EFFECT OF CASEIN PHOSPHOPEPTIDES-AMORPHOUS CALCIUM PHOSPHATE ON REMINERLIZATION OF DEMINERALIZED ENAMEL SURFACES .

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This work is dedicated to…

The loving memory of my father…

My dear mother…

My beloved husband…

My precious sons Muhammed& Ahmed…

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Abstract

Aim: This study aimed to determine in vitro the effect of Tooth Mousse [a commercial paste based on Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) complex] on the remineralization of demineralized enamel surfaces.

Materials and methods: Thirty human enamel specimens were divided into two equal groups (n=15).Group A was topically treated with CPP-ACP paste (Tooth Mousse) while Group B was used as a control group with no agent applied. Specimens of the two groups were subjected to a 10 days pH cycling model. The effect of CPP-ACP paste in preventing demineralization and promoting remineralization was evaluated by recording the changes in the microhardness values of the specimen's surface enamel.

Results: The results of the present study showed that both the Tooth Mousse and the control group showed reduction in the microhardness mean valuesof the enamel surfaces after ten days pH cycling. However specimens of group A (Tooth Mousse) recorded a lower reduction in microhardness mean value than obtained with the control group (Group B).

Conclusions: The present study showed that the triple daily application of the CPP-ACP paste as a topical Coating for ten days had a significant effect in reducing demineralization of the enamel surface.

Recommendations: It is recommended that CPP-ACP should be used as a self-applied topical coating after teeth brushing by children who have a high caries risk.

Keywords: Casein, Remineralization, pH cycling, Microhardness.

INTRODUCTION

The conventional treatment concept for all caries-attacked teeth included the removal of the affected tissues and the replacement with a restorative material **(Burke, 2003).**

Scientific advances in restorative materials and techniques as well as in understanding the pathogenesis and prevention of caries, have led to more efficient oral health management **(Oschiro et al; 2007).**

Modern dentistry has evolved to a minimally invasive approach, in which caries is managed as an infectious disease, deferring operative intervention as long as possible. The focus is on maximum conservation of demineralized, noncavitated enamel and dentin.

Miles Markley, one of several great leaders in preventive dentistry, summarized in this statement the central concept in the modern approach to the dentist's role in the treatment of dental caries: that the loss of even a part of a human tooth should be considered "a serious injury," and that dentistry's goal should be to preserve healthy, natural tooth structure. His words are perhaps even more relevant today than when he wrote them half a century ago, now that we have the scientific understanding and the means to realize his vision **(Murdoch-Kinch and Mclean, 2003).**

In the noncavitated lesion, to take advantage of the tooth's capacity to remineralize, one must first alter the oral environment, to tip the balance in favor of remineralization and away from demineralization **(Tyas et al; 2000).**

Early studies demonstrated that dairy products have anti-caries activity. The dairy products that were determined to be responsible were milk, casein, caseinates and cheese **(Shaw, 1950).**

When used as an ingredient in toothpaste, acid casein was able to reduce caries. Unfortunately, the levels of casein needed made the toothpaste unpalatable **(Bavetta and McClure, 1957).**

Therefore, the early studies demonstrated that, while casein was an effective anticariogenic substance, the levels needed for activity precluded its use as a food or toothpaste additive due to its adverse effects on taste **(Reynolds, 1998).**

Fortunately, it was discovered that trypsin digestion of caseinate did not destroy the protein's ability to prevent enamel demineralization **(Reynolds, 1987).**

In a study by Reynolds, it was found that the tryptic peptides of casein were incorporated into the plaque of humans and that they were associated with a substantial increase in the content of calcium and phosphate in the plaque. It was concluded that the tryptic peptides responsible for the anticariogenic activity were the calcium-phosphate-stabilizing phosphopeptides (CPP) because the non-phosphorylated tryptic peptides, which do not stabilize calcium and phosphate, did not affect caries activity **(Reynolds et al; 1995).**

The CPP from the tryptic digestion is not associated with unpalatability and have a potential anticariogenic effect at least ten times greater on a weight basis **(Reynolds, 1998)**.

Reynolds in an in vitro study, found that CPP can stabilize over one hundred times more calcium phosphate than is normally possible in aqueous solution before spontaneous precipitation **(Reynolds, 1987).**

This discovery focused studies on casein peptides **(Aimutis, 2004).**

It has been proposed that the mechanism of anticariogenicity for casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) is that it substantially increases the level of calcium phosphate in plaque **(Reynolds, 1987).**

The increased calcium phosphate in the plaque buffers free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization **(Reynolds, 1998).**

In recent years CPP-ACP nanocomplexes have been demonstrated to have anticariogenic properties in both laboratory animal and human in situ experiments **(Kumar et al; 2008).**

The CPP-ACP unlike fluoride can be added to sugar-containing foods since it is a natural derivative of milk and therefore have commercial potential as an additive to foods as well as to toothpastes and mouthwashes for the control of dental caries **(Reynolds, 1998).**

High solubility, the ability to rapidly hydrolyze to form apatite under oral conditions and the fact that CPP-ACP does not have adverse effects on taste make CPP-ACP a prime candidate for remineralization therapy **(Tung, 1999).**

CPP-ACP is currently marketed under the trade name Recaldent. It was discovered and patented by the school of Dental Science at the University of Melbourne in Australia. It contains xylitol and CPP-ACP, available as sticks and tablets. Because Recaldent is milk derived, it isn't recommended for people with milk allergies, but Recaldent will not affect people with lactose intolerance. Studies have shown that Recaldent will improve the effect of fluoride, since Recaldent provides an excellent source of soluble calcium and phosphates and fluoride requires calcium and phosphate to work, so rather being better than fluoride, or replacing fluoride, Recaldent and fluoride work together to rebuild and strengthen tooth enamel **(Moezizadeh and Moayedi, 2009).**

CPP-ACP is also found in PROSPEC MI paste (GC America, Alsip, IL) and Trident chewing gum.

The commercial paste based on Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) complex that will be used in this study is Tooth Mousse.

REVIEW OF LITERATURE

Enamel is the hardest tissue in the animal body. It is a high minerlalized tissue composed mainly of calcium phosphate (hydroxyapatite) and a little proportion of calcium carbonate. Certain other elements are present in trace amounts (**Moheb, 2003).**

Enamel forms a protective covering of variable thickness covering the entire surface of tooth crown provide the shape and contour of the crown. As it covers that part of the tooth exposed to the oral environment it is the primary site of defense against dental caries therefore of special interest in dental research **(Sharawy and Yeager, 1991).**

Although macroscopically dental enamel may seem very solid, at high magnification it is relatively porous.

The mineral phase is about 96% of the total weight, 85% by volume, the remaining 15% by volume 11% water and 4% protein and lipid (fatty material) present in approximately equal amounts, constitutes the diffusion channels between crystals and prisms allowing acid, minerals, and fluoride to pass in or out of the enamel during demineralization or remineralization **(Axelsson, 2004).**

The hardness of enamel reflects its degree of mineralization, it is greatest at the surface, decreases close to the surface and remains relatively constant until just before the amelodentinal junction where it drops considerably. It is also higher at the incisal edge or cusp tip, decreases towards the cervical margin **(Scott and Symon, 1974).**

Enamel hardness was reported to be 270-380 knoop hardness number (KHN). Primary human incisors enamel showed microhardness of 308.6 ± 28.0 von hardness number (VHN) (**EL-Motayam and EL Motyam, 1985).**

The surface enamel has received particular attention, because of its significant resistance to the initiation of dental caries, and for adhesion of polymeric restorative materials **(Palmara et al; 1980).**

Both physically and chemically, surface enamel differs markedly from subsurface enamel. Surface enamel is harder, less porous, less soluble and more radiopaque than subsurface enamel. It is richer in trace elements especially fluoride, zinc, lead, chlorides as well as low carbonate content, low water content and higher degree of mineralization. These properties may contribute to the ability to resist acid dissolution and caries initiation as well as to the character of the early enamel lesion **(Anderson and Elliot, 1992).**

The physicochemical integrity of dental enamel in the oral environment is entirely dependent on the composition and chemical behavior of the surrounding fluids as saliva and plaque fluids.The main factors governing the stability of enamel apatite are PH and the free active concentrations of calcium, phosphate and fluoride in solution **(Axelsson, 2004).**

Studies with radioactive isotopes confirm the belief that little replacement of the enamel is accomplished via normal metabolic pathways, progressively through the pulp and dentin. The same techniques reveal that the mineral components of the enamel surface are being constantly replaced or added by salivary ions.

These replacements and / or additions to the surface enamel can come from three major sources, the normal oral environment including saliva, materials that are introduced into the mouth ordinarily in eating and drinking and materials that are introduced for therapeutic purposes as for example, dental restorations and soluble therapeutic agents **(Saunders, 2004) .**

Decalcification can be defined as the reduction of mineral elements, specifically calcium and phosphate in tooth structure that occurs during a period of decreased oral pH. Decalcification occurs in relation to the retention of bacterial plaque on the enamel surface for extended periods of time. The bacterial plaque produces organic acids which can cause dissolution of the mineral content of the enamel **(Mitchell, 1992, Gorelick et al; 1982 and Vivaldi-Rodrigues et al; 2006).**

As the dissolution continues, the decalcification becomes clinically evident as a white spot lesion, so called because of its chalky appearance.

The white spot lesion is the beginning of a carious lesion. Demineralization is an episodic process with phases of demineralization and remineralization. These phases cycle back and forth as intraoral conditions change. Lesions develop if the balance of the equilibrium shifts towards demineralization for extended periods of time (**O'Reilly and Featherstone, 1987, Sturdevant, 1995 and Zaura and Ten Cate, 2004).**

Factors that affect the phase of mineralization include the oral pH, the contents and concentration of saliva, the oral bacteria present, frequency of sucrose ingestion, presence of fluoride or other chemicals and the duration of time all of these factors are present **(Van Palenstein Helderman et al, 1996).**

Both of the de- and remineralization phases can occur at the same time in different parts of the mouth.

During periods of high bacterial metabolic activity and low pH conditions, demineralization and remineralization can occur at various depths in the early carious lesion **(Sturdevant, 1995).**

Streptococcus mutans is the bacteria most commonly associated with the initiation of caries due to its ability to rapidly produce acid from fermentable carbohydrates. Caries rates are directly affected by the increase in the population of S. mutans. Lactobacillus is involved in the further development of the lesion **(Loesche, 1986).**

There are three factors that necessary to cause caries: acid-producing bacteria, time and a source of nutrition for the bacteria as sucrose.

The relationship between dietary sucrose and caries shows that frequent consumption of sucrose is directly associated with caries. Acid produced by the fermentation of dietary sugars results in a pH drop in the plaque. This drop in pH initiates the decalcification of the enamel. The intake of dietary sucrose has two effects on plaque, first, the frequent ingestion of foods containing sucrose provides a strong potential for colonization of S. mutans, enhancing the caries potential of plaque and second, mature plaque exposed frequently to sucrose rapidly metabolizes it into organic acids. This results in a profound and prolonged drop in plaque pH. Bacterial plaque is a soft, sticky material that is composed of bacteria and bacterial byproducts. Plaque accumulation is not a random process but rather a highly organized and ordered sequence **(Keyes and Fitzgerald, 1961 and Sturtevant, 1995).**

The pellicle, which is formed from proteins and other salivary components, reforms almost immediately after a cleaned tooth surface is exposed to saliva. Adherent bacteria attach to receptors within the acquired pellicle on the tooth surface. Once there, they secrete a sticky matrix that allows them to cohere to each other. Adhesion and cohesion allow the bacteria to colonize the enamel surface within minutes of the formation of the pellicle **(Loesche, 1986, Gorelick et al; 1982 and Sturdevant, 1995).**

The colony, in the presence of a fermentable carbohydrate, will progress into a thin plaque mass in approximately twenty four hours. One colonizer of the plaque mass, S. mutans, metabolizes sucrose into lactic acid. As the level of lactic acid increases, the oral pH begins to decrease. If the plaque mass is not removed, the pH can decrease below the critical level of 5.5. When pH reaches 5.5, conditions favor the dissolution, or demineralization, of the enamel. The pH of plaque can drop below 5.5 in 4-8 minutes following a sugar challenge **(Loesche, 1986 and Zaura and Ten Cate, 2004).**

In a study by Thylstrup using scanning electron microscope, some enamel crystal dissolution was found after two days of plaque accumulation. Within eight to fourteen days following the elimination of plaque removal, all of the participants in the study demonstrated enamel decalcification despite the presence of a fluoridated community water supply **(Thylstrup et al; 1990).**

Several studies have described the characteristics of the early enamel carious lesion **(Gorelick et al; 1982, Ogaard et al; 1988, Arends and Christoffersen, 1986).**

Evidence from these studies supports the concept that the early lesion consists of an intact surface with a demineralized sub-surface area **(Haikel et al; 1983).**

The mineral-rich enamel surface of the early lesion is slightly softer and more porous than the surrounding enamel. In contrast, the sub-surface zone has a reduced mineral content (10-70 volume %) **(Arends and Christoffersen, 1986).**

It is well-recognized that it is possible to arrest and even reverse the mineral loss associated with caries at an early stage, before cavitation takes place **(Murdoch-Kinch and Mclean, 2003).**