Amorphous Calcium Phosphates for Tooth Mineralization

Abstract: The destruction of tooth structure through caries and erosive processes is due to two types of acidic challenges that affect the tooth in different ways. Acidic attack by cariogenic bacteria initially produces subsurface lesions that weaken the enamel and, if left unchecked, can progress through the enamel and dentin and eventually into the pulpal cavity. Erosive attack by acidic foods and beverages removes mineral from the surface of enamel and initially causes dulling and loss of tooth luster; if left unchecked, it can progress to a more severe loss of enamel thickness and contour. This article focuses on the potential means of improving the cosmetic appearance of teeth by depositing mineral into surface defects. Several approaches use the unique properties of amorphous calcium phosphate (ACP) compounds, which have the highest rates of formation and dissolution among all the calcium phosphates. ACP has been shown to rapidly hydrolyze to form apatite, similar to carbonated apatite, the tooth mineral. Products containing ACP or ingredients that form ACP can include toothpastes, mouth rinses, artificial saliva, chewing gums, topically applied coatings, and other vehicles for topical use. When applied, they readily precipitate ACPs on and into tooth-surface defects. These products hopefully will provide users with new tools to restore and enhance the smoothness and luster of their teeth.

Tooth enamel is composed almost entirely (97% by weight) of a calcium phosphate mineral in the form of carbonated hydroxyapatite (HAP). HAP is essentially insoluble at neutral and alkaline pH. However, it becomes increasingly soluble as the pH is lowered. Two sources of acidic challenges can affect the integrity of tooth enamel but in different ways.

On one hand, acids—generated by acidogenic plaque bacteria—cause a loss of mineral from beneath the enamel surface, the softening of the enamel, and the formation of subsurface carious lesions. Continued exposure to plaque acids results in more mineral loss and in the development of full-blown caries. If allowed to progress, this can extend through the dentin into the pulpal cavity. Dental caries continues to be the most prevalent disease affecting humans. The World Oral Health Report 2003, issued by the World Health Organization, estimates that 5 billion people worldwide have experienced dental caries. In recent years, significant reductions in caries have been observed in some segments of the population. This reduction in caries is attributed to the widespread use of therapeutic fluorides.

On the other hand, erosion of tooth enamel can be caused by such things as acid reflux, acidic foods, and acidic beverages, which can remove the surface mineral. The presence of these surface defects is believed to cause a dull appearance and loss of luster of the tooth. An ideal way to repair such defects, and perhaps other crevices, would be to put calcium and phosphate back onto the surface of the tooth. Such a process also may be able to fill or smooth other enamel-surface defects, such as scratches, to restore tooth luster.

Factors affecting the ability of fluoride to prevent caries and remineralize early carious lesions, and the effect of calcium and phosphate ions on caries repair and prevention, have been extensively discussed in the literature. This article will discuss the potential cosmetic benefits available from calcium and phosphate systems through the deposition of mineral onto surface defects in tooth enamel.

Learning Objectives

After reading this article, the reader should be able to:

- discuss two kinds of acidic challenges on the integrity of the tooth.
- discuss how and why products containing calcium and phosphate may help enhance tooth remineralization.
- describe two methods for delivery of amorphous calcium phosphate to the oral cavity.
- explain the chemistry of amorphous calcium phosphate as related to tooth remineralization.
- describe possible roles of carbonate in remineralization.
The Use of ACPs to Fill Surface-Enamel Defects

Any method to enhance the surface deposition of mineral should be capable of being applied quickly and conveniently. The ingredients should contain all of the essential ions, such as calcium and phosphate, necessary to fill surface defects with mineral similar to that present in tooth enamel. Several new approaches using the unique properties of amorphous calcium phosphate (ACP) compounds have the potential to achieve this effect. The vehicles for such surface deposition include mouth rinses, dentifrices, prophylactic pastes, coatings, and dental flosses.

The method for applying ACPs is limited to two classes of systems. In the first class, preformed ACPs can be applied as a single-phase system. Examples of currently available single-phase systems are ACP-containing chewing gum and dental composites. These products can be formulated without water or stabilized to keep the ACP from reacting and forming apatite before use.

The second class comprises two-phase packaging systems that can be used to separate the calcium component from the phosphate and, if present, the fluoride component until they both are applied. The physical separation of the reactive components prevents the formation of calcium phosphate until the product is dispensed and used. An example of a two-phase system is a toothpaste in which the calcium component is separated from the phosphate and fluoride-containing components; these two components are mixed during dispensing and brushing. Another example of a two-phase system is a two-step application of a calcium solution and a phosphate solution in high concentrations. The calcium solution is applied to the tooth surface first, followed by the application of the phosphate solution; ACP forms immediately and converts within a short period of time to apatite in vivo.

Bicarbonate can be used to regulate the formation rate of ACP, and casein phosphopeptide can be used to localize ACP at the tooth surface. Both help to maintain a state of supersaturation of calcium and phosphate ions for a prolonged period of time to facilitate mineral deposition.

The Chemistry of Calcium Phosphates

The dissolution and formation of tooth mineral in the mouth are primarily controlled by the acidity (pH) of the oral environment. The fluids in contact with the teeth become undersaturated with HAP as the pH decreases and supersaturated as the pH increases. The solubility of HAP and ACP as a function of pH is illustrated in Figure 1. Under physiological conditions, apatite is the most stable of the calcium phosphate compounds and ACP is the least stable. The left curve represents the dissolution behavior of apatite as a function of pH, while the right curve represents the dissolution of ACP. Areas to the right of the two curves represent regions where ion concentrations would be high enough to precipitate the corresponding mineral phase. Areas to the left represent where the mineral phase would be actively dissolved into ionic calcium and phosphates. Point B designates where apatite would be the stable mineral phase at normal oral pH. The direct formation of apatite above point B, however, is kinetically a very slow process. Adding ACP-forming components to the system can rapidly shift conditions to point C, where ACP rapidly precipitates. The further conversion of ACP to apatite will drive the system to point B much faster than the natural precipitation process.

Under oral conditions, ACPs have the fastest rates of formation and dissolution, and—with the exception of the highly acidic monocalcium phosphate—the highest solubility among all the calcium phosphates. ACPs lack the long-range, periodic atomic order of crystalline materials.
Thus, in the presence of carbon dioxide, the solution can contain high concentrations of calcium and phosphate because of the lower pH. Conversely, as carbon dioxide evaporates from the solution, the pH increases and the calcium-phosphate solutions can precipitate. This is illustrated in Figure 1. Point A indicates a stable calcium-phosphate solution of high concentration in equilibrium with HAP in a carbon-dioxide–pressurized aerosol at a pH of 4.5 (caused by the presence of carboxylic acid and formed by dissolved carbon dioxide). When this solution is applied to the tooth, the carbon dioxide evaporates and the pH increases to point C according to reaction 2. The solution is above the ACP solubility line and is supersaturated with ACP, causing the precipitation of ACP according to reaction 1 (indicated by the vertical arrow moving from point C to the ACP solubility line). The ACP then converts to HAP, indicated by the second vertical arrow moving from the ACP solubility line to point B.

One method to take advantage of this property is to package calcium-phosphate solutions as carbon-dioxide–pressurized aerosols. This method contains high concentrations of calcium and phosphate as a stable solution. Carbonated solutions with high concentrations of calcium and phosphate also can be made by mixing two stable solutions: one containing a high concentration of acidic calcium, and the other containing a high concentration of basic phosphate and carbonate. When these solutions are combined and applied to the teeth, the pH of the applied solution rapidly increases because of the evaporation of carbon dioxide, and the calcium and phosphate ions in the solution precipitate as ACPs. The precipitated ACPs then convert to form apatite mineral similar to that present in the teeth.

Figure 3 shows the solution pH as a function of time during precipitation of a carbonated calcium-phosphate solution (CCPS) containing 17.5
mmol/L of calcium, 10 mmol/L of phosphate, and 25.3 mmol/L of carbon dioxide at a pH of 5.94. In this figure, curve 2 shows the change in pH of a control solution caused by the carbon-dioxide evaporation of reaction 2. The control solution is the same as the CCPs except that calcium ions have been replaced by potassium ions, resulting in a stable solution where no precipitation can occur. Curve 1 + 2 represents the combination of reactions 2 and 1, the carbon-dioxide evaporation and the ACP formation. The rapid drop in pH beginning at about 8 minutes is caused by the consumption of phosphate as it combines with calcium and precipitates as ACP. In Figure 4, the difference in pH change between curve 1 + 2 and curve 1 represents reaction 1, the precipitation kinetics of ACP. The induction time and precipitation rate can be controlled by the amount of carbonate, fluoride, magnesium, and strontium in the solution. In this way, CCPs overcome the instability of solutions containing high concentrations of calcium and phosphate.

Conversion of ACPs to Apatite

The conversion of ACPs to crystalline calcium phosphates is an important part of the proposed surface-enhancement process. It is believed that after deposition onto the tooth surface, precipitated ACPs convert in situ into apatite, filling microporosities and microscopic surface defects.

The conversion of ACPs is complex and depends on the composition of the ACPs and the solution’s conditions.13 The hydrolysis of pure ACP to apatite at the physiological pH of 7.4 and 37°C is fast, occurring within 6 minutes, and goes through an intermediate octacalcium phosphate-like pathway.15 The conversion process is affected by inorganic ions such as fluoride, carbonate, magnesium, and strontium, as well as organic compounds such as proteins and biomolecular species present in the oral environment. ACP-forming solutions may contain these ions, which can regulate the formation of ACP, incorporate into the ACP, and control the conversion to apatite.

Toothpaste Containing Calcium Phosphate

The precipitation of calcium phosphate from toothpaste containing fluoride, calcium, and phosphate was studied to elucidate the kinetics and mechanism of precipitation.6 The two individual components of the toothpaste (one containing calcium and the other containing phosphate) were mixed separately with saliva (one part toothpaste with one part saliva by mass). The calcium–saliva component was then added to the phosphate–saliva component while stirring. The pH and fluoride concentrations were followed over time using electrodes (Figure 5). The pH of the mixture decreased within 1 minute, indicating the rapid precipitation of calcium phosphate. The fluoride concentration also decreased after mixing, indicating the incorporation of fluoride into the precipitates. Because the precipitation and the incorporation of fluoride into the precipitates occurred within minutes, this emphasizes the importance of applying the mixed components to the tooth as soon after mixing as possible.

Discussion

Incorporating new mineral on the surface of enamel involves the diffusion of calcium and phosphate ions into the irregularities in the enamel, followed by the precipitation of calcium phosphate and the formation of apatite.16 The approach of using ACP increases both the diffusion and the precipitation rates because of the high localized concentrations of calcium and phosphate. The calcium and phosphate concentrations can be in the range of 18 mmol/L to 1.5 mmol/L. Carbonate can be used to regulate the system pH, ensure stability, and control the precipitation rate. The formation of ACP is about 20,000 times faster than that of HAP at equivalent saturation levels. The ability to deliver ACP materials in the form of topical gels, toothpastes, mouth rinses, chewing gums, prophylaxis pastes, varnishes, dental floss, and perhaps even lozenges may provide a broad range of products that can be
used to enhance the surface of the teeth. It would seem possible that someday clinicians will prescribe not only fluoride therapies that will more effectively prevent the formation of new lesions but also cosmetic treatments to enhance the esthetics of tooth enamel.

**Disclosure**

Certain commercial materials and equipment are identified in this article to specify the experimental procedure. In no instance does such identification imply recognition or endorsement by the National Institute of Standards and Technology or by the American Dental Association Foundation, or that the material or equipment identified is necessarily the best available for the purpose. Official contribution of the National Institute of Standards and Technology; not subject to copyright in the United States.

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**References**