

Effect of CPP-ACP paste on tooth mineralization: an FE-SEM study

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Abstract: Milk and milk products, such as cheese, have been shown to exhibit anticariogenic properties in human and animal models. CPP-ACP shows an anti-caries effect by suppressing demineralization, enhancing remineralization, or possibly a combination of both. The purpose of this study was to evaluate the effect of CPP-ACP paste on demineralization by observing the treated tooth surface using an FE-SEM. The specimens were prepared by cutting enamel and dentin of bovine teeth into blocks. A few specimens were stored in 0.1 M lactic acid buffer solution for 10 min and then in artificial saliva (negative control). The remaining specimens were stored in a 10 times-diluted solution of CPP-ACP paste or a placebo paste containing no CPP-ACP for 10 min, followed by 10 min immersion in a demineralizing solution (pH = 4.75, Ca) twice a day before storage in artificial saliva. After treatment of the specimens for 3, 7, 21 and 28 days, they were fixed in 2.5% glutaraldehyde in cacodylate buffer solution, dehydrated in ascending grades of *tert*-butyl alcohol, and then transferred to a critical-point dryer. The surfaces were coated with a thin film of Au in a vacuum evaporator, and were observed under field emission-scanning electron microscopy (FE-SEM). The SEM observations revealed different morphological features

brought about by the various storage conditions. Demineralization of the enamel and dentin surfaces was more pronounced with the longer test period in the control and negative control specimens. On the other hand, enamel and dentin specimens treated with CPP-ACP paste revealed slight changes in their morphological features. From the morphological observations of the enamel and dentin surfaces, it could be considered that the CPP-ACP paste might prevent demineralization of the tooth structure. (J. Oral Sci. 49, 115-120, 2007)

Keywords: CPP-ACP; demineralization; enamel; dentin.

Introduction

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 (1). A remarkable decline in the prevalence of caries has been reported (2), which can be attributed to the increase in scientific knowledge about the etiology of this disease coupled with the utilization of a wide range of adhesive materials. According to the principles of minimal intervention dentistry, optimal invasive strategies are preferred for the treatment of decayed lesions (3). Efforts have focused on reducing the risk of caries in patients, and have highlighted the importance of a 'partnership' approach between patients and dentists in order to ensure ultimate success in the control of caries.

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It has been reported that the consumption of soft drinks might lead to pathological tooth wear, which is termed dental erosion (4). The development of erosion involves a chemical process in which the inorganic phase of the tooth is demineralised, thereby reducing the hardness of the tooth substrates (5). Subsequent abrasive challenges through brushing increase the loss of the tooth substrates (6). Paradoxically, lifelong exposure to the oral environment is thought by many to be largely responsible for changes in the pattern and progression of tooth wear (7). Previously, erosion lesions in the cervical area were reported mostly in elderly people, whose teeth had become non-functional over a number of years. However, dietary changes and inadequate oral hygiene have led to erosion becoming more frequent among young people in the UK (8). This phenomenon is largely due to physical and chemical factors that act in the cervical area of the tooth, resulting in enamel loss, dentin exposure and dentin erosion. Tooth wear is almost a universal condition (9). Even in populations with a decreased prevalence of caries, it seems that the relative importance of tooth wear has been increasing. Any irritation that exceeds the dentin tolerance level creates erosion (10). The dentin surface is more susceptible to demineralization than the enamel, because of its higher critical pH (11). Thus, there is an urgent need for measures to prevent dental erosion.

The anticariogenic properties of milk and milk products, such as cheese, have been studied previously in animal models (12,13). This activity has been attributed to the direct chemical effects of phosphoprotein casein and calcium phosphate components in cheese (14,15). It has been suggested that casein phosphopeptides (CPPs) have the ability to stabilize calcium phosphate in solution by binding amorphous calcium phosphate (ACP) with their multiple phosphoserine residues, thereby allowing the formation of small CPP-ACP clusters (15). CPP-ACP might prevent tooth erosion by suppressing demineralization, enhancing remineralization or a combination of these two processes.

In a previous study conducted to detect changes in the re- and demineralization status of tooth structure (16), we concluded that the inorganic components contained in high concentrations in CPP-ACP enhanced re-mineralization. This study employed an ultrasound device to monitor the sound velocities through tooth substrates as indicators of mineral content, however, the morphological changes need to be examined in detail. Therefore, the purpose of the current study was to evaluate the effect of CPP-ACP paste on the demineralization process in enamel and dentin, by microscopic observation using FE-SEM.

Materials and Methods

Preparation of the specimens

Freshly extracted bovine incisors, without cracks or erosion, were cleaned and stored in physiological saline for up to 2 weeks. Enamel or dentin was sliced into 1-mm thick sections from different directions with a low-speed diamond saw (Buehler Ltd., Lake Bluff, IL, USA). Each slab was carefully shaped into a rectangular form ($4 \times 4 \times 1$ mm) using a super-fine diamond finishing point (ISO #021, Shofu Inc., Kyoto, Japan). Each specimen surface was ground successively on wet silicon carbide (SiC) paper with a grit size ranging from #600 to #2,000. The thickness and size of the specimens were measured using a dial gauge micrometer (CPM15-25DM, Mitutoyo, Tokyo, Japan).

One group of specimens was treated with 0.1 M lactic-acid buffer solution (pH 4.75, 0.75 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.45 mM KH_2PO_4) for 10 min before being stored in artificial saliva (pH 7.0; Table 1, group DE). Two additional groups of specimens were stored in a 10-fold diluted solution of CPP-ACP paste (Tooth Mousse, GC Corp. Tokyo, Japan) or placebo paste without CPP-ACP (Table 1, groups TM and PP, respectively) for 10 min prior to storage in a demineralising solution. The specimens were stored in 0.1 M lactic-acid buffer solution and artificial saliva at 37°C. They were exposed to the demineralising solution twice a day throughout the 4-week test period. An additional group (Control group) was not treated, but was simply stored in artificial saliva (Table 1) for the same period of time. Observations were carried out daily for the first

Table 1 Composition of the topical paste and the artificial saliva

Topical paste	
Code	Paste
TM	1% (w/v) CPP-ACP-containing paste
PP	CPP-ACP-free placebo paste

Artificial saliva	
Component	wt %
NaCl	0.08
KCl	0.12
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.01
K_2HPO_4	0.03
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.01
CMC-Na	0.10
IEW	99.6

CMC-Na: sodium carboxymethyl cellulose

IEW: ion-exchanged water

7 days and subsequently at 14, 21 and 28 days after the start of the test period.

FE-SEM observation

For the ultrastructural examination of the tooth surfaces by FE-SEM, specimens that were stored under each condition were dehydrated with ascending concentrations of *tert*-butanol (50% for 20 min, 75% for 20 min, 95% for 20 min and 100% for 2 h) and then transferred to a critical-point dryer for 30 min. The surfaces were coated with a thin film of Au in a vacuum evaporator (Quick Coater Type SC-701; Sanyu Denshi Inc., Tokyo, Japan). The specimens were then observed under FE-SEM (ERA 8800FE, Elionix Ltd., Tokyo, Japan).

Results

Representative SEM images of the enamel and dentin specimens are shown in Figs. 1, 2.

SEM observations revealed different morphological features brought about by various storage conditions.

Demineralization of the enamel surfaces was more pronounced with the longer test period in the demineralization and negative control specimens (DE and PP groups). On the other hand, enamel specimens treated with CPP-ACP paste (TM group) revealed slight changes in their morphological features.

The demineralization of the dentin surfaces was more pronounced over the test period in the demineralization (DE) and negative control (PP) groups. By contrast, the dentin specimens in the TM group showed relatively minor changes in their morphological features.

Discussion

Although human teeth are generally used for *in vitro* studies, bovine teeth were used in the current work as they are easy to obtain in large quantities and in good condition, and show less variability in composition than human teeth (17). Moreover, bovine teeth have larger flat surfaces that have not been subjected to previous caries challenges before testing. The mineral distribution in carious lesions

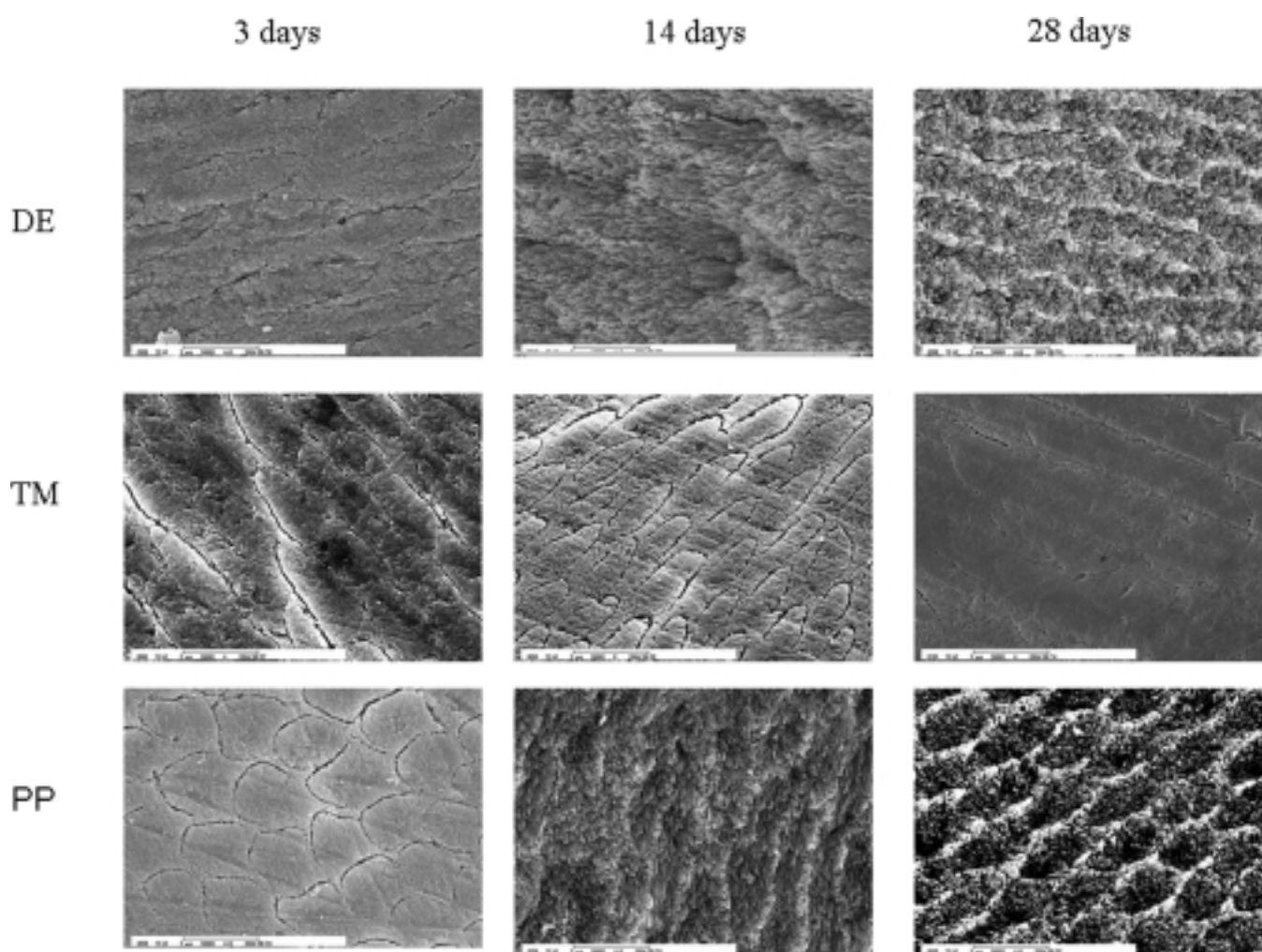


Fig. 1 FE-SEM observation of enamel surfaces (original magnification: $\times 5,000$).

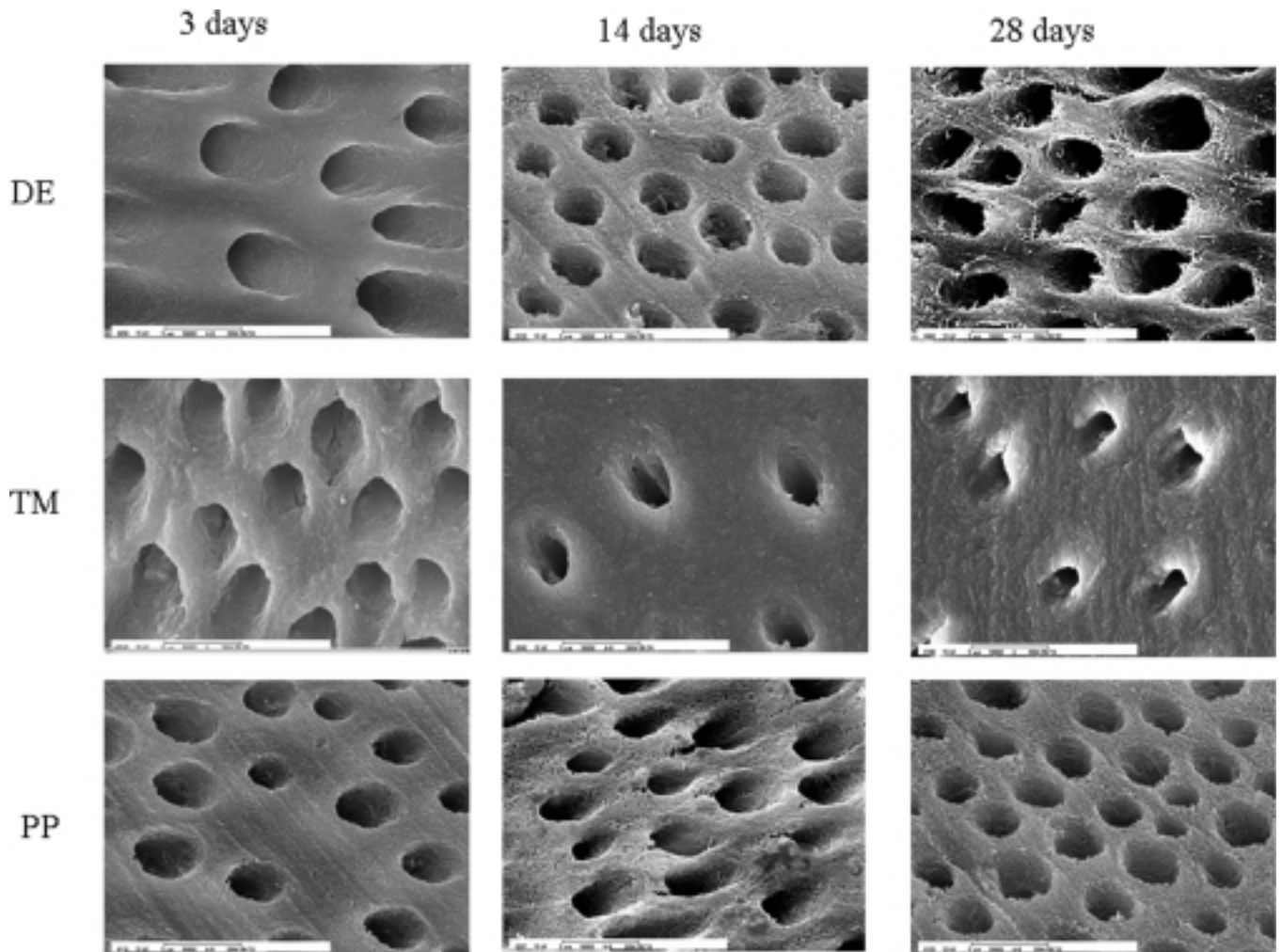


Fig. 2 FE-SEM observation of dentin surfaces (original magnification: $\times 5,000$).

in bovine teeth is reportedly similar to that found in human teeth, and the structural changes in human and bovine teeth are comparable (17). The experimental parameters that must be considered in this type of experiment are the study design, the type of substrate and the method that is used to assess the mineral status. Each parameter must be carefully considered in relation to the objectives of the research (18). Care should be taken when drawing conclusions from the data that are generated, as many factors can affect the results that are obtained *in vitro*.

This study revealed significant alterations in surface morphologies of both enamel and dentin after 21 days of storage in the DE and PP groups. The erosion of tooth substrate depends on the number and duration of the acidic attacks, as well as the pH-value of the acidic agent (11). The role of CPP-ACP has been described as localization of ACP on the tooth surface which buffers the free calcium and phosphate ion activities, helping to maintain a state of supersaturation with respect to enamel by suppressing demineralization and enhancing remineralization (19).

The presence of CPP-ACP might permit a rapid return to resting calcium concentrations and allow earlier remineralization of the enamel substrate.

The non-linear demineralization of dentin due to the rapid initial loss of the smear layer has been reported previously (20). On exposure to acidic solutions, the smear layer is removed and numerous open dentinal tubules become apparent on the surface. The early stages of dentin demineralization seem to be similar to the erosion pattern of enamel. The mineral component of dentin is lost due to the acidic attack and erosion progresses at a relatively high rate. However, it has been reported that dentin is less susceptible to erosion than enamel (21). This difference in susceptibility has been attributed to the higher organic content of dentin, which might play an important role in slowing the demineralization process (22). The collagen matrix of dentin is assumed to play a protective role in maintaining the integrity of the softened dentin surface (23). This layer might not only function as a diffusion barrier but it also exhibits buffering properties and serves as a polar

membrane, which modifies the diffusion process.

The surface morphologies of the specimens in the TM group showed no apparent differences among the different storage periods. Previously, it has been reported that CPP-ACP functions by localising ACP on the tooth surface, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation by suppressing demineralization and enhancing remineralization (19). Other studies demonstrated that CPP-ACP in a mouthwash significantly increased the level of calcium and inorganic phosphate ions in supragingival plaque; the CPP bound both to the salivary pellicle and to the surface of bacteria in the plaque biofilm (24). The present study showed that the twice-daily application of 10-fold diluted CPP-ACP paste resulted in no alteration in the surface morphological changes of the tooth substrate, thus indicating that CPP-ACP prevented demineralization of the tooth. Accordingly, the placebo paste without CPP-ACP showed no protective effects on demineralization.

In the oral environment, host factors (such as the mineral concentration of the tooth, and pellicle and plaque formation) can influence the progression of demineralization. Salivary factors, such as the salivary flow rate, composition and buffering capacity, might exert protective action on dental surfaces (11). Enhancing the demineralization capability of saliva is important from the clinical point of view. Therefore, the influence of CPP-ACP on the prevention of demineralization observed in the present study would be clinically beneficial.

Within the limitations of this *in vitro* study, the following conclusions were drawn. The CPP-ACP paste was effective in preventing demineralization of enamel and dentin more effectively than the placebo paste (CPP-ACP free). Further studies are needed to know the morphological changes of tooth substrates *in vivo* conditions.

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