risk associated with mature biofilms which are cariogenic in nature.

The proposed solution to this issue, is by use of combination therapy:

1. Use an alkaline biofilm penetrant (BFP) which can create channels in the plaque biofilm.
2. Utilize combination neutralizing agent(s) which can enter the plaque mass alongside the biofilm penetrant.
3. Create a starved state using a polyol and calcium acetate, in addition to helping to stabilize calcium and phosphate levels in plaque.

It is critical that the biofilm penetrant (BFP) be chosen with the greatest care. The properties required are multiple:

1. The biofilm penetrant must be able to easily enter into the biofilm.
2. The biofilm penetrant must be less than 100nm in order to effectively enter and bypass the biofilm channel network to reach plaque fluid.
3. The biofilm penetrant must be alkaline to neutralize acids.
4. The biofilm penetrant cannot interfere with other neutralizing agent/remineralization process.
5. The biofilm penetrant must ideally be useable in minute concentrations.
6. The biofilm penetrant must be minimally toxic to human cells.
7. The biofilm penetrant must be able to interfere with biofilm mechanisms of action: (attachment, acid release, glycolysis).

In addition, the neutralizing agent has certain criteria:

1. Must be the predominant ion which contacts tooth surfaces.
2. Must not interfere with the biofilm penetrant.
3. Must be alkaline and satisfy criteria for HA's ionic product.
4. Must contribute to degree of saturation.
5. Remineralizes teeth (adsorption/absorption).

Additionally, It is recommended since there is high benefit in multiple modes of action to use a polyol, which can inhibit biofilms, act as a secondary biofilm penetrant in addition to contributing to neutralization. This is why combination use with xylitol is recommended and a calcium compound. As has been mentioned, these benefits include:

1. Breaking biofilm attachment to tooth structure.
2. Displays strong antibacterial activity towards cariogenic organisms.
3. Reduces lactic acid output from cariogenic organisms.
4. Reduces production of biofilm attachment glycoproteins .
5. Penetrates biofilm and tooth structure, and acts as a delivery system for calcium and phosphate.

6. Stabilizes calcium and phosphate in solution (statherin mimic).
7. Prevents new biofilm attachments from occurring.
8. Raises the pH and acts as a pH neutralizing agent (saliva and plaque fluid).

Therefore, the proposed solution involves:

1. A primary biofilm penetrant (nanosilver particles).
2. A secondary biofilm penetrant (xylitol).
3. A primary, secondary, and tertiary neutralizing agents (calcium acetate, xylitol, nanosilver).
4. Remineralizing agents: calcium salts, xylitol, nanosilver.

VII: Conclusions: Biofilms Must be Controlled in Order to Reduce Risk Factors for Oral Disease:

When considering the primary obstacle to controlling risk factors for disease, the oral biofilm cannot be overlooked[5,6]. Due to the nature of oral biofilms and their tendency to shift towards pathogenesis as they mature, interrupting this cycle becomes critical[10]. As these shifts and maturation occur, their ability to prevent salivary entry over time increases, delaying the neutralization and clearance times required to prevent demineralization and decay[5,6,44,45,91]. Biofilms should always be the first point of contention when considering strategies to prevent dental caries.

pH-based Concepts are Critical for Success: As can be illustrated from the high number of literature studies and concepts cited, pH is an absolutely fundamental aspect of controlling cariogenic disease, and oral disease for that matter. Without considering all aspects of how clinical disease occurs, we cannot find many examples which exclude how vital pH is to these processes. It is therefore undeniable that without the consideration of pH, you cannot employ effective prevention strategies that work with the body's natural defense systems. It is always important to consider how pH can ultimately affect all systems, including saliva, plaque fluid, oral bacteria, and how all these factors interact with tooth structure and host processes.

New Combination Methods Show Great Potential Against Oral Biofilms and Prevention of Caries:

By combining the diverse modes of action against cariogenic organisms, it is possible to target a wide variety of bacterial mechanisms involved in the decay process:

1. Biofilm attachments.
2. Biofilm production of acids.
3. Biofilm sequestering of plaque fluid.
4. Interrupting biofilm shifts and cariogenic glycolysis.

5. Suppression and elimination of cariogenic bacteria.

Due to the diverse nature of all the agents involved in disrupting bacterial modes of action and promoting the remineralization cycle, it is suggested that the best solution involves utilizing all available modes of action in tandem. All agents have a proven mechanism of action shown by multiple literature reviews and studies. Thus, by combining these multiple modes of action, together these agents stand the best chance to dismantle the pathogenicity of oral biofilms.

References:

1. Dental Caries (Tooth Decay) | National Institute of Dental and Craniofacial Research. (n.d.). Retrieved January 10, 2019, from https://www.nidcr.nih.gov/research/data-statistics/dental-caries.
2. Harris, R., Nicoll, A. D., Adair, P. M., & Pine, C. M. (2004). *Risk factors for dental caries in young children: a systematic review of the literature*. *Community Dental Health* (Vol. 21).
3. Dawes, C. (2003). What is the critical pH and why does a tooth dissolve in acid? *Journal (Canadian Dental Association)*, *69*(11), 722–724. http://doi.org/10.1016/S1072-3498(37)80084-9
4. Featherstone, J. (2008). Dental caries: a dynamic disease process. *Australian Dental Journal*, *53*(3), 286–291. http://doi.org/10.1111/j.1834-7819.2008.00064.x
5. Uzel, N. G., Teles, F. R., Teles, R. P., Song, X. Q., Torresyap, G., Socransky, S. S., & Haffajee, A. D. (2011). Microbial shifts during dental biofilm re-development in the absence of oral hygiene in periodontal health and disease. *Journal of Clinical Periodontology*, *38*(7), 612–20. http://doi.org/10.1111/j.1600-051X.2011.01730.x
6. Bowen, W. H., Burne, R. A., Wu, H., & Koo, H. (2018). Oral Biofilms: Pathogens, Matrix, and Polymicrobial Interactions in Microenvironments. *Trends in Microbiology*, *26*(3), 229–242. http://doi.org/10.1016/j.tim.2017.09.008
7. Dawes, C. (2008). Salivary flow patterns and the health of hard and soft oral tissues. *Journal of the American Dental Association (1939)*, *139 Suppl*, 18S–24S. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18460676
8. Walsh, L. J. (n.d.). *DENTAL PLAQUE FERMENTATION AND ITS ROLE IN CARIES RISK ASSESSMENT*. *34 INTERNATIONAL DENTISTRY SA* (Vol. 8).
9. Loesche, W. J. (1986). *Role of Streptococcus mutans in Human Dental Decay*. *MICROBIOLOGICAL REVIEWS* (Vol. 50).
10. Qayyum, S., & Khan, A. U. (2016). Nanoparticles vs. biofilms: a battle against another paradigm of antibiotic resistance. *MedChemComm*, *7*(8), 1479–1498. http://doi.org/10.1039/C6MD00124F

11. Bell, L. , Posner, A. ., & Quirk, J. . (1973). The point of zero charge of hydroxyapatite and fluorapatite in aqueous solutions. *Journal of Colloid and Interface Science*, *42*(2), 250–261. http://doi.org/10.1016/0021-9797(73)90288-9
12. Marsh, P. D. (2006). Dental plaque as a biofilm and a microbial community - implications for health and disease. *BMC Oral Health*, *6 Suppl 1*(Suppl 1), S14. http://doi.org/10.1186/1472-6831-6-S1-S14
13. Autio-Gold, J. (2008). The Role of Chlorhexidine in Caries Prevention. *Operative Dentistry*, *33*(6), 710–716. http://doi.org/10.2341/08-3
14. Thuptimdang, P., Limpiyakorn, T., Mcevoy, J., Prüß, B. M., Khan, E., & Khan, E. (2015). Effect of silver nanoparticles on Pseudomonas putida biofilms at different stages of maturity. *Journal of Hazardous Materials*, *290*, 127–133. http://doi.org/10.1016/j.jhazmat.2015.02.073
15. Kubyshkin, A., Chegodar, D., Katsev, A., Petrosyan, A., Krivorutchenko, Y., & Postnikova, O. (2016). Antimicrobial Effects of Silver Nanoparticles Stabilized in Solution by Sodium Alginate. *Biochemistry & Molecular Biology Journal*, *2*(2). https://doi.org/10.21767/2471-8084.100022
16. Behra, R., Sigg, L., Clift, M. J. D., Herzog, F., Minghetti, M., Johnston, B., … Rothen-Rutishauser, B. (2013). Bioavailability of silver nanoparticles and ions: from a chemical and biochemical perspective. *Journal of the Royal Society, Interface*, *10*(87), 20130396. https://doi.org/10.1098/rsif.2013.0396
17. Beyer, K. (2012). *Influence of Capping Agents on Silver Nanoparticle (Ag--NP) Toxicity to Nitrifying Bacteria*.
18. Radmerikhi, S., Azul, E., Fajardo, K., & Formantes, B. (2013). Antimicrobial effect of different xylitol concentrations on Streptococcus mutans and Lactobacillus acidophilus count. *Journal of Restorative Dentistry*, *1*(3), 95. https://doi.org/10.4103/2321-4619.118907
19. Vogel, G. L., Schumacher, G. E., Chow, L. C., Takagi, S., & Carey, C. M. (2008). Ca pre-rinse greatly increases plaque and plaque fluid F. *Journal of Dental Research*, *87*(5), 466–469. https://doi.org/10.1177/154405910808700513
20. Chandki, R., Banthia, P., & Banthia, R. (2011). Biofilms: A microbial home. *Journal of Indian Society of Periodontology*, *15*(2), 111–114. https://doi.org/10.4103/0972-124X.84377
21. Huang, R., Li, M., & Gregory, R. L. (2011). Bacterial interactions in dental biofilm. *Virulence*, *2*(5), 435–444. https://doi.org/10.4161/viru.2.5.16140
22. Watson, P. S., Pontefract, H. A., Devine, D. A., Shore, R. C., Nattress, B. R., Kirkham, J., & Robinson, C. (2005). Penetration of Fluoride into Natural Plaque Biofilms. *Journal of Dental Research*, *84*(5), 451–455. https://doi.org/10.1177/154405910508400510
23. Kawada-Matsuo, M., Oogai, Y., & Komatsuzawa, H. (2016). Sugar Allocation to Metabolic Pathways is Tightly Regulated and Affects the Virulence of Streptococcus mutans. *Genes*, *8*(1). https://doi.org/10.3390/genes8010011

24. Hall, C. W., & Mah, T.-F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews*, *41*(3), 276–301. https://doi.org/10.1093/femsre/fux010
25. Marsh, P. D. (2003). Are dental diseases examples of ecological catastrophes? *Microbiology*, *149*(2), 279–294. https://doi.org/10.1099/mic.0.26082-0
26. Loesche, W. J. (1996). *Microbiology of Dental Decay and Periodontal Disease*. *Medical Microbiology*. University of Texas Medical Branch at Galveston. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21413316
27. Carlsson, J., & Hamilton, I. R. (1996). Differential toxic effects of lactate and acetate on the metabolism of Streptococcus mutans and Streptococcus sanguis. *Oral Microbiology and Immunology*, *11*(6), 412–419.
28. Carbon Dioxide and Carbonic Acid [Internet]. 2006 December 21st; 2013 May 17th. Available from: http://ion.chem.usu.edu/~sbialkow/Classes/3650/Carbonate/Carbonic%20Acid.ht ht
29. Antony RajSO, P., Johnssonllll, M., Levine, M. J., & NancollasllII, G. H. (1992). *THE JOURNAL OP BIOLOGICAL CHEMISTRY Salivary Statherin DEPENDENCE ON SEQUENCE, CHARGE, HYDROGEN BONDING POTENCY, AND HELICAL CONFORMATION FOR ADSORPTION TO HYDROXYAPATITE AND INHIBITION OF MINERALIZATION* mation at the N-terminal region of statherin are important for its surface interaction with HAP* (Vol. 267).
30. Dawes, C. (2008). Salivary flow patterns and the health of hard and soft oral tissues. *The Journal of the American Dental Association*, *139*, 18S–24S. https://doi.org/10.14219/jada.archive.2008.0351
31. Eslinger E, Pevear DR. *Clay Minerals for Petroleum Geologists and Engineers*. SEPM; 1988.
32. Astasov-Frauenhoffer M, Varenganayil MM, Decho AW, Waltimo T, Braissant O. Exopolysaccharides regulate calcium flow in cariogenic biofilms. *PLoS One*. 2017;12(10):e0186256.doi:10.1371/journal.pone.0186256
33. Islas-Granillo, H., Borges-Yañez, S. A., Medina-Solís, C. E., Galan-Vidal, C. A., Navarrete-Hernández, J. J., Escoffié-Ramirez, M., & Maupomé, G. (2014). Salivary Parameters (Salivary Flow, pH and Buffering Capacity) in Stimulated Saliva of Mexican Elders 60 Years Old and Older. *The West Indian Medical Journal*, *63*(7), 758–765. https://doi.org/10.7727/wimj.2014.036
34. Zhang, Z., Nadezhina, E., & Wilkinson, K. J. (2011). Quantifying diffusion in a biofilm of Streptococcus mutans. *Antimicrobial Agents and Chemotherapy*, *55*(3), 1075–1081. https://doi.org/10.1128/AAC.01329-10
35. Shimotoyodome, A., Koudate, T., Kobayashi, H., Nakamura, J., Tokimitsu, I., Hase, T., … Takaesu, Y. (2007). Reduction of Streptococcus mutans adherence and dental biofilm formation by surface treatment with phosphorylated polyethylene glycol.

Antimicrobial Agents and Chemotherapy, *51*(10), 3634–3641. https://doi.org/10.1128/AAC.00380-07
36. Vinogradov, J., Jaafar, M. Z., & Jackson, M. D. (2010). Measurement of streaming potential coupling coefficient in sandstones saturated with natural and artificial brines at high salinity. *Journal of Geophysical Research*, *115*(B12), B12204. https://doi.org/10.1029/2010JB007593
37. JOHNSTON CT, Schoonheydt R a. *Surface and Interface Chemistry of Clay Minerals*. Vol 1.; 2006. doi:10.1016/S1572-4352(05)01003-2
38. *Surface Chemistry and Ion Exchange*. (n.d.). Retrieved from http://www.geo.utexas.edu/courses/376m/LectureNotes/Surface.pdf
39. Lxiv, V., Janusz, W., & Skwarek, E. (n.d.). I E-S K Ł O D O W S K A L U B L I N-P O L O N I A The study of the properties of the hydroxyapatite/electrolyte interface. https://doi.org/10.2478/v10063-008-0003-x
40. Atwa, A.-D. A., AbuShahba, R. Y., Mostafa, M., & Hashem, M. I. (2014). Effect of honey in preventing gingivitis and dental caries in patients undergoing orthodontic treatment. *The Saudi Dental Journal*, *26*(3), 108–114. https://doi.org/10.1016/j.sdentj.2014.03.001
41. Kilian, M., Chapple, I. L. C., Hannig, M., Marsh, P. D., Meuric, V., Pedersen, A. M. L., … Zaura, E. (2016). The oral microbiome – an update for oral healthcare professionals. *British Dental Journal*, *221*(10), 657–666. https://doi.org/10.1038/sj.bdj.2016.865
42. Chow, L. C. (2001). *Octacalcium Phosplrate*. *Monogl Oral Sci. Basel. Karger* (Vol. 13).
43. *The Beneficial Effects of a Supersaturated Calcium Phosphate Rinse on the Oral Cavity in Xerostomia Patients*. (n.d.).
44. Tokura, T., Robinson, C., Watson, P., Abudiak, H., Nakano, T., Higashi, K., … Nakagaki, H. (2012). *Effect of pH on fluoride penetration into natural human plaque*. *PEDIATRIC DENTAL JOURNAL* (Vol. 22).
45. Tokura, T., Robinson, C., Watson, P., Abudiak, H., Nakano, T., Higashi, K., … Nakagaki, H. (2012). *Effect of pH on fluoride penetration into natural human plaque*. *PEDIATRIC DENTAL JOURNAL* (Vol.22).
46. Dominguez-Benetton, Xochitl. (2007). Biocomplexity and Bioelectrochemical Influence of Gasoline Pipelines Biofilms in Carbon Steel Deterioration: A Transmission Lines and Transfer Functions Approach.
47. Mei, M. L., Nudelman, F., Marzec, B., Walker, J. M., Lo, E. C. M., Walls, A. W., & Chu, C. H. (2017). Formation of Fluorohydroxyapatite with Silver Diamine Fluoride. *Journal of Dental Research*, *96*(10), 1122–1128. https://doi.org/10.1177/0022034517709738
48. Niska, K., Knap, N., Kędzia, A., Jaskiewicz, M., Kamysz, W., & Inkielewicz-Stepniak, I. (2016). Capping Agent-Dependent Toxicity and Antimicrobial Activity of Silver Nanoparticles: An In Vitro Study. Concerns about Potential Application in Dental

Practice. *International Journal of Medical Sciences*, *13*(10), 772–782. https://doi.org/10.7150/ijms.16011
49. McShan, D., Ray, P. C., & Yu, H. (2014). Molecular toxicity mechanism of nanosilver. *Journal of Food and Drug Analysis*, *22*(1), 116–127. https://doi.org/10.1016/J.JFDA.2014.01.010
50. Mariotti, A. J., & Rumpf, D. A. H. (1999). Chlorhexidine-Induced Changes to Human Gingival Fibroblast Collagen and Non-Collagen Protein Production. *Journal of Periodontology*, *70*(12), 1443–1448. https://doi.org/10.1902/jop.1999.70.12.1443
51. Pavlin, M., & Bregar, V. B. (n.d.). *STABILITY OF NANOPARTICLE SUSPENSIONS IN DIFFERENT BIOLOGICALLY RELEVANT MEDIA*. *Digest Journal of Nanomaterials and Biostructures* (Vol. 7). Retrieved from http://chalcogen.ro/1389_Bregar.pdf
52. Rajan, R., Chandran, K., Harper, S. L., Yun, S.-I., & Kalaichelvan, P. T. (2015). Plant extract synthesized silver nanoparticles: An ongoing source of novel biocompatible materials. *Industrial Crops and Products*, *70*, 356–373. https://doi.org/10.1016/j.indcrop.2015.03.015
53. Tran, Q. H., Nguyen, V. Q., & Le, A.-T. (2011). Conference Series To cite this article: L Kvitek et al. *J. Phys.: Conf. Ser*, *304*, 12029. https://doi.org/10.1088/1742-6596/304/1/012029
54. Loza K, Sengstock C, Chernousova S, Köller M, Epple M. The predominant species of ionic silver in biological media is colloidally dispersed nanoparticulate silver chloride. *RSC Adv*. 2014;4(67):35290. doi:10.1039/C4RA04764H
55. Swathy JR, Udhaya Sankar M, Chaudhary A, Aigal S, Anshup, Pradeep T. Antimicrobial silver: An unprecedented anion effect. *Sci Rep*. 2014;11(1):7161. doi:10.1038/srep07161
56. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *Int J Nanomedicine*. 2017;12:1227-1249. doi:10.2147/IJN.S121956
57. Laroo H. Colloidal Nano Silver-Its Production Method, Properties, Standards and its Bio-efficacy as an Inorganic Antibiotic. *J Phys Chem Biophys*. 2013;03(05):1-9. doi:10.4172/2161-0398.1000130
58. Niska K, Knap N, Kędzia A, Jaskiewicz M, Kamysz W, Inkielewicz-Stepniak I. Capping Agent-Dependent Toxicity and Antimicrobial Activity of Silver Nanoparticles: An In Vitro Study. Concerns about Potential Application in Dental Practice. *Int J Med Sci*. 2016;13(10):772-782. doi:10.7150/ijms.16011
59. Das RK, Brar SK, Verma M. Checking the Biocompatibility of Plant-Derived Metallic Nanoparticles: Molecular Perspectives. *Trends Biotechnol*. 2016;34(6):440-449. doi:10.1016/J.TIBTECH.2016.02.005
60. Freire PLL, Albuquerque AJR, Sampaio FC, et al. AgNPs: The New Allies Against S. Mutans Biofilm - A Pilot Clinical Trial and Microbiological Assay. *Braz Dent J*. 2017;28(4):417-422. doi:10.1590/0103-6440201600994

61. Abadi MFD, Mehrabian S, Asghari B, Namvar AE, Ezzatifar F, Lari AR. Silver nanoparticles as active ingredient used for alcohol-free mouthwash. *GMS Hyg Infect Control*. 2013;8(1):Doc05. doi:10.3205/dgkh000205
62. Thuptimdang P, Limpiyakorn T, McEvoy J, Prüß BM, Khan E. Effect of silver nanoparticles on Pseudomonas putida biofilms at different stages of maturity. *J Hazard Mater*. 2015;290:127-133. doi:10.1016/J.JHAZMAT.2015.02.073
63. Jayanthi S, Shanthi S, Vaseeharan B, et al. Growth inhibition and antibiofilm potential of Ag nanoparticles coated with lectin, an arthropod immune molecule. *J Photochem Photobiol B Biol*. 2017;170:208-216. doi:10.1016/J.JPHOTOBIOL.2017.04.011
64. Sakaue Y, Takenaka S, Ohsumi T, Domon H, Terao Y, Noiri Y. The effect of chlorhexidine on dental calculus formation: an in vitro study. doi:10.1186/s12903-018-0517-3
65. Cheng L, Zhang K, Weir MD, Melo MAS, Zhou X, Xu HHK. Nanotechnology strategies for antibacterial and remineralizing composites and adhesives to tackle dental caries. *Nanomedicine (Lond)*. 2015;10(4):627-641. doi:10.2217/nnm.14.191
66. Railsback's Some Fundamentals of Mineralogy and Geochemistry X-ray diffraction (XRD) of aragonite and calcite. 2012.
67. Mäkinen KK. Sugar alcohols, caries incidence, and remineralization of caries lesions: a literature review. *Int J Dent*. 2010;2010:981072. doi:10.1155/2010/981072
68. K. K. Mäkinen, "Can the pentitol-hexitol theory explain the clinical observations made with xylitol?" Medical Hypotheses, vol. 54, no. 4, pp. 603–613, 2000.
69. K. K. Mäkinen, "New biochemical aspects of sweeteners," The International Dental Journal, vol. 35, no. 1, pp. 23–35, 1985.
70. C. Badet, A. Furiga, and N. Thébaud, "Effect of xylitol on an in vitro model of oral biofilm," Oral Health & Preventive Dentistry, vol. 6, no. 4, pp. 337–341, 2008.
71. Bahador A, Lesan S, Kashi N. Effect of xylitol on cariogenic and beneficial oral streptococci: a randomized, double-blind crossover trial. *Iran J Microbiol*. 2012;4(2):75-81.
72. K. Kiyosawa, "Volumetric properties of polyols (ethylene glycol, glycerol, meso-erythritol, xylitol and mannitol) in relation to their membrane permeability: group additivity and estimation of the maximum radius of their molecules," Biochimica et Biophysica Acta, vol. 1064, no. 2, pp. 251–255, 1991.
73. A. Scheinin and K. K. Mäkinen, "The effect of various sugars on the formation and chemical composition of dental plaque," The International Dental Journal, vol. 21, no. 3, pp. 302–321, 1971.
74. Zabner J, Seiler MP, Launspach JL, et al. The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing. *Proc Natl Acad Sci*. 2000;97(21):11614-11619. doi:10.1073/PNAS.97.21.11614

75. Bahador A, Lesan S, Kashi N. Effect of xylitol on cariogenic and beneficial oral streptococci: a randomized, double-blind crossover trial. *Iran J Mic*
76. Badet, Cecile & Furiga, Aurelie & Thébaud, Noélie. (2008). Effect of Xylitol on an In Vitro Model of Oral Biofilm. Oral health & preventive dentistry. 6. 337-41.
77. Mirzakhani M, Amir Siavashani M, Seyyed Akhavan P, Siavashani AM, Akhavan SP. *The Effect of Different Chewing Gum on PH of Dental Plaque The Effect of Different Chewing Gum on PH of Dental Plaque Introduction*. Vol 32.; 2014.
78. T. Yanagisawa, "Ultrastructure of crystals in enamel carious lesions," Journal of Japanese Dental Association, vol. 46, pp. 1167–1176, 1994.
79. T. Yanagisawa, Y. Miake, Y. Saeki, and M. Takahashi, "Remineralization in enamel caries and restoration of carious lesions by enhanced remineralization induced by saliva and xylitol," Dentistry in Japan, vol. 39, pp. 208–215, 2003.
80. Margolis, H., & Moreno, E. (1992). Composition of Pooled Plaque Fluid from Caries-free and Caries-positive Individuals Following Composition of Pooled Plaque Fluid from Caries-free and Caries-positive Individuals Following Sucrose Exposure. *J Dent Res*, *71*(11), 1776–1784. https://doi.org/10.1177/00220345920710110301
81. Solubility of Calcium Fluoride | Image and Video Exchange Forum. (n.d.). Retrieved January 14, 2019, from https://community.asdlib.org/imageandvideoexchangeforum/2013/07/24/solubility-of-calcium-fluoride/
82. Pan, H.-B., & Darvell, B. W. (2007). Solubility of calcium fluoride and fluorapatite by solid titration. *Archives of Oral Biology*, *52*(9), 861–868. https://doi.org/10.1016/J.ARCHORALBIO.2007.03.002
83. Sheikh, M. S., Santa Ana, C. A., Nicar, M. J., Schiller, L. R., & Fordtran, J. S. (1987). Gastrointestinal Absorption of Calcium from Milk and Calcium Salts. *New England Journal of Medicine*, *317*(9), 532–536. https://doi.org/10.1056/NEJM198708273170903
84. Chapter 16 Aqueous Equilibria - ppt download. (n.d.). Retrieved January 14, 2019, from https://slideplayer.com/slide/6189864/
85. Dashper, S., & Reynolds, E. (2000). Effects of Organic Acid Anions on Growth, Glycolysis, and Intracellular pH of Oral Streptococci. *J Dent Res*, *79*(1), 90–96. https://doi.org/10.1177/00220345000790011601
86. Marsh, P., & Martin, M. (Michael V. . (n.d.). *Marsh and Martin's oral microbiology*.
87. Lemos, J. A., Quivey, R. G., Koo, H., & Correspondence, J. A. (n.d.). Streptococcus mutans: a new Gram-positive paradigm? https://doi.org/10.1099/mic.0.066134-0
89. Leroy, Philippe & Li, Shuai & Jougnot, Damien & Revil, André & Wu, Yuxin. (2017). Modeling the evolution of complex conductivity during calcite precipitation on glass beads. Geophysical Journal International. 209.
90. Hyvonen L, Koivistoinen P, Voirol F. Food technological evaluation of xylitol. Adv Food Res.1982;28:373-403.

91. Callister C, Callister M, Nolan M, Nolan R. Anti-caries Potential of Silver Nanoparticles via Modulation of Free Calcium Activity within the Plaque Fluid of the Oral Biofilm: A Pilot Study. 2019. doi:10.4172/2161-1122.1000529
92. Masadeh, M. M., Gharaibeh, S. F., Alzoubi, K. H., Al-Azzam, S. I., & Obeidat, W. M. (2013). Antimicrobial Activity of Common Mouthwash Solutions on Multidrug-Resistance Bacterial Biofilms. *Journal of Clinical Medicine Research*, *5*(5), 389–394. https://doi.org/10.4021/jocmr1535w
93. Salas-López, E. K., Pierdant-Pérez, M., Hernández-Sierra, J. F., Ruíz, F., Mandeville, P., & Pozos-Guillén, A. J. (2017). Effect of Silver Nanoparticle-Added Pit and Fissure Sealant in the Prevention of Dental Caries in Children. *Journal of Clinical Pediatric Dentistry*, *41*(1), 48–52. https://doi.org/10.17796/1053-4628-41.1.48
94. Scarpelli, B. B., Punhagui, M. F., Hoeppner, M. G., Couto De Almeida, R. S., Juliani, F. A., Danil Guiraldo, R., & Berger, S. B. (2017). Antimicrobial Activity of a Cariostatic Agent with Silver Nanoparticles. *Brazilian Dental Journal*, *28*(6), 738–743. https://doi.org/10.1590/0103-6440201701365