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Effect of application time of colloidal platinum nanoparticles on the microtensile bond strength to dentin

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The purpose of this study was to investigate the effect of application time of colloidal platinum nanoparticles (CPN) on bond strength. Dentin surfaces were subjected to one of the following treatments: (A) Etching with 10% citric acid-3% $FeCl_3$ solution (10-3 solution); (B) Etching with 10-3 solution followed by applying CPN as a primer solution for 10, 20, 30, or 60 seconds; and (C) Priming with CPN for 10, 20, 30, or 60 seconds followed by etching with 10-3 solution. An acrylic rod was bonded to each treated dentin surface using 4-META/MMA-TBB resin. Bonded specimens were sectioned into beams for microtensile bond strength testing. In groups (B) and (C), highest bond strength was obtained when dentin surfaces were treated with CPN for 30 seconds. This meant that the CPN primer solution either enhanced the penetration of resin into dentin or the degree of conversion of 4-META/MMA-TBB resin. Within the limitations of this study, treatment with 0.1 mN CPN primer solution followed by 20 seconds of water rinsing resulted in high bond strength.

Keywords: Colloidal platinum nanoparticles, Microtensile bond strength, 4-META/MMA-TBB resin

INTRODUCTION

Nanotechnology is widely believed to be the future technology which could have countless potential and innumerable applications. In medical sciences, biocompatible nanomaterials such as carbon nanotubes, fullerene, and imogolite have already found a niche application area as scaffold materials to promote cell growth and tissue regeneration[1-4]. In dentistry, nanotechnology has led to the development of nanofilled resin composites which contain nanosized filler particles that can be dissolved in higher concentrations[5-9], enabled the deposition of hydroxyapatite on enamel[10], and currently being investigated for the potential of improving and prolonging bond durability between tooth structure and adhesive resin[11-13].

Amongst the synthesized nanomaterials are the colloidal platinum nanoparticles (CPN), a colloidal suspension of nanosized platinum particles in water and with sodium citrate as a protectant. CPN is currently used in the manufacture of cosmetics and has been investigated as a therapeutic agent against pulmonary inflammation[14]. CPN is a new class of antioxidant that has been shown to efficiently quench reactive oxygen species[14]. Oxidation reactions can produce free radicals, which in turn can start chain reactions that damage cells. Antioxidants terminate these chain reactions by getting rid of free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. In a recent research by Kajita *et al.*[15], it was demonstrated that CPN suppressed the propagation of lipid peroxidation. Apart from exhibiting antioxidant properties, CPN is a SOD/catalase mimetic. Hence, CPN may also be useful for the treatment of oxidative stress- and aging-related diseases[16].

Besides, platinum is a low-allergy metal and is widely used as a catalyst in diverse applications. For example, it is used to convert methyl alcohol vapors (CH_4O) into formaldehyde (CH_2O) upon contact, glowing red hot in the process —an effect used to make small hand warmers. In catalytic converters, a device found in the exhaust system of most cars, platinum is used to combine carbon monoxide and unburned fuel from a car's exhaust with oxygen from the air, forming carbon dioxide and water[17]. In fuel cells, platinum is used as a catalyst to combine hydrogen and oxygen to produce electricity and water. Further, in the quest to increase catalytic activity, metal nanoparticles have been developed due to the advantage of larger surface area which can be attained with smaller particles[15].

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Hence, when the inherent catalytic properties of platinum are coupled with the nanoparticles' larger surface area for reactivity, CPN works as a strong catalyst.

On the possible use of nanotechnology to improve dentin bond strength, our previous study showed that the bond strength nearly doubled when 4-META-MMA/TBB resin was used in combination with CPN[18]. However, the highest bond strength was obtained when 0.1 mM CPN as a primer solution was applied for 60 seconds followed by water rinsing of 20 seconds[18]. In any clinical setting, a primer application time of 60 seconds is deemed unrealistically long. Typically, the clinical primer application time ranges between 10 to 30 seconds.

Therefore, the purpose of this study was to investigate the effect of a clinically shorter application time of CPN (10, 20, or 30 seconds) on the bond strength between dentin and 4-META/MMA-TBB resin. The null hypothesis tested was that there would be no statistically significant differences in bond strength among the different application times of CPN examined in this study (10, 20, 30, and 60 seconds).

MATERIALS AND METHODS

Materials used

The materials used in this study are shown in Table 1. The surface treatment agents were Green Conditioner (10-3 solution) (Sun Medical, Moriyama, Japan) and CPN (Apt., Tokyo, Japan). The 10-3 solution was composed of 10% citric acid and 3% $FeCl_3$, while CPN was composed of 0.02% platinum, 0.29% sodium citric acid, and 99.69% water. CPN was weakly acidic with a pH value of 6.5−6.7 and that it did not have any second phase of aggregation. Average diameter of the platinum particles was approximately 2 nm.

The resin luting agent used was Super-Bond C&B (Sun Medical, Moriyama, Japan), which contained tri-*n*-butylborane (TBB) as the initiator. Decomposition of TBB resulted in free radicals being formed in the presence of oxygen and water. These free radicals, which acted as polymerization initiators, then promoted polymerization on the dentin surface which contained water. The monomer mixture consisted of 5% 4-META and 95% MMA, and the PMMA polymer was in powder form.

Specimen preparation

1. Tooth specimens

Eighteen extracted human third molars were stored in 0.5% chloramine T and used within 6 months after extraction. The protocol of this research was approved by the Hokkaido University Ethics Committee.

Using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, USA), the extracted molars were sectioned above the mid-coronal portion to create crown segments. The occlusal dentin surface of each crown segment was then polished with 600-grit silicon carbide paper under running water. All the 18 tooth specimens were randomly divided into one control group and eight experimental groups, with two tooth specimens per group.

2. Surface treatments and bonding procedure

The polished dentin surfaces were subjected to one of the surface treatments:

A) Etching with 10-3 solution followed by water rinsing (Control);
B) Etching with 10-3 solution and then water-rinsing. This is followed by application of 0.1 mM CPN as a primer solution for 10, 20, 30, or 60 seconds and then water-rinsing for 20 seconds (Etch-Pt);
C) Priming with 0.1 mM CPN for 10, 20, 30, or 60 seconds and then water-rinsing for 20 seconds. This is followed by application of 10-3 solution (Pt-Etch).

Dentin specimens subjected to one of the surface treatments above were each bonded to an acrylic rod using hand-mixed Super-Bond C&B.

Microtensile bond strength test

After storage in 37°C water for 24 hours, each bonded specimen per group was sectioned into 15 beams (cross-sectional area: 1 mm^2) using an Isomet diamond saw. Microtensile bond strength testing was carried out using a portable test machine (EZ Test, SHIMADZU, Kyoto, Japan) at a crosshead speed of 1 mm/min until failure occurred. Microtensile bond strength was expressed in MPa, and all data were analyzed by the

Table 1 Materials used in this study

Product (Manufacturer)	Composition (batch number)
Green Conditioner (10-3 solution) (Sun Medical, Moriyama, Japan)	10% citric acid, 3% ferric chloride, water (RT1)
Colloidal Platinum Nanoparticles (Apt., Tokyo, Japan)	0.02% platinum, 0.29% sodium citric acid, 99.69% water (231080821)
Super-Bond C&B (Sun Medical, Moriyama, Japan)	Initiator: Tri-*n*-butylborane (RX31) Monomer: 5% 4-META, 95% MMA (SE2) Powder (Clear): PMMA powder (RV32)

Tukey's and Games-Howell method (n=15; $p<0.05$).

SEM observation

1. Observation of the resin-dentin interface

To observe the morphology of the resin-dentin interface, dentin surfaces from nine tooth specimens were prepared and applied with hand-mixed Super-Bond C&B resin in the same manner as for microtensile bond strength testing. The resin-bonded specimens were sectioned perpendicular to the adhesive interface, using an Isomet saw, to obtain slabs of 2 mm thickness. The cut surfaces were sequentially polished with 600-, 800-, and 1000-grit silicon carbide papers under running water. This was followed by polishing sequentially with 6-, 3-, and 1-µm diamond pastes (DP-Paste, Struers, Tokyo, Japan), whereby cleaning with an ultrasonic device was done between each diamond paste polish.

After polishing, the specimens were immersed in 1 M hydrochloric acid for 30 seconds and 5% sodium hypochlorite for 5 minutes, followed by rinsing with water. After drying, the specimens were sputter-coated with Pt-Pd for 150 seconds. The resin-dentin interfaces were then observed using a scanning electron microscope (SEM; S-4000, Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV.

2. Observation of the conditioned surface

Approximately 1-mm-thick occlusal dentin slices from nine tooth specimens were sectioned perpendicular to the long axis of each tooth using an Isomet saw. The cut surfaces were polished with 600-grit silicon carbide paper under running water. Surface treatments were as per those performed for microtensile bond strength testing. After surface conditioning, the specimens were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for at least 12 hours at 4°C. Rinsing was done with 0.2 M sodium cacodylate buffer at pH 7.4 for three changes of 1 hour each, following by rinsing with distilled water for 1 minute.

Dehydration was performed in ascending concentrations of ethanol (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 95% for 30 minutes, and 100% for 60 minutes), followed by immersion in hexamethyldisilazane (HMDS)[19-21] for 10 minutes. Air-drying was done at room temperature on a filter paper inside a covered glass dish underneath the hood. Air-dried specimens were sputter-coated with Pt-Pd for 150 seconds and then observed using a SEM (S-4000, Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV.

X-ray photoelectron spectroscopy analysis

To analyze the intensity ratio of Pt on the dentin surface, approximately 1-mm-thick occlusal dentin slices were sectioned parallel to the adhesive interface using Isomet. After the dentin surfaces were polished with 600-grit silicon carbide paper under running water, surface treatments as per those for microtensile bond strength testing were performed. After air-drying at room temperature, surface composition was determined by X-ray photoelectron spectroscopy (XPS; JPC-9010, JEOL). XPS was used because it reveals the composition and chemical state of a material's surface.

XPS measurements were performed using a monochromatic Mg Kα X-ray source under these conditions: operating pressure at 10^{-6} Pa, emission current at 10 mA, and accelerating voltage at 10 kV. Binding energy was calibrated using the C 1s peak at 284.5 eV.

RESULTS

Microtensile bond strength

Figures 1 and 2 show the microtensile bond strength results. In Fig. 1, Etch-Pt(30) and Etch-Pt(60) showed significantly higher bond strengths than the control, Etch-Pt(10), and Etch-Pt(20). However, there were no significant differences in bond strength between Etch-Pt(30) and Etch-Pt(60).

In Fig. 2, Pt(30)-Etch and Pt(60)-Etch showed significantly higher bond strengths than the control and Pt(10)-Etch. There were no significant differences in bond strength between Pt(30)-Etch and Pt(60)-Etch, but the control showed a significantly lower bond strength than all the Pt-Etch groups.

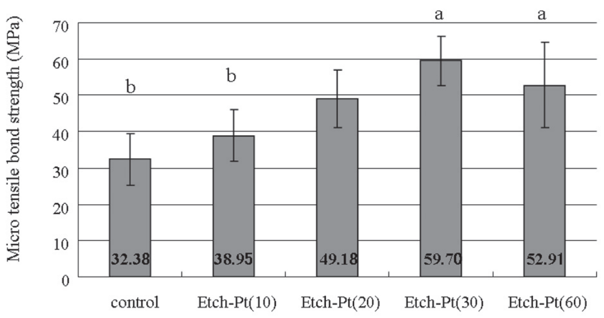

Fig. 1 Microtensile bond strength results of Group (B). Same letters indicate no statistically significant differences ($p>0.05$).

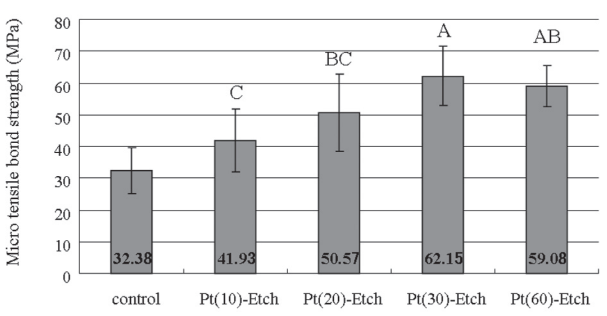

Fig. 2 Microtensile bond strength results of Group (C). Same letters indicate no statistically significant differences ($p>0.05$).

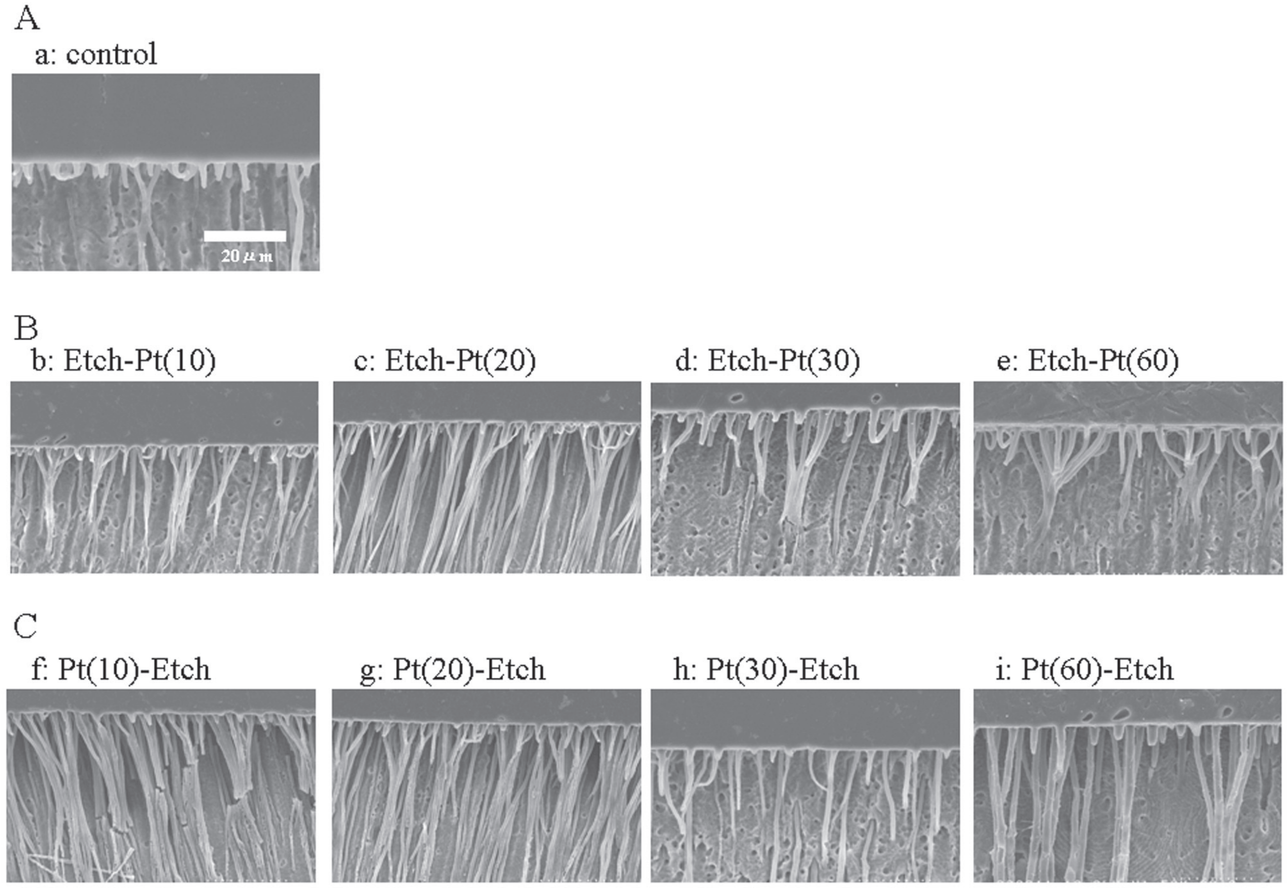

Fig. 3 SEM images of the resin-dentin interfaces of: (a) control; (b) Etch-Pt(10); (c) Etch-Pt(20); (d) Etch-Pt(30); (e) Etch-Pt(60); (f) Pt(10)-Etch; (g) Pt(20)-Etch; (h) Pt(30)-Etch; and (i) Pt(60)-Etch (magnification ×1,500)

SEM observation

Figure 3 shows the SEM images of the resin-dentin interfaces. For the control (Fig. 3a), the hybrid layer was about 2–3 μm thick. For groups (B) and (C), the hybrid layer was also 2–3 μm thick —regardless of the application time of CPN, and no morphological differences could be observed at this magnification. On resin tag formation, denser and longer resin tags that penetrated deeper were formed in group (B) (Figs. 3b–e) and group (C) (Figs. 3f–i) when compared to the control (Fig. 3a). Therefore, in general, CPN application seemed to result in denser and longer resin tag formation than the control surface treatment.

Figure 4 shows the SEM images of the conditioned surfaces. For the control (Fig. 4a), the smear layer was removed with the 10-3 solution. The dentinal tubules were exposed, and collagen fibrils were seen on the intertubular dentin surface. For group (B), the intertubular dentin surface was smooth compared to the control and that it appeared to be partially covered with a film-like structure (Figs. 4b–e). However, surface roughness could not be clearly determined at this magnification. As for the peritubular dentin layers of Etch-Pt(20) and Etch-Pt(30), they were slightly dissolved.

For group (C), their surface morphology (Figs. 4f–i) seemed to be similar to that of group (B).

XPS analysis

Figure 5 shows the XPS wide scan spectra of the conditioned dentin surfaces. The peak of Pt 4f7/2 was expected to appear around 71.2 eV, but no Pt peak was detected on the control surface. The intensity ratios of Pt to O and Pt to N of groups (B) and (C) are shown in Figs. 6 and 7 respectively. Although Pt/O is the intensity ratio generally used, the Pt/N intensity ratio was also used in this study. This was because O was also present in the 10-3 solution, but N was specfically present in collagen.

Amongst the conditioned dentin surfaces, Etch-Pt(60) exhibited the strongest Pt peak. As for Etch-Pt(10), Etch-Pt(20), Etch-Pt(30), Pt(30)-Etch, and Pt(60)-Etch, they exhibited weaker Pt peaks than Etch-Pt(60) but clearly stronger Pt peaks than Pt(10)-Etch and Pt(20)-Etch. Since Pt peak was detected in all the groups except the control, it showed that platinum remained on the dentin surface after water rinsing.

In a preliminary study, the presence of platinum

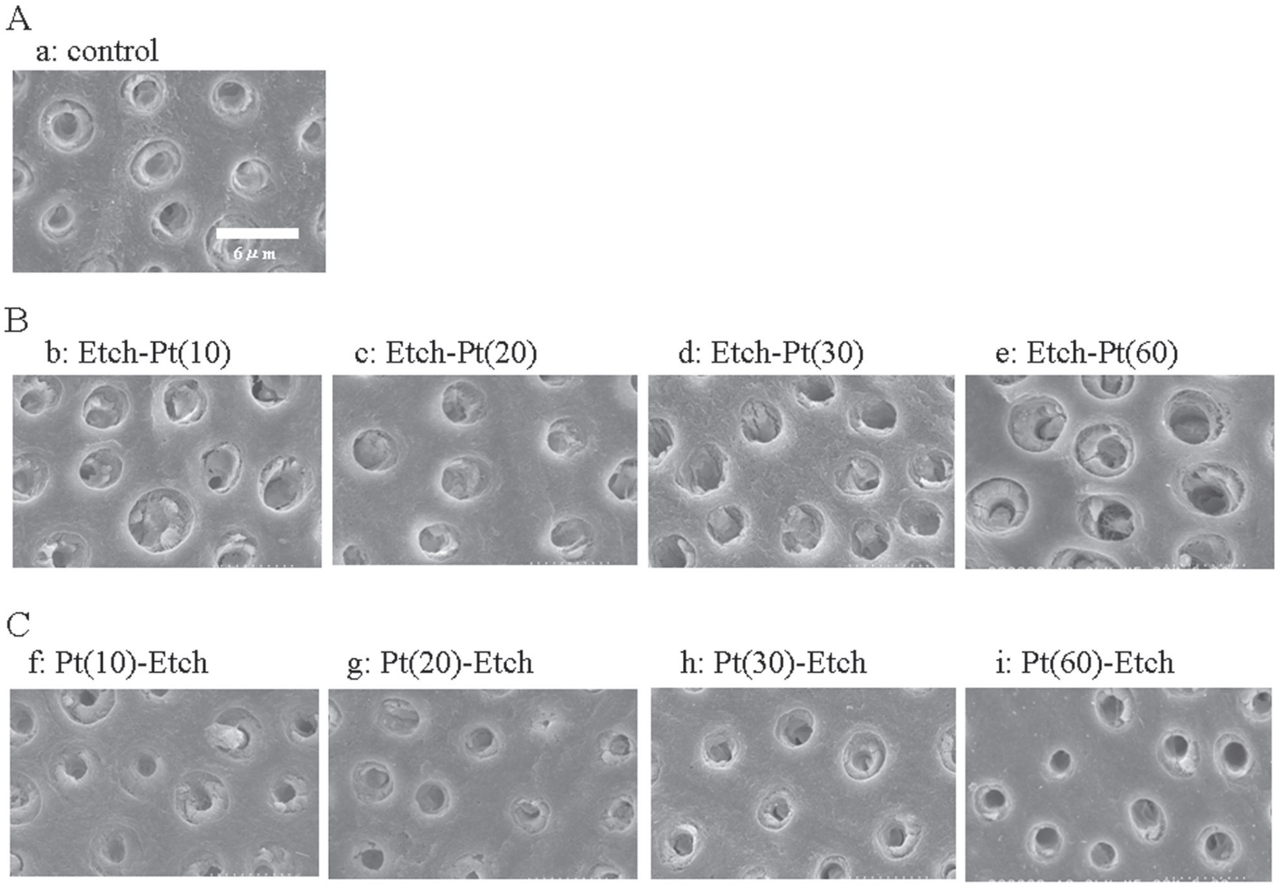

Fig. 4 SEM images of the conditioned surfaces of: (a) control; (b) Etch-Pt(10); (c) Etch-Pt(20); (d) Etch-Pt(30); (e) Etch-Pt(60); (f) Pt(10)-Etch; (g) Pt(20)-Etch; (h) Pt(30)-Etch; and (i) Pt(60)-Etch (magnification ×5,000)

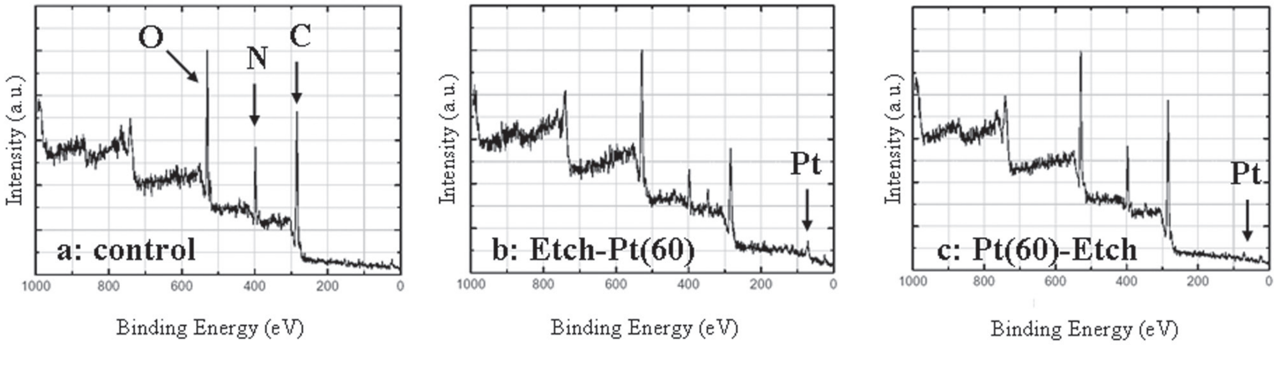

Fig. 5 XPS wide scan spectra of: (a) control; (b) Etch-Pt(60) as representative of group (B); and (c) Pt(60)-Etch as representative of group (C)

on the dentin surfaces failed to be detected using energy dispersive X-ray spectroscopy (Genesis Spectrum, EDAX Inc., Mahwah, USA). This showed that XPS was an appropriate and useful technique in the detection of small amounts of platinum in this study.

DISCUSSION

Based on the microtensile bond strength results, the use of CPN seemed to significantly ($p<0.05$) increase the bond strength between 4-META-MMA/TBB resin and the dentin substrate, except for Etch-Pt(10). This improvement in resin-dentin bond strength was even

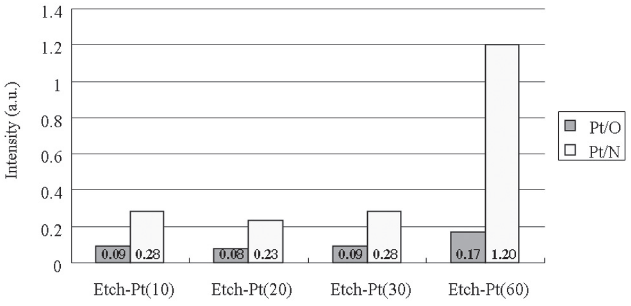

Fig. 6 XPS peak intensity ratios of Pt/O and Pt/N of group (B)

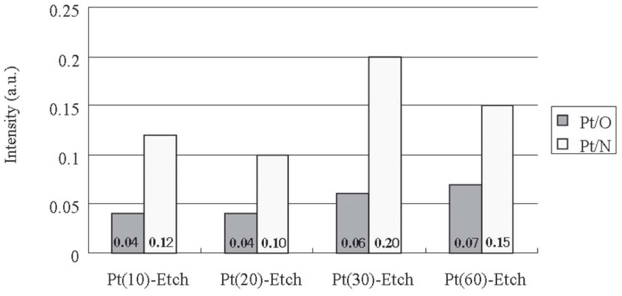

Fig. 7 XPS peak intensity ratios of Pt/O and Pt/N of group (C)

observed for group (C) specimens, which meant that the effect of CPN primer pretreatment remained even after etching with 10-3 solution.

For adhesion between 4-META/MMA-TBB resin and dentin, the hybrid layer plays an important role in the adhesion mechanism[22]. However, no morphological differences between the control and CPN application groups were observed from the relatively low magnification SEM images. Therefore, the increased bond strength with CPN application might be due to one or more of these factors: increased infiltration of resin, enhanced conversion of adhesive resin in the hybrid layer, or denser and longer resin tag formation.

Increased adhesive resin infiltration into the hybrid layer might be caused by an increased wettability of the resin[23,24]. As for the enhanced conversion of adhesive resin in the hybrid layer, it might be caused by the platinum catalyst in CPN. For example, polysiloxane with a vinyl group (CH_2=CH−) is used as the basic material of silicone rubber, and the material with silicon hydride (Si-H) is utilized as a hardening agent. The addition cure mechanism, known as hydrosilylation, involves the addition of a silicon hydride to an unsaturated carbon-carbon bond in the presence of platinum catalysts[25,26]. The vinyl double bond on the polymer interacts with the platinum center, which has one free coordination site, and activates the double bond[27]. In the case of CPN, the platinum nanoparticles were capable of exhibiting stronger catalytic activity due to the advantage of a larger surface area that was attained with smaller particles. It is noteworthy that for a resin composite, the degree of conversion affects and accounts for its many properties, such as mechanical properties, solubility, dimensional stability, and biocompatibility[28]. Thus, an increase in the degree of conversion would correlate to improvements not only in the physical and mechanical properties of a resin composite, but also in bond durability. However, it is very difficult to measure wettability and conversion of resin within a hybrid layer. Therefore, further studies are needed to confirm this hypothesis that CPN primer application resulted in increased resin infiltration and conversion of adhesive resin.

On resin tag formation, the resin tags observed for CPN application groups (B) and (C) seemed to be longer and penetrate deeper into the dentinal tubules[29] than the control. Apart from hybrid layer thickness, resin tag length and density are also important factors that affect the quality of the bond[30,31]. However in this study, there seemed to be an apparent lack of direct correlation between resin tag formation and bonding performance. When comparing between Etch-Pt(20) (Fig. 3c) and Etch-Pt(30) (Fig. 3d), the resin tags in Etch-Pt(20) seemed to be longer and denser than in Etch-Pt(30) —but bond strength in Etch-Pt(30) was higher than in Etch-Pt(20). Therefore, further research seems inevitable to clarify this relationship.

On the adsorption behavior of platinum on superficial dentin, XPS analysis confirmed the presence of platinum on the dentin surfaces of CPN application groups (B) and (C). For group (B), it is interesting to observe that 10 seconds of CPN application time seemed to be sufficient to attain Pt/O and Pt/N intensity ratios which were similar to the other application times, such that 60 seconds of CPN application time might not be necessary to ensure a higher adsorbed amount of platinum on the conditioned dentin surface. As for group (C), 10 and 20 seconds of CPN application time might be too short for adequate platinum adsorption. This difference in platinum adsorption behavior for the same CPN application time was further seen in the XPS wide scans of groups (B) and (C). At the same CPN application time, the Pt peak of group (B) was of a higher intensity than group (C). This could be because CPN was applied across the smear layer in group (C). In other words, apart from CPN application time, the application protocol (*i.e.*, CPN primer treatment after 10-3 solution etching *versus* CPN primer pretreatment before 10-3 solution etching) had an effect on platinum adsorption to the dentin surface.

Putting together the microtensile bond strength results and XPS analysis results, this study demonstrated that bond durability was not influenced by the amount of platinum adsorbed on the dentin surface. However, it was clear that a longer CPN application time might result in a higher bond strength than a relatively short application time of 10 seconds. Thus, it remained to be established the relationship between the amount of platinum adsorbed on the dentin surface and CPN application time —and their combined effect on bond strength. Further on bond durability, it was reported that CPN application increased the bond strength of a Bis-GMA-based resin— although the mechanism of how bond strength was improved remained unclear[32,33]. The Bis-GMA-based resin comprised a camphorquinone initiator which began the polymerization reaction when irradiated by a light of 470 nm wavelength, thereby generating free radicals. It was probable that the free radical polymerization was catalyzed by the platinum nanoparticles of CPN. Indeed, while the results of the present study and published studies[11-13,16,33] unanimously showed that CPN application increased bond strength, further studies and different tools would be needed to investigate if dentin bonding improvement arose from increased resin conversion (*i.e.*, evaluation by Raman spectroscopy) or increased resin infiltration (*i.e.*, evaluation by transmission electron microscopy), or other factors that are yet to be identified.

Notably, microtensile bond strength results in Figs. 1 and 2 seemed to suggest the likelihood of an appropriate CPN application time, which would be longer than 10 or 20 seconds, in order to achieve high bond strength. Thus, the null hypothesis that there would be no differences in bond strength among the different application times of CPN was rejected. While the mechanism for long-term durability[34,35] remains to be investigated, short CPN application time and rinsing time would continue for daily clinic use for practical reasons. Besides, it is also important to examine the biocompatibility of CPN. Although platinum is a food additive certified for consumption by the Ministry of Health, Labor and Welfare in Japan, nanosized platinum particles might acquire a new chemical character. This meant that CPN should be treated as a new material, just like radioisotopes are treated as a different material from the other material forms of the selfsame element.

CONCLUSIONS

Results of this study showed that:

1. Higher bond strengths than the control were obtained in this surface treatment regime: etching with 10-3 solution followed by water rinsing; application of 0.1 mM CPN as a primer solution for 10, 20, 30, or 60 seconds followed by water rinsing for 20 seconds.
2. Higher bond strengths than the control were obtained in this surface treatment regime: primer pretreatment with 0.1 mM CPN for 20, 30, or 60 seconds, water rinsing for 20 seconds, followed by etching with 10-3 solution.

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