Effects of different anti-caries procedures on microhardness and micromorphology of irradiated permanent enamel

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To explore the effects of infiltration resin, casein phosphate polypeptide-amorphous calcium phosphate (CPP-ACP) and fluoride on microhardness and micromorphology of irradiated enamel. Sixty human permanent teeth were mesiodistally sectioned, yielding 120 enamel samples, which randomly divided into 8 groups: G1: blank control; G2: irradiation control; G3: irradiation+fluoride; G4: irradiation+CPP-ACP; G5: irradiation+CPP-ACP+fluoride; G6: irradiation+infiltration resin; G7: irradiation+infiltration resin+fluoride; G8: irradiation+infiltration resin+CPP-ACP. A progressive improvement was observed on the superficial morphology of enamel treated with different anti-caries procedures. The order of microhardness values after irradiation from the highest to the lowest was as follows: G1>G8~G7>G6>G5>G4~G3>G2. CPP-ACP, infiltration resin and fluoride can effectively restore the direct destruction of enamel caused by irradiation and promote the occurrence of remineralization. Infiltration resin and its combined effects with fluoride or CPP-ACP have the most potential anti-caries agent to resist radiation-caries.

Keywords: Radiotherapy, Permanent enamel, Infiltration resin, Casein phosphate polypeptide-amorphous calcium phosphate (CPP-ACP), Remineralization

INTRODUCTION

One of the most common complications in irradiated head and neck cancers (HNC) patients is a well-known devastating clinical finding of dental hard tissues known as radiation-related caries (RRC)¹. RRC is a rapidly progressing and highly destructive type of dental caries, which is circumferential at the cervical part of the tooth, affecting smooth enamel surfaces that are normally resistant to decay, and then lead to the complete crown amputation of part of, or the whole, dentition². There is evidence that the direct effects of radiotherapy on teeth are one of the important pathogenesis of RRC, which includes changes in microstructure and the mechanical properties of dental hard tissues³. Fränzel and Gerlach⁴ found that enamel demineralization was shown after irradiation, accompanied with significantly changes of morphology and structure of enamel as well as the decrease of acid-resistance, which resulted in the increased sensitivity to caries. Because of the rapid progression of RRC and its difficulty of controlling, RRC is also known as 'rampant caries', whose treatment is mainly focus on prevention in clinical.

Kumar and Rastogi⁵ found that oral hygiene instructions before and after radiotherapy in HNC patients can effectively reduce the incidence and severity of RRC. Fluorides, whose effects on caries has been well established in clinical trials and systematic reviews, can effectively prevent the damage of enamel caused by radiotherapy and be a feasible measure to prevent the occurrence of RRC in irradiated HNC patients⁶. For the last decade, a novel product casein phosphate polypeptide-amorphous calcium phosphate (CPP-ACP), which is a nanocomplex derived from milk protein and

usually used for the management of incipient caries, has become commercially available⁷. CPP contains the cluster sequence of Ser(P)-Ser(P)-Ser(P)-Glu-Glu from casein and is reported to have topical anti-caries effects due to its ability to stabilize calcium and phosphate in an amorphous state. CPP-ACP affects the demineralization-remineralization processes by maintaining an hypersaturated state on the tooth surface and a reservoir of calcium phosphate⁸. Its anti-cariogenic properties and remineralized effects have been demonstrated in animal laboratory⁹, *in vitro* studies¹⁰ and human *in situ* experiments⁷. However, the reported anti-caries efficacy of CPP-ACP in comparison with traditional fluorides was controversial⁷. In a randomized clinical trial¹¹ of 24 patients with nasopharyngeal carcinoma (NPC), it was found that patients with CPP-ACP plus fluorides showed lower caries progressive behavior than those with fluorides alone. However, this synergistic remineralization effects of fluorides and CPP-ACP was not observed in other studies^{12,13}. Therefore, whether fluorides and CPP-ACP have combined effects on remineralization is still controversial.

Infiltration resin is a recent approach to limit enamel demineralization. This material was originally used in clinical to prevent the progression of non-cavitated dental caries and later considered as an alternative treatment for fluorides and CPP-ACP¹⁴. Resin infiltration technique can slow down or reverse the progress of non-cavitated enamel lesions and has preventive effects on rampant caries caused by Sjogren syndrome¹⁵. In addition, infiltration resin has combined effects with CPP-ACP and fluorides in caries-prevention. Inhibition of caries progression in permanent teeth was significantly effective after treatment of resin

infiltration and fluoride varnish than that of resin infiltration or fluoride alone¹⁶⁾. Chen¹⁷⁾ found that the combined treatment better improved the effects both in remineralization and inhibition of demineralization than the application of resin infiltration or CPP-ACP alone. To the best of our knowledge, whether this effect will be the same when infiltration resin applied alone or combined with CPP-ACP or fluoride to irradiated enamel is still unknown.

Therefore, the purpose of this study was to evaluate, by scanning electron microscopy (SEM) and micro indentation hardness test, the *in vitro* ability of different anti-caries procedures to prevent damage of dental hard tissues caused by radiotherapy. The null hypothesis tested was that different anti-cariogenic agents does not influence the micromorphology and microhardness of irradiated permanent enamel.

MATERIALS AND METHODS

A total of sixty healthy human permanent premolars and molars were collected from Center of Stomatology of Xiangya Hospital of Central South University after suitable approval from the Human Research Ethics Committee of the same institution (20171117) and performed as a double-blind study. The teeth were extracted for orthodontic reasons and none of them had any caries, restorations, surface defects, cracks, or enamel hypoplastic. The teeth were thoroughly washed, scraped, and ultrasonically scaled, to remove plaque and calculus, then stored in physiological saline solution at a temperature of 4°C (DW-25W322, Aucma, Qingdao, China) and used not more than 3 months post-extraction¹⁸⁾.

Sample preparation¹⁹⁾

The teeth were cleaned and had their roots removed at the cementum-enamel junction (CEJ) using a high-speed diamond disc (MANI, Tochigi, Japan) under cooling-water. Crowns were sectioned longitudinally with a water-cooled diamond saw into two halves in the mesiodistal direction, yielding 120 enamel samples. The buccal or lingual surfaces were used as tested surfaces and ground with an increasing sequence of silicon carbide (SiC) paper (1500, 2000, 2500, 3000, 5000 and 7000 grit) for 60 s each under running water to produce flat enamel. Between each polishing stage, samples were cleaned using physiological saline solution in an ultrasonic cleaner (JP-010T, Jiemeng, Shenzhen, China) for 8 min to remove any debris. These 120 samples were randomly divided into eight groups ($n=15$). All samples were embedded with medical red wax and exposed the tested surface, which was covered with two layers of acid-resistant nail polish, leaving a 3×3 mm window exposed for microhardness test and SEM observation.

Irradiation procedure

The radiation dose was calculated based on a mean that were applied on 166 HNC patients collected from Oncology Department of Xiangya Hospital. The

radiotherapy protocols consisted of a total of 35 fractions equal to 68.25 Gy, with 1.95 Gy exposure per day applied 5 days per week, during 7 weeks with X-rays from a linear accelerator (Clinac 23EX, Varian, Lincolnshire, IL, USA) in the Department of Radiotherapy at Xiangya Hospital. Moreover, all irradiations were done in the container of the same size with the enamel samples submerged in physiological saline water. The distance between the “radiation source” and the samples would be 100 cm.

Test groups and interventions

For each group, the open window area was treated with different protocols. All enamels were submerged in physiological saline solution and the normal saline was changed every day. The researcher who conducted the interventions of 8 experimental groups was not participated in the statistical analysis.

Group 1 (Control group): The enamels were not treated with irradiation or any anti-caries agents.

Group 2 (Irradiation): The enamels were irradiated but not treated with any anti-caries agents.

Group 3 (Irradiation+Fluoride): Every-time before irradiation, fluoride varnish (Clinpro™ White Varnish, 3M, Saint Paul, MN, USA) was applied on enamels and left for 3 min then cleaned with normal saline. When the maximum cumulative radiation dose of 68.25 Gy was delivered, fluoride varnish was continued applied 5 days per week for 4 weeks.

Group 4 (Irradiation+CPP-ACP): Every-time before irradiation, CPP-ACP (GC Tooth Mousse™, Tokyo, Japan) was applied on enamels and left for 3 min then cleaned with normal saline. When the maximum cumulative radiation dose of 68.25 Gy was delivered, fluoride varnish was continued applied 5 days per week for 4 weeks.

Group 5 (Irradiation+Fluoride+CPP-ACP): Every-time before irradiation, fluoride varnish was applied on enamels and 5 min later CPP-ACP was used. When the maximum cumulative radiation dose of 68.25 Gy was delivered, fluoride varnish and CPP-ACP was continued applied 5 days per week for 4 weeks.

Group 6 (Irradiation+Infiltration resin): When the maximum cumulative radiation dose of 68.25 Gy is reached, in accordance with the manufacturer's instructions²⁰⁾, the enamels were etched with 15% HCL gel for 120 s, rinsed for 30 s, and dehydrated with 100% ethanol for 30 s. Infiltration resin (Icon™, DMG, Hamburg, Germany) was applied with a micro brush for 3 min, excess material was gently removed by air blowing and flossing; then the resin was light-cured for 40 s. Infiltration resin was applied a second time for an additional 1

min, light curing for 40 s.

Group 7 (Irradiation+Infiltration resin+Fluoride): When the maximum cumulative radiation dose of 68.25 Gy was delivered, infiltration resin was applied accordance to the manufacturer's instructions then fluoride varnish was used 5 days per week for 4 weeks.

Group 8 (Irradiation+Infiltration resin+CPP-ACP): When the maximum cumulative radiation dose of 68.25 Gy was delivered, infiltration resin was applied accordance to the manufacturer's instructions then CPP-ACP was used 5 days per week for 4 weeks.

SEM analysis²¹⁾

Five enamels from each group were fixed in 2% glutaraldehyde in cacodylate buffer for 2 h and were dehydrated in a series of increasing ethanol concentrations (30, 50, 75, 95, and 100%) for 10 min. Subsequently, specimens were sputter-coated with gold in a vacuum metallizing machine (SCD500, BAL-TEC, Leica, Switzerland) and examined with a scanning electron microscope (JSM-6490LV, Neptune Tex's HP, Shimadzu, Kyoto, Japan). Two images were obtained of each sample at two magnifications ($\times 2,000$ and $\times 5,000$).

Microhardness testing

Surface hardness of 10 enamels from each group was determined using a microhardness tester with a Vickers diamond indenter¹⁸⁾ (HMV-2T Microhardness Tester, Shimadzu, Kyoto, Japan). The baseline surface microhardness (SMH₀) and microhardness post-irradiation (SMH₁) of all enamels were measured.

Statistical analysis

All tests employed $\alpha=0.05$ as level of significance and statistical analysis was performed with IBM SPSS Statistics Version 22 for Windows. Student's *t* test was used to compare the microhardness pre-and post-irradiation within groups. One-way analysis of variance (ANOVA) was used for comparison between groups. LSD test was used for pairwise comparison between groups when ANOVA test was significant.

RESULTS

SEM analysis

Figure 1 shows the representative SEM photographs ($\times 2,000$, insets $\times 5,000$) of enamel surface from different groups. SEM images show that in sound enamel, it is possible to note normal organization of enamel, showing a flat and smooth surface with some striation due to the grinding procedure (Fig. 1a). The well-defined structure of enamel was destroyed after irradiation exposure with 68.25 Gy, showing enamel suffered increased porosity and cracks as depicted in Fig. 1b. Moderate superficial alterations were found in enamel applied with fluoride (Fig. 1c) and CPP-ACP (Fig. 1d), which was characterized by superficial porosities and slight depressions. Following application of fluoride with CPP-ACP (Fig. 1e), the enamel surface was distributed with small globular or rod-shaped mineral particles with high-density, forming irregular cloud or snow-like mineralized structure. The enamel morphology of infiltration resin group was slightly changed (Fig. 1f). The surface was well-distributed and smooth and only a few shallow pits and cracks were observed. The micrographs for infiltration resin plus fluoride (Fig. 1g) or CPP-ACP (Fig. 1h) were similar. The enamel surfaces were slightly changed and covered by large number of

Fig. 1 Representative SEM photographs of enamel surface appearance from different groups ($\times 2,000$, insets $\times 5,000$): a. Control group; b. Irradiation group; c. Irradiation+Fluoride; d. Irradiation+CPP-ACP; e. Irradiation+Fluoride+CPP-ACP; f. Irradiation+Infiltration resin; g. Irradiation+Infiltration resin+Fluoride; h. Irradiation+Infiltration resin+CPP-ACP.

Table 1 Microhardness value of permanent enamel treated with different anti-caries agents, and results of Student's *t* test pre-and post-irradiation intra-groups ($\bar{X} \pm S$, $n=10$, MHV)

Groups	SMH ₀ (Kg/mm ²)	SMH ₁ (Kg/mm ²)	<i>t</i>	<i>p</i>
G1	336.80±9.61	336.92±9.82	−0.13	0.905
G2	332.53±7.55	263.86±7.88	28.37	0.00*
G3	335.55±4.35	286.70±10.97	16.15	0.00*
G4	336.29±10.35	292.25±10.60	8.26	0.00*
G5	337.30±10.82	315.43±10.50	10.61	0.00*
G6	335.79±8.47	304.81±13.58	5.90	0.00*
G7	336.28±7.06	320.42±5.77	4.57	0.00*
G8	338.02±9.92	320.04±9.89	4.75	0.00*

*means that there was significant difference in the enamel microhardness pre- and post-irradiation intra-group ($p < 0.05$). SMH: surface microhardness.

Table 2 Microhardness value of permanent enamel treated with different anti-caries agents, and results of one-way ANOVA analysis pre-and post-irradiation between-groups ($\bar{X} \pm S$, $n=10$, MHV)

Groups	SMH ₀ (Kg/mm ²)	SMH ₁ (Kg/mm ²)	<i>F</i> ₁	<i>P</i> ₁	<i>F</i> ₂	<i>P</i> ₂
G1	336.80±9.61	336.92±9.82	0.23	0.98	37.04	0.00*
G2	332.53±7.55	263.86±7.88				
G3	335.55±4.35	286.70±10.97				
G4	336.29±10.35	292.25±10.60				
G5	337.30±10.82	315.43±10.50				
G6	335.79±8.47	304.81±13.58				
G7	336.28±7.06	320.42±5.77				
G8	338.02±9.92	320.04±9.89				

*F*₁, *P*₁: One-way ANOVA analysis in enamel SMH between 8 groups before irradiation. *F*₂, *P*₂: One-way ANOVA analysis in enamel SMH between 8 groups after irradiation. SMH: surface microhardness. *means that there was significant difference in enamel SMH post-irradiation between 8 groups ($p < 0.05$).

Table 3 Microhardness value of permanent enamel treated with different anti-caries agents, and results of LSD-*t* pairwise comparison test post-irradiation between-groups ($\bar{X} \pm S$, $n=10$, MHV)

Groups	SMH ₁ (Kg/mm ²)	<i>P</i> ₁	<i>P</i> ₂	<i>P</i> ₃	<i>P</i> ₄	<i>P</i> ₅	<i>P</i> ₆	<i>P</i> ₇	<i>P</i> ₈
G1	336.92±9.82	—	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G2	263.86±7.88	0.00	—	0.00	0.00	0.00	0.00	0.00	0.00
G3	286.70±10.97	0.00	0.00	—	0.31	0.00	0.00	0.00	0.00
G4	292.25±10.60	0.00	0.00	0.31	—	0.02	0.00	0.00	0.00
G5	315.43±10.50	0.00	0.00	0.00	0.02	—	0.06	0.01	0.01
G6	304.81±13.58	0.00	0.00	0.00	0.00	0.06	—	0.36	0.40
G7	320.42±5.77	0.00	0.00	0.00	0.00	0.01	0.36	—	0.94
G8	320.04±9.89	0.00	0.00	0.00	0.00	0.01	0.40	0.94	—

*P*_{*n*} means that results of LSD-*t* test between Gn and other 7 groups ($n=1-8$). SMH: surface microhardness. *means that there was significant difference in enamel SMH post-irradiation between-groups ($p < 0.05$).

irregular high-density minerals.

SMH analysis

The SMH values of enamel for all groups pre-and post-irradiation were presented in Table 1. The results of Student's *t* test pre-and post-irradiation intra-groups showed that irradiation significantly reduced the SMH of enamel ($p < 0.05$). One-way ANOVA analysis showed that no significant difference was found in enamel SMH between 8 groups pre-irradiation (Table 2, $p > 0.05$). However, a statistically significant difference was found in SMH post-irradiation between the groups (Table 3, $p < 0.05$). Six different anti-caries agents (three applications alone and three pairwise combined applications) significantly increased the SMH of irradiated enamel ($p < 0.05$). However, they cannot restore the SMH of irradiated enamel to non-irradiation level. The order of enamel SMH after radiotherapy from the highest to the lowest was as follows: G1 (control group) > G8 (infiltration resin+CPP-ACP) ~ G7 (infiltration resin+ fluoride) ~ G6 (infiltration resin) ~ G5 (fluoride+CPP-ACP) > G4 (CPP-ACP) ~ G3 (fluoride) > G2 (irradiation group). The SMH values of G5–G8 were the highest among 7 irradiated enamel group, and LSD-*t* test showed that no statistically significant difference was detected between them (Table 3, $p > 0.05$). G3 and G4 groups were not statistically different as well (Table 3, $p > 0.05$).

DISCUSSION

The aim of this study was to investigate the preventive effects of different anti-caries procedures on radiation-caries by simulating the influences of irradiation on the micromorphology and mechanical properties of enamel *in vitro*. It is indeed that the radiation affected the structural and mechanical properties of enamel. The infiltration resin, CPP-ACP and fluoride, whether used alone or in combination with each other, can effectively improve the destruction of enamel surface caused by irradiation, and increase its surface microhardness. Among which the infiltration resin and its combination with CPP-ACP or fluoride had the most remarkable effects on the prevention of radiation-caries. The null hypothesis was rejected.

Radiation-caries is the most common oral complication after radiotherapy in HNC patients. SEM is widely used to elucidate microstructural changes in the surface of dental hard tissues after being treated with different anti-caries procedures²². Microhardness is one of the important physical properties of tooth surface, which reflects the ability to resist plastic deformation and persistent indentation. Featherstone²³ found that a linear correlation was between microhardness and enamel mineral content. Therefore, the determination of enamel microhardness and SEM has become important measures and indexes to estimate the demineralization and remineralization of dental hard tissues in the studies of caries researches.

The Vickers microhardness of enamel is generally

in the range of 254–363 (kg/mm²)²⁴. In this study, the baseline SMH of enamel was 321.34–353.68 kg/mm², which was in accordance with the range of normal enamel microhardness. After irradiation, decreases in microhardness values accompanied with superficial morphology alterations were found, which was consistent with previous studies^{3,19}. Radiotherapy has significantly destructive effects on the organic matrix in enamel, which influences the mechanical properties of enamel¹⁹. Liang²⁵ showed that the mechanism of weakened microhardness caused by irradiation was the decarboxylic reaction of dental hard tissues. Radiation induces carboxylate decarboxylation on the side chain of collagen, resulting in the loss of acidic phosphates. In addition, decrease of apatite crystals-organic matrix interaction and increase of carbon dioxide production create microcracks and fractures in hydroxyapatite minerals, resulting in rough enamel surface and decreased microhardness²⁶. Fränzel and Gerlach⁴ reported that the deleterious effects of irradiated dental hard tissues seemed like the process of demineralization. These changes of micromorphology led to the reduced microhardness values.

If these lesions are properly treated, enamel caries can be arrested, hardened and reverted to healthy enamel condition through a remineralization process involving the diffusion of minerals into defective tooth structures. Early detection and remineralization of demineralized dental tissues through application of oral hygiene instruction are important methods to prevent radiation-caries in HNC patients. We would like to encourage different protocols to minimize damages caused by radiotherapy on enamel and reduce this impact during and after radiotherapy. Three agents were applied solely or combined with each other on irradiated enamel and we have noted that each treatment was to some extent effective. Infiltration resin is a new method for limiting demineralization of dental hard tissues. After light curing, the resin-porous hydroxyapatite complex was formed, which occludes micro-porosities within the caries lesion, forming a diffusion barrier to prevent further demineralization²⁷. After the application of infiltration resin, the mineral content of the demineralized enamel was only 2.1% less than that of sound enamel²⁸. In this study, infiltration resin was used for the first time in preventing radiation-caries and achieved significant curative effects. When the tested enamel was treated with one of infiltration resin, CPP-ACP or fluoride, it was found that infiltration resin had the strongest effects on the maintenance of micromorphology and the increasement of microhardness of irradiated enamel. In agreement with our findings, previous studies reported that the effects of infiltration resin on the surface microhardness of demineralized enamel was better than that of fluoride²⁹ and CPP-ACP³⁰, which was closely related to the highly permeability of infiltration resin.

It is well known that the remineralized layer formed by fluoride or CPP-ACP can seal the enamel surface, but only a small amount can enter into the demineralized micropores of enamel with a very shallow permeation depth³¹. However, infiltration resin can

penetrate deep into the inner space of demineralized enamel. Its average penetration depth is 516.8 μm , and the deepest penetration depth is 973 μm ²⁷⁾, which forms a diffusion barrier and blocks the passage of acid into enamel. Then the structure of demineralized enamel was significantly improved and the mechanical properties strengthened. Souza³²⁾ determined the surface microhardness of infiltration resin samples and found that its microhardness was not only lower than that of sound enamel, but also lower than that of demineralized enamel. Therefore, the effectiveness of infiltration resin on irradiated enamel is not because of the increase in microhardness of infiltration resin material after curing itself, but its penetration into the micropores within the enamel crystals and formation of a mixture layer of enamel-infiltration resin. The restoration of enamel superficial morphology and strengthening of mechanical supports resulting in the rise of microhardness.

In this study, CPP-ACP and fluoride had protective effects on irradiated enamel. Fluoride ions combine with hydroxyapatite to produce fluorapatite, a strong deterrent to demineralization, which deposits on the surface or micropores of enamel. The crystal structure of fluorapatite is more stable than that of pure hydroxyapatite, which increases the ability of fluoride to resist incipient caries³³⁾. CPP-ACP has been shown to localize and stabilize calcium and phosphate ions at the enamel surface in bioavailable forms that can promote remineralization of enamel lesions⁹⁾. Zhou³⁴⁾ have demonstrated that by increasing the grain size of hydroxyapatite and the mol ratio of calcium to phosphorus, CPP-ACP effectively repaired the microstructure of enamel. In our study, no statistically significant differences were found between the commercially available CPP-ACP and fluoride on microhardness of irradiated enamel. Hay and Thomson³⁵⁾ compared the anti-carries effects of CPP-ACP and fluoride for HNC patients after radiotherapy and believed that both CPP-ACP and fluoride can effectively prevent radiation-carries, but no significant differences were found between them. In addition, because CPP-ACP was as effective as fluoride, the application of CPP-ACP may have advantages in HNC patients due to the fact that it can help reduce the risk of radiation-carries without increasing the risk of fluorosis.

In this study, the protective effects of infiltration resin combined with fluoride or with CPP-ACP on irradiated enamel was the most obvious among 8 groups. Previous studies^{16,36)} have combined infiltration resin with fluorides to treat early caries and found their synergetic anti-carries effects. And the combination of infiltration resin with CPP-ACP was better than that of CPP-ACP in reducing demineralization and facilitating remineralization of dental hard tissue¹⁷⁾. In this study, the protective effects of infiltration resin combined with fluoride or with CPP-ACP on irradiated enamel were stronger than infiltration resin monotherapy, but no significant difference were found between them. This may be due to the permanent occlusion of enamel micropores and surface by infiltration resin, which makes fluoride

and CPP-ACP unable to interact with hydroxyapatite. As a result, fluoride or CPP-ACP are mainly deposited on the surface of light-cured infiltration resin. Therefore, when infiltration resin combined with fluoride or with CPP-ACP, the infiltration resin plays a leading role in protecting irradiated enamel.

Studies^{11,37)} demonstrating that adjunct use of CPP-ACP with stannous fluorides achieved significantly clinical advantages over CPP-ACP or fluorides alone in reducing the progression of radiation-carries in patients with NPC, which was consistent with our study. In a randomized clinical trial¹¹⁾ of 24 NPC patients, the patients treated with both CPP-ACP and fluorides were underwent lower progressive caries behavior after a follow-up period of 3 months, which confirmed that the simultaneous application of CPP-ACP with fluorides has synergistic effects in preventing radiation-carries. In the presence of fluoride, CPP-ACP mixed with fluoride ions to form a new complex: casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP). Patil³⁸⁾ believed that CPP-ACFP nanocomposites may produce more effective remineralization components than fluorides or CPP-ACP. In this study, fluoride was firstly applied, making the concentration of fluoride ion increased, which provides a stable substrate for CPP-ACP. Therefore, the current study argues that CPP-ACP should be used clinically in collaboration with fluorides rather than applied as an alternative to fluorides.

The appearance of dental fluorosis and osteofluorosis in the process of application also limited the clinical use of fluorides to some extent. No allergies or severe side effects were recorded when evaluating the clinical safety of CPP-ACP³⁹⁾. CPP-ACP were derived from milk protein casein, with the results that people with milk allergies are not allowed to use CPP-ACP. No side effects associated with infiltration resin have been observed so far⁴⁰⁾. Infiltration resin and CPP-ACP have great biosafety and can be effectively used for irradiated enamel, so both of them could be widely applied in clinical as preventive procedures for radiation-carries.

This study was an *in vitro* experiment mainly based on permanent teeth. The conditions *in vitro* are crucially different from those *in vivo* with dynamic biological system. Therefore, this study cannot completely simulate the actual oral cavity of HNC patients after radiotherapy. As to the mechanical properties of dental hard tissues, Vickers microhardness was selected. However, other parameters such as fracture resistance, ultimate tensile strength and elastic modulus, needed to be further studied. Therefore, more *in vitro*, *in vivo* studies and clinical trials are needed to further demonstrate the remineralized potential and long-term anti-carries efficacy of infiltration resin, CPP-ACP, fluoride and their combined application of each other for the radiation-carries.

CONCLUSIONS

Based on the data from this *in vitro* study, we make the following conclusions: (1) The exposure of 68.95 Gy

radiotherapy was able to affects micromorphological structure changes of enamel accompanied with reduced microhardness. (2) Infiltration resin, CPP-ACP and fluorides can effectively promote the remineralization of irradiated enamel and preserve the integrity of enamel morphology. Among them, infiltration resin has the strongest ability to prevent radiation-carries. (3) Fluoride and CPP-ACP have synergistic effect on remineralization of radiation-carries. The synergistic effects of infiltration resin combined with fluoride or CPP-ACP on radiation-carries were stronger than combined effects of fluoride and CPP-ACP, but the same with that of infiltration resin monotherapy.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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