Original article

Clinical evaluation of remineralization potential of casein phosphopeptide amorphous calcium phosphate nanocomplexes for enamel decalcification in orthodontics

WANG Jun-xiang, YAN Yan and WANG Xiu-jing

Keywords: *casein phosphopeptide amorphous calcium phosphate; enamel remineralization; orthodontics*

Background Enamel decalcification in orthodontics is a concern for dentists and methods to remineralize these lesions are the focus of intense research. The aim of this study was to evaluate the remineralizing effect of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) nanocomplexes on enamel decalcification in orthodontics.

Methods Twenty orthodontic patients with decalcified enamel lesions during fixed orthodontic therapy were recruited to this study as test group and twenty orthodontic patients with the similar condition as control group. GC Tooth Mousse, the main component of which is CPP-ACP, was used by each patient of test group every night after tooth-brushing for six months. For control group, each patient was asked to brush teeth with toothpaste containing 1100 parts per million (ppm) of fluoride twice a day. Standardized intraoral images were taken for all patients and the extent of enamel decalcification was evaluated before and after treatment over this study period. Measurements were statistically compared by *t* test.

Results After using CPP-ACP for six months, the enamel decalcification index (EDI) of all patients had decreased; the mean EDI before using CPP-ACP was 0.191±0.025 and that after using CPP-ACP was 0.183±0.023, the difference was significant (t=5.169, P <0.01). For control group, the mean EDI before treatment was 0.188±0.037 and that after treatment was 0.187±0.046, the difference was not significant (t=1.711, P >0.05).

Conclusion CPP-ACP can effectively improve the demineralized enamel lesions during orthodontic treatment, so it has some remineralization potential for enamel decalcification in orthodontics.

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The demineralization of enamel adjacent to orthodontic brackets is a considerable clinical problem, with reports of a significant increase in the prevalence and severity of enamel demineralization after orthodontic treatment when compared with untreated control subjects. The prevalence of white spot lesions in orthodontic patients has been reported as being between 2%–96%.[1-4] These decalcified lesions not only result in an esthetically unacceptable appearance, but may also require subsequent restorative treatment in severe cases.

During the last decade, bioactive agents based on milk products have been developed that, under cariogenic conditions, can release elements enhancing enamel and dentin remineralization. As a result, if used in the early stages of disease, they can arrest the progression of carious lesions and allow healing of the affected tissue.[5] Recently, a novel product known as Tooth Mousse has become commercially available. Tooth Mousse is based on a nanocomplex of the milk protein casein-phosphopeptide (CPP) and amorphous calcium phosphate (ACP). CPP-ACP is a casein-derived peptide in which ACP is stabilized by CPP, and these nanocomplexes act as a calcium and phosphate reservoir when incorporated into the dental plaque or on the tooth surface.[6] CPP-ACP has been shown to reduce demineralization and promote remineralization of carious lesions both *in vitro* and in clinical situations.[7-10]

Although many studies have greatly improved our understanding of the de-/re-mineralization process, none has yet simulated the complex nature of the oral environment and few have focused on the remineralization of enamel decalcification during orthodontic treatments. In this study, we used GC Tooth Mousse, the main component of which is CPP-ACP, to evaluate the remineralization potential of CPP-ACP in young patients undergoing orthodontic treatment.

METHODS

Clinical data

Selection criteria were that patients were below 18 years old and displayed enamel decalcification of at least one tooth during orthodontic treatment. Twenty patients (12 male, 8 female; average age: 14 years) requiring fixed orthodontic treatment were selected as test group and twenty patients (10 male, 10 female; average age: 16 years) as control group. Patients with progressive

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Department of Orthodontics, Peking University School of Stomatology, Outpatient Dental Center, Beijing 100034, China (Wang JX, Yan Y and Wang XJ)
Correspondence to: Dr. WANG Xiu-jing, Department of Orthodontics, Peking University School of Stomatology, Outpatient Dental Center, Beijing 100034, China (Tel: 86-10-58595075. Email: Wang_xiu_jing@163.com)

periodontitis, tetracycline pigmentation of teeth or dental fluorosis were excluded. None of the patients were using antimicrobials or medications that could affect saliva quality and flow. All patients were informed of the study objectives and informed consent for participation in this study was signed by each.

Study methods

Straight wire appliances were used for all patients with bracket slots measuring 0.022×0.028 inches (1 inch=25.4 mm). Ligature wires were used for all the patients. Before appliance application, standardized intraoral images were taken with a single lens reflex camera (Canon, Japan) and the extent of enamel decalcification was quantified. After orthodontic treatment lasting 8–12 months, further intraoral images of all patients with enamel decalcification were taken.

For test group, these patients were instructed in the use of GC Tooth Mousse (Recaldent, Japan) once a day. They were asked to brush their teeth carefully before sleep (all patients used the same brand of toothpaste without fluoride) and to then apply Tooth Mousse on the tooth surface adjacent to their orthodontic brackets. During routine check-up examinations, the method and frequency of use was monitored. For control group, these patients were asked to brush their teeth with toothpaste (Colgate, China) containing 1100 parts per million (ppm) of fluoride. They were required to brush twice a day, brush all surfaces for at least 3 minutes and avoid drinking or eating for 30 minutes after brushing. After six months, further standardized intraoral images were taken for all the patients and the extent of enamel decalcification was quantified and compared with the values prior to treatment.

Clinical evaluation

All images were taken by a single doctor using a single lens reflex camera. The aperture and speed of the shutter were uniform across all images. The extent of enamel decalcification for six upper anterior and six lower anterior teeth were evaluated by a single dentist, in a blinded fashion. After one month, the 30 intraoral images taken at the start of the study were re-evaluated to test the reliability and reproducibility of this study.

An enamel decalcification index (EDI) was used for evaluation of changes in enamel mineralization over the course of the study. Each tooth was divided into nine areas (Figure 1), with the bracket occupying the central area. Any area with evident enamel decalcification was marked "1", whereas areas with no apparent decalcification were marked "0". The EDI for a single tooth was the sum of the eight peripheral areas (i.e. excluding the bracket area) divided by 8. The overall patient EDI was the average of the individual tooth EDI values from the 12 anterior teeth studied.

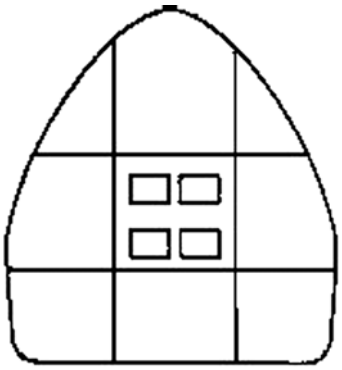

Figure 1. Subdivision of each tooth into nine subareas.

Statistical analysis

EDI values of two groups were compared by Student's *t* test using SPSS 11.0 for Windows statistical software (SPSS Inc., USA). A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Before the application of the appliances, the mean EDI of test group was 0.075±0.041 and that of control group was 0.073±0.042, the difference was not significant (t=0.481, P >0.05). These results indicated that the patients of two groups had the similar baseline before application of the appliances. Table 1 shows the result of comparison of EDI between two groups before and after treatment for six months. These results indicated that the mean EDI decreased significantly (P <0.01) after using Tooth Mousse over the six-month study period; however, the mean EDI of control group had no significant difference before and after treatment for six months (P >0.05).

Case report

To reiterate the efficacy of Tooth Mousse, Figures 2–4 show a representative case from test group of remineralization of white spot lesions occurring during orthodontic treatment. The patient was a 12-year-old boy requiring orthodontic treatment to correct crowding. Before application of straight-wire appliances, his overall EDI score was 0.031. After orthodontic treatment for about 10 months, the enamel of several teeth was decalcified and the EDI value had increased to 0.156. After using Tooth Mousse for six months, some of these white spot lesions had remineralized and the EDI had fallen to 0.135.

DISCUSSION

The increased prevalence of enamel decalcification during fixed appliance therapy is partly due to the creation of plaque stagnation areas around the irregular

Table 1. Comparison of EDI of two groups before and after treatment for six months

Groups	Cases (n)	EDI (before)	EDI (after)	t value	P value
Test group	20	0.191±0.045	0.183±0.063	5.169	0.000
Control group	20	0.188±0.037	0.187±0.046	1.711	0.115

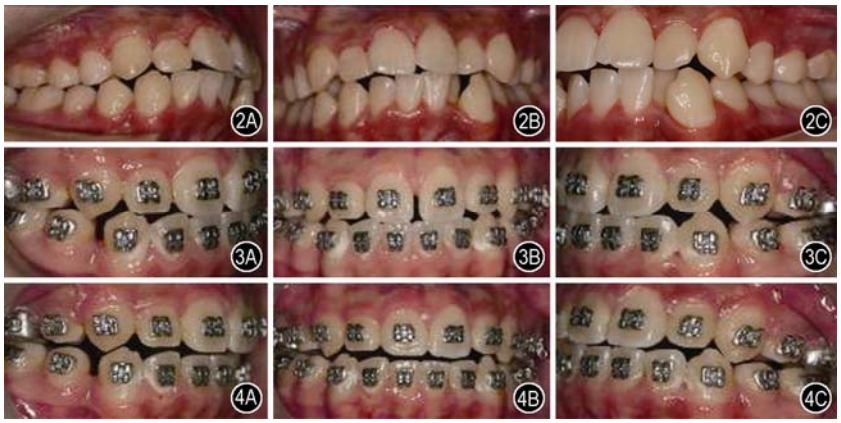

Figure 2. Intraoral images before application of appliances. **2A:** right side; **2B:** centric occlusion; **2C:** left side.
Figure 3. Intraoral images after orthodontic treatment for about 10 months. **3A:** right side; **3B:** centric occlusion; **3C:** left side.
Figure 4. Intraoral images after using Tooth Mousse for six months. **4A:** right side; **4B:** centric occlusion; **4C:** left side.

surfaces of the brackets, bands, wires, and other attachments used in these techniques. These areas are notoriously hard to clean and limit the physiological oral self-cleansing mechanisms, such as the movement of the oral musculature and saliva.[11] The accumulation of plaque causes a drop in pH in the presence of fermentable carbohydrates, which accelerates the rate of plaque accumulation and maturation, and promotes the survival of aciduric bacteria such as *Streptococcus mutans* and *lactobacilli*.[1-4,11] Adolescents generally have poor oral hygiene because of their limited power of self-governance, so enamel decalcification occurs more frequently in young orthodontic patients. Dentists are thus compelled to understand what measures are available to treat these lesions in adolescent orthodontic patients.

However, remineralization of enamel decalcification has proven a stubborn problem in the clinic. Fluoride, the traditional method, is proven to effectively prevent decalcification and promote remineralization,[12] and low fluoride preparations have been recommended to promote remineralization of enamel decalcification.[13] However, some scholars found that the use of high local doses of fluoride have no effect on the size of the arrested lesion and can allow it to acquire unsightly staining with organic debris. Ogaard and co-workers[3] warned against treating visible white lesions on labial surfaces with concentrated fluoride agents, since this arrests both demineralization and remineralization in the lesion by surface hypermineralization. Excessive use of fluoride can also be systemically harmful, thus most fluoride agents such as fluoride foam, fluoride gel and fluoride varnish were recommended to be provided for patients every 6 months, so we selected fluoride toothpaste as control group in this study which can be used every day to make a better comparison with test group.

CPP containing the cluster sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- have a remarkable ability to stabilize ACP in a metastable solution. Through its multiple phosphoseryl residues, CPP binds to forming nanoclusters of ACP, preventing their growth to the critical size required for nucleation and phase transformation. CPP-ACP has been demonstrated to have anticariogenic activity in laboratory, animal and human *in situ* experiments and has been shown to remineralize enamel subsurface lesions significantly *in vitro*.[7-10,14] It has been claimed that it promotes remineralization of carious lesions by maintaining enamel minerals in a supersaturated state while hindering the colonization of dental surfaces by cariogenic bacteria.[15] Furthermore, it has been shown to act as a buffering agent, preventing the pH reductions in the oral micro-environment that are so damaging to the enamel integrity.[16] Srinivasan et al[17] found that both CPP-ACP and fluoride (900 ppm)-supplemented CPP-ACP substantially remineralized softened enamel, with the fluoride-supplemented CPP-ACP being the most efficacious. This study confirms the synergistic effect of fluoride with CPP-ACP on remineralization of eroded enamel. CPP-ACP is marketed under the trade name “Recaldent”, and several different vehicles have been produced to deliver it, including a water-based mousse, a topical cream, chewing gum, mouth rinses, and sugar-free lozenges.[7,8,11] Studies of the effects of CCP-ACP have shown promising dose-related increases in enamel remineralization within already-demineralized lesions.[18,19] Clinical experience has been developed using the mousse (GC Tooth Mousse; Recaldent) to treat postorthodontic lesions using thermoplastic retainers as the delivery method.

Saliva plays important roles in remineralization of enamel decalcification. Studies[20,21] have reported the remineralization potential of stimulated saliva on erosion; however, it is proved that the remineralization of saliva was much lower compared with that of CPP-ACP.[17] Meanwhile, it has been documented the synergistic effect of CPP-ACP and saliva in remineralization.[22] pH of the stimulated saliva marginally improved due to the buffering effects of CPP-ACP. The patients all tended to exhibit a low resting salivary flow rate; however, an increase in flow rate was noted after baseline and this again may be attributed to the regular use of CPP-ACP.

Accurate quantification of enamel decalcification is a conundrum that has long exercised researchers. The ideal method of assessment should be simple, noninvasive, reproducible, and precise. Measurement of the rate of enamel decalcification has been used previously but is too

imprecise to evaluate the extent of enamel decalcification. The evaluation criterion used in this study is EDI, which is relatively more precise because the areas are more finely sub-divided and the borderlines are more clearly defined. However, errors are inevitable and more studies are required to refine this and other methods. Nevertheless, Willmot et al[23] and Benson et al[24] have concluded that computerized, standardized intraoral images are more reproducible and more credible in assessing enamel decalcification than direct visual observation in the clinic. We therefore chose to record the extent of enamel decalcification using intraoral images to increase the precision and convenience of study.

We found that CPP-ACP has the potential as a treatment to remineralize areas of enamel decalcification in orthodontic patients. However, because the patients with enamel decalcification are generally those with poor oral hygiene, good oral hygiene instruction is still the foundation of our work in preventing enamel decalcification in orthodontic patients, with CPP-ACP used as an adjunct therapy.

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