Caries-preventative effect of mouthguards containing a surface pre-reacted glass-ionomer filler

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Abstract

This study aimed to provide an *in vitro* demonstration that incorporating surface pre-reacted glass-ionomer (S-PRG) fillers into ethylene vinyl acetate (EVA) copolymers, which are used in mouthguards, could be effective in preventing enamel demineralization during mouthguard use.

A bovine incisor was cut into enamel blocks. EVA plates containing and lacking S-PRG fillers were formed into eight mouthguards of each type using a thermoplastic material molding device to fit enamel blocks. Mouthguards were attached to each enamel block and immersed in a demineralization solution (37°C, 24 h). The degree of demineralization was assessed by micro-CT. Using the mouthguard lacking S-PRG filler (control), enamel blocks revealed a major demineralization over a wide area, including areas immediately under the mouthguard, and uncovered areas. In contrast, enamel blocks covered with mouthguards containing S-PRG filler revealed only mild demineralization, both directly under the mouthguard and in uncovered areas. CT values revealed that scores for enamel surfaces at depths from 0 to 252 μm were significantly higher in enamel blocks that were fitted with S-PRG filler mouthguard than in those of the controls ($p < 0.05$). These results indicate that mouthguards containing S-PRG filler could inhibit demineralization.

Introduction

A meta-analysis of orofacial sports injuries revealed a 1.6- to 1.9-fold increased risk of trauma when mouthguards were not used [20]. Mouthguard use is recommended worldwide to prevent orofacial injuries during sports activities [23]. However, mouthguards can enable sugars contained in various beverages, such as sports drinks, to remain in contact with dental surfaces for extended periods, possibly leading to the formation of dental caries [22,24]. In particular, school-aged children are at an increased risk [5] because they have immature permanent teeth that are highly susceptible to caries, and mouthguard use in this population is believed to increase the risk of caries. Developing a sports mouthguard material with anti-caries properties could promote mouthguard use. This study aimed to investigate mouthguards that were composed of ethylene vinyl acetate (EVA) and surface pre-reacted glass-ionomer (S-PRG) filler, which releases fluoride ions, to determine *in vitro* whether these mouthguards could be effective in preventing caries.

Materials and Methods

S-PRG filler (20 wt%; diameter: 3 μm) [8,15,17] was homogeneously mixed with copolymer pellets that were made of ethylene (75 wt%) and vinyl acetate (25 wt%). EVA containing S-PRG filler and control EVA lacking S-PRG filler were both molded into sheets of 4-mm thickness.

An extracted bovine incisor was cut into blocks (6 × 6 mm) using a SECOTOM-50® precision cutting machine (Struers, A/S, Ballerup, Denmark) with a MOD13® diamond cutting wheel (Struers). The dentin side of each block was polished to a final thickness of 1.5 mm with water-resistant sandpaper (#80) and then masked with nail varnish. The enamel surface was gradually sanded with a progressive series of sandpapers (#320, #600, #1200, and #2000) to use as a test side. A 1 × 1-mm square was cut into the surface of the enamel at each of the four corners of the blocks using a cutting disk following the method of Watanabe et al. [31]. Nail varnish was used to mask these areas, which served as non-demineralized standard surfaces during micro CT. A 3-mm square polypropylene film (thickness, 0.22 mm) was placed as a spacer atop the enamel block, and EVA sheets containing S-PRG filler and control EVA sheets were formed into mouthguards using a thermoplastic material molding device (Model capture try®, Matsukaze, Kyoto, Japan) to make eight mouthguards of each type. These mouthguards were attached to each enamel block and immersed for 24 h in a demineralization solution (37°C, 160 ml 0.2 mol/l acetate, 0.2 mol/l sodium acetate, pH 4.5; Wako Pure Chemical Industries, Osaka, Japan). After the mouthguards were placed in a constant-temperature water bath (WTB-9®, IWAKI, Tokyo, Japan) that was set at 37°C, the temperature was maintained and a magnetic stirrer (Octopus®, AS ONE, Osaka, Japan) was set at 300 rpm to create an environment with the continuously circulating solution. A thermometer and pH meter were placed in each beaker to ensure that a temperature of 37°C and a pH of 4.5 was maintained (Fig. 1).

A micro-CT scanner (SkyScan 1174®, Kontich, Belgium) was used to acquire cross-sectional X-ray images of enamel blocks before and after demineralization. The imaging conditions were as follows: X-ray tube voltage, 50 kV; tube current, 800 μA; 0.25 mm Al filter plate; slice thickness, 18 μm; unit pixel size, 18 μm; stage turning pitch, 3°; and cross-section image resolution, 512 × 512 pixels. Reconstruction software (NRecon, Kontich, Belgium) was used to reconstruct 3D images from the tomographic images. As a qualitative assessment, 3D CT values of the enamel surface were first color mapped and the demineralization effects of mouthguards containing or lacking S-PRG filler were compared. As a quantitative assessment, the uppermost surface of the non-demineralized enamel was next set as the baseline surface and assessed with the reconstruction software using a 1 × 1-mm square established as the region of interest in the center of each enamel block. Using numerical analysis software (CTAn, Kontich, Belgium), CT values were measured in 18-µm slices starting at the baseline and successively to a depth of 504 µm towards the dentin side.

All data were analyzed with IBM SPSS version 23, and the two groups were compared with Mann–Whitney U-test. A significance level of 0.05 was considered statistically significant.

Results

Figure 2 shows color mapping of 3D CT values of enamel blocks before and after demineralization (Fig. 2). For mouthguards lacking S-PRG (controls), demineralization occurred over a wide area, including those areas immediately under the mouthguard, and uncovered areas marked with a * (Fig. 2b). In contrast, samples covered with mouthguards containing S-PRG filler revealed only mild demineralization in the enamel block, both directly under the mouthguard and in uncovered areas marked with a *. (Fig. 2d).

Figure 3 shows CT values of enamel blocks from the surface down to a 504-µm depth in 18-µm increments. It demonstrated that relative to the control samples, enamel blocks that were covered with mouthguards containing S-PRG filler had CT values that were significantly higher for enamel surfaces at depths from 0 to 252 μm ($p < 0.05$).

Discussion

The World Dental Federation recommends that contact sport athletes wear mouthguards to prevent orofacial injuries. Indeed, many studies have reported regarding mouthguard use and the ability of these devices to prevent oral trauma [20], minimize concussion impact [2], and improve sports performance [4,25]. However, several side effects are associated with mouthguard use, including impeded oxygen intake and difficulties in pronunciation, although these issues can be resolved through proper adjustment and fitting to match individual dentitions [3]. Another study reported infections [9] resulting from dirty mouthguards. Moreover, mouthguard use can promote the progress of initial caries [5,22,24]. Thus, developing a mouthguard material that prevents caries is an important research topic that may ultimately increase mouthguard use in athletic activities.

Given that EVA sheets are widely used to create sports mouthguards [20], we hypothesized that adding a caries-preventive compound to EVA would be an effective means to prevent caries formation arising from mouthguard use.

Given the ability of fluoride to prevent caries, we focused on S-PRG fillers with the ability to release and recharge fluoride ions [12]. S-PRG fillers with a three-layer structure can provide sustained release of multiple ions, such as fluoride, strontium, sodium, aluminum, silicate, and borate [8]. S-PRG fillers strengthen teeth [17], inhibit demineralization [19], buffer acidity [8], prevent plaque formation [10,14,28], and have been widely reported to exert various bioactive effects. S-PRG fillers have been already applied in composite resin [26], fissure sealants[27], and tooth surface-coating agents [13,19], and are used as a dental material. S-PRG fillers have no reported cytotoxicity [16], and thus, may be an optimal material for mouthguard use with anti-caries properties.

The thickness and hardness of mouthguard materials play an important role in shock and energy absorption [1,6,29,30,32]. Thus, mouthguards must be of a certain thickness and strength to absorb impacts. Although there is concern that adding a filler to mouthguards may affect shock-absorbing properties, a preliminary experiment revealed that adding up to 40% of filler did not affect Shore hardness in any way, and thus, S-PRG filler would not be predicted to affect the shock-absorption capacity of mouthguards. We concluded that the addition of an anti-caries filler would not weaken mouthguard strength.

The Plaque has a tendency to accumulate on teeth and the border of removable appliance, and the high incidence of demineralization in those areas is clinically documented [21]. In our study, 3D CT-value color mapping of the enamel surface revealed that for mouthguards containing a filler, demineralization was also effectively inhibited at the edges of the mouthguard. Thus, the anti-caries effects of S-PRG fillers in mouthguards appeared to protect not only areas directly under the mouthguard but also sections that were on the fringes of the mouthguard and would be at a highest risk for caries. This inhibitory effect has been attributed to pH modulation by S-PRG filler of the surrounding solution, shifting pH to neutral along with the release of multiple ions [8]. However, future studies will be required to determine the actual concentrations of ions released from this product.

Fluoride and strontium released from S-PRG filler may also enhance the acid resistance of teeth by converting hydroxyapatite to fluoroapatite and strontium apatite, respectively [7]. Only one time point was evaluated in this study (24 h of immersion in demineralization solution), but considering the actual length of time that mouthguards would be used in practice, these enamel blocks will also need to be studied after immersions for 72 and 120 h, as indicated by a study of Hamba et al.[11] . S-PRG fillers are rechargeable [12,18], such that storage by immersion of sports mouthguards in a solution containing various ions could recharge the mouthguard to enable continuous ion release and provide potential long-term prophylaxis against caries.

Our study suggests that adding S-PRG filler to EVA sheets can provide preventative effects against caries when mouthguards containing this filler are used. EVA sheets containing S-PRG filler may be useful for applications beyond sports mouthguards, including incorporation into Dental Drug Delivery Systems or orthodontic devices, that use EVA sheets.

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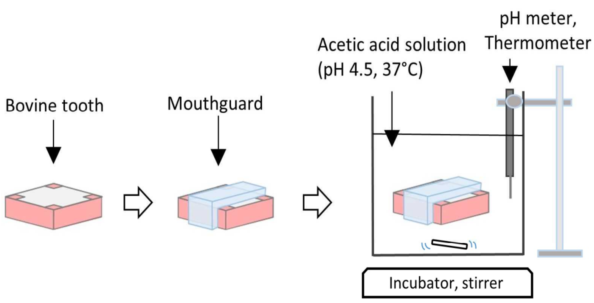

Fig. 1 Demineralization study protocol.

Red-colored area on each enamel block was covered with acid-resistant nail varnish.

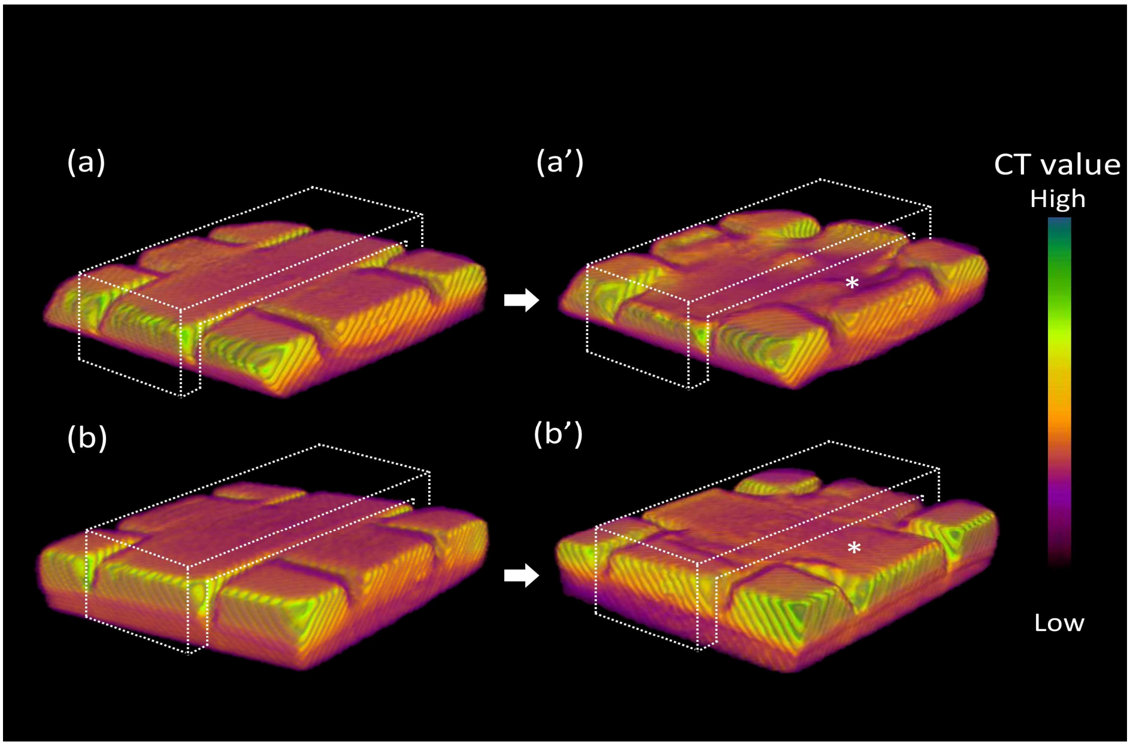

Fig. 2 Color mapping of 3D CT values of enamel blocks before and after demineralization

Block (a)/(b) and block (c)/(d) are the same pieces before/after demineralization, respectively. For control mouthguards, demineralization occurred in a widespread area, including areas immediately under the mouthguard, and uncovered areas marked with an *(b). In contrast, samples covered with mouthguards containing S-PRG filler showed only mild demineralization in the enamel block, both directly under the mouthguard and in uncovered areas marked with an * (d)

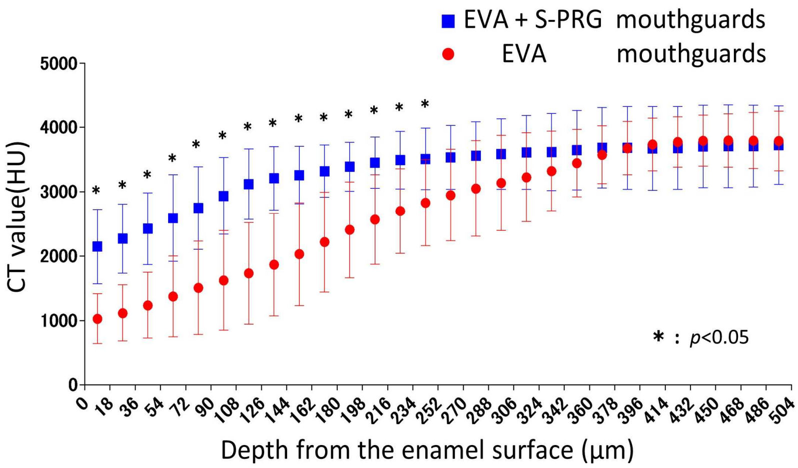

Fig. 3 Depth from the enamel surface and CT values