SEM Observation of Human Enamel and Resin Tag Formation After Phosphoric Acid Etching: Influence of Saliva Contamination

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Abstract
The objectives of this study were to observe the surface appearance as well as resin tag formation on acid-etched human enamel using field-emission scanning microscopy (FE-SEM) and to study the effects of saliva contamination and repeat etching. Phosphoric acid etching produced a finely roughened enamel surface with random arrangement of enamel crystals. Dissolution of both enamel prisms and peripheries was observed. FE-SEM observation reveals a large amount of remaining contaminated saliva on the etched enamel and subsequently no resin tag formation. Repeat etching after saliva contamination incompletely removed the contaminated saliva and produced less density of resin tags. The results of SEM observation may provide an explanation for the results of shear bond strengths; that is, a large amount of contaminated saliva remaining on the etched enamel surface and incomplete removal of contaminated saliva after repeat etching result in the decrease of shear bond strength of 4-META/MMA-TBB resin to enamel.

Keywords:
SEM, human enamel, resin tag, phosphoric acid etching, saliva

Introduction
Superbond C&B (Sunmedic Co Ltd, Shiga, Japan), a 4-methacyloxyethyl trimellitate anhydride (4-META)/methyl methacrylate (MMA)-tri-n-butyl borane (TBB) resin, is a unique MMA-based adhesive resin cement that has been widely used for bonding orthodontic brackets to enamel (1-5). This resin cement is known as C&B Metabond (Parkell Inc, Farmingdale, NY) in North America. Tight bonding is achieved by 65 wt% phosphoric acid etching. Previous study showed that variation of the concentration of phosphoric acid from 20 to 65 wt% produced no difference in strength between 4-META/MMA-TBB resin and etched enamel although demineralization decreased with increasing concentration of phosphoric acid (6). Thus, the manufacturer recommended the application of 65 wt% phosphoric acid etchant for the adhesion of 4-META. MMA-TBB resin to enamel to minimize the enamel loss.

It is very difficult to avoid completely saliva and blood contamination of cleansed and dried enamel surfaces when bonding attachments to impacted or lingual tooth surfaces. Various studies have reported the reduction of shear bond strength when resin is directly bonded to saliva-contaminated etched enamel (7-9). Itoh et al. (10) reported that saliva and blood contamination decreased the bond strength of 4-META/MMA-TBB resin in orthodontic brackets bonded to the anterior surface of bovine teeth etched with phosphoric acid. Arita et al. (11) also reported that saliva contamination decreased the shear bond strength of orthodontic brackets with 4-META/MMA-TBB resin to etched bovine or human enamel.

On the basis of aforementioned results, we designed an experiment to investigate the effect of saliva contamination in bonding orthodontic brackets to human enamel using 4-META/MMA-
TBB resin. In a previous study (12), we demonstrated that saliva contamination significantly decreased the bond strength of 4-META/MMA-TBB resin to etched enamel, and repeat acid etching did not completely recover the initial bond strength.

To learn more about the influence of saliva contamination, we observed the surface of acid-etched and saliva-contaminated human enamel as well as resin tag formation on etched and saliva-contaminated enamel using field-emission scanning microscopy (FE-SEM). The influence of repeat etching after saliva contamination on the surface appearance and resin tag formation was also investigated by FE-SEM observation.

**Materials and Methods**

This study used human premolar teeth. The tooth surfaces were cleansed and then polished with pumice and rubber prophylactic cups for 10 seconds. The phosphoric acid etching gel (65 wt%) was included in the kit of 4-META/MMA-TBB resin (Sunmedical Co., Ltd. Shiga, Japan).

**Observation of human enamel surface**

Protocol 1: Phosphoric acid etching.

The teeth were etched with 65% phosphoric acid gel for 30 seconds, washed for 20 seconds, and air-dried.

Protocol 2: Phosphoric acid etching followed by saliva contamination.

The teeth were etched with 65% phosphoric acid gel for 30 seconds. After rinsing and drying, the etched surface was contaminated with 20 μl of fresh human saliva. The contaminant was left on the surface for 30 seconds to simulate extremely severe clinical conditions. The saliva was then blown off with air for 5 seconds.

Protocol 3: Phosphoric acid etching followed by saliva contamination and repeat phosphoric acid etching.

The teeth were etched with 65% phosphoric acid gel for 30 seconds. After rinsing and drying, the etched surface was contaminated with 20 μl of fresh human saliva, which was left on the surface for 30 seconds. After blowing off the saliva with air for 5 seconds, the contaminated teeth were re-etched with 65% phosphoric acid gel for 30 seconds.

After each treatment, the specimen was dehydrated through a graded series of ethanol, dried in a critical drying apparatus, and ion-coated with platinum. The surface appearance of human enamel after each treatment was observed using a field-emission scanning electron microscope (FE-SEM; JSM-6340-F, JEOL, Tokyo, Japan).

**Observation of resin tags formation**

After each treatment, as mentioned above (Protocol 1, 2 and 3), 4-META/MMA-TBB resin cement was applied to each enamel surface. The catalyst, a partly oxidized TBB initiator, was added to the monomer mixture of 4-META and MMA to prepare an activated polymerized monomer liquid and mixed. Then the monomer-polymer mixture was applied on the enamel surface using the brush-dip technique. After the resin was cured, the specimen was immersed into 4mol/L HCl for 4 days and then immersed in 10 wt% NaOCl solution. The enamel part was completely dissolved, and the remaining bonded resin was rinsed with water and dried in a desiccator under the vacuum. The resin specimens were ion-coated with platinum, and the formation of resin tag was observed using FE-SEM.

**Results**

**Observation of human enamel surfaces**

Figure 1 shows the FE-SEM micrographs of enamel surfaces that had been (a) etched with phosphoric acid, (b) etched with phosphoric acid followed by saliva contamination, and (c) etched with phosphoric acid followed by saliva contamination and repeat etching with phosphoric acid.

Phosphoric acid etching produced a finely roughened enamel surface with random arrangement of enamel crystals (Fig. 1a). Dissolution of both enamel prisms and peripheries was observed. When etched enamel was contaminated with saliva after phosphoric acid etching, most of the enamel surface was
covered with saliva but not completely. The finely roughened enamel surface was observed in some areas (Fig. 1b). Repeat phosphoric acid etching after the saliva contamination did not remove the contaminated saliva on the enamel surface. The repeat-etched surface showed greater roughness than the contaminated surface (Fig. 1c), but was less rough than the original etched surface.

**Discussion**

Few reports have examined the surface appearance of enamel and formation of resin tags after phosphoric acid etching with the saliva contamination observed by scanning electron microscopy. Itoh et al. (10) reported that resin tag formation was influenced by saliva contamination. They used bovine enamel polished with 600-grit SiC paper and not the outer human enamel surface, which was bonded to brackets.

Silverstone et al. (13) classified the etched enamel into three types. Type 1 shows the preferential dissolution of the enamel prism core after phosphoric acid etching. Type 2 shows the preferential dissolution of prism peripheries, and type 3 shows the random
pattern and the mixture of type 1 and type 2. In the present study, the human premolar enamel surface shows Silverstone’s type 3 pattern after 65 wt% phosphoric acid etching.

We investigated the influence of saliva contamination and repeat phosphoric acid–etching after saliva contamination on the enamel surface appearance and resin tag formation. It is generally recognized that the main contribution to the bond strength of dental resin materials to enamel is the mechanical interlocking of cured resin that is formed on the roughened enamel surface (14, 15).

SEM observation revealed the presence of contaminated saliva on phosphoric acid–etched enamel. Our previous study (12) showed that saliva contamination significantly decreased the shear bond strengths of the phosphoric acid–etched enamel surface. The present findings suggest that the decrease in shear bond strength is due to the remaining saliva on the etched enamel and, subsequently, no resin tag formation. Repeat etching after saliva contamination did not significantly improve the bond strength. This may be due to the incomplete removal of contaminated saliva by repeat phosphoric acid etching and also incomplete formation of resin tags.

The results of SEM observation may provide an explanation for the results of shear bond strengths; that is, the large amount of contaminated saliva remaining on the etched enamel surface and incomplete removal of contaminated saliva after repeat etching resulted in the decrease in the shear bond strengths under three conditions.

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References


