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Expansion of Nanotechnology for Dentistry: Effect of Colloidal Platinum Nanoparticles on Dentin Adhesion Mediated by 4-META/MMA-TBB

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Purpose: To investigate the effect of Colloidal Platinum Nanoparticles (CPN) on the bond strength between dentin and 4-META/MMA-TBB resin using different concentrations of CPN.

Materials and Methods: Twenty-five extracted human third molars were stored in 0.5% chloramine T. The occlusal dentin slices were prepared by grinding occlusal surfaces of each tooth and polishing with 600-grit silicon carbide paper under running water. One control and four experimental groups (2 specimens per group) were used as follows: a) dentin surfaces treated with 10-3 solution, followed by rinsing with water and subsequently an acrylic rod bonded with hand-mixed 4META/MMA-TBB resin (Super-Bond C&B, Sun Medical) (control); b) dentin surfaces treated with 10-3 etching solution, followed by rinsing with water and application of CPN (100% or 10%) as a primer solution for 60 s and rinsed with water for 20 s, then an acrylic rod bonded with Super-Bond C&B(Etch-CPN [100% or 10%]); c) dentin surfaces treated with CPN (100% or 10%) for 60 s, rinsed with water for 20 s, followed by application of 10-3 solution, then an acrylic rod bonded with Super-Bond C&B (CPN-Etch [100% or 10%]). After storage in 37°C water, specimens were sectioned into beams (cross-sectional area: 1 mm^2) for microtensile bond strength testing at a crosshead speed of 1mm/min. The data were analyzed using the Games-Howell method ($p < 0.05$; $n = 15$).

Results: Etch-CPN (100), CPN-Etch(100) and CPN-Etch (10) showed significantly higher bond strengths compared to the control. When using 10% CPN, the highest bond strength was demonstrated. The bond strength of 4META/MMA-TBB resin was approximately doubled by CPN application.

Conclusion: The results of this study showed that higher bond strengths are obtained when treating dentin with a lower concentration of CPN. Further evaluation to optimize conditions such as the application time and rinsing time are required.

Keywords: dentin adhesion, nano-technology, microtensile bond strength, 4META/MMA-TBB resin.

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Nanotechnology is increasingly considered to be the future of technology,[14] and its possible applications are immense. Nanotechnology is considered to be the fourth technological advance after information technology, biotechnology and ecology. Nanotechnology is now used in the chemical,[7] machine,[2] electronic,[23] environmental,[6] energy,[5] and medical[17] industries. In dentistry, nanotechnology is applied to fillers of composite resins,[1,8,22] carbon nanotubes for living organisms,[24] as well as in tooth adhesion. The possibility of doubling the bond strength of 4META-MMA/TBB resin by using Colloidal Platinum Nanoparticles (CPN, Apt; Tokyo, Japan) has been previously suggested.[10]

Platinum (Pt) is known as a low-allergenic metal and it is also a strong catalyst in chemical reactions. It has been employed in this application since the early 1800s, when platinum powder was used to catalyze the ignition of hydrogen. Platinum metal also strongly catalyzes the decomposition of hydrogen peroxide into water and oxygen gas. In the laboratory, platinum wire is used for electrodes, and platinum pans are used in thermogravimetric analysis. Platinum is also used as an alloying agent for various metal products, including fine wires, noncorrosive laboratory containers, medical instruments, dental prostheses, electrical contacts, and thermocouples.

CPN quench reactive oxygen species, and are expected to be one of the next generation of antioxidants.[4] However, Nagano et al[9] showed that CPN did not work as a scavenger in tooth adhesion, although there are some reports about the scavenger effect of sodium ascorbic acid causing recovery of the bond strength between 4-META/MMA-TBB resin and dentin which had been reduced by using sodium hypochlorite. When considering the effect of catalytic activity of platinum, CPN could potentially work as an excellent catalyst for bonding of 4META/MMA-TBB resin. CPN would accelerate chemical reactions of addition polymerization.

The null hypothesis to be tested is that there is no difference in the mean bond strength between the use of 100% CPN and 10% CPN. Therefore, the purpose of this study was to investigate the effect of CPN on the bond strength between dentin and 4-META/MMA-TBB resin using different concentrations of CPN.

MATERIALS AND METHODS

Specimen Preparation

The materials used in this study are shown in Table 1.

Table 1 Materials used in this study

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

The protocol was approved by the Hokkaido University Ethics Committee. Twenty-five extracted human third molars were stored in 0.5% chloramine T and used within 6 months after extraction. The occlusal dentin surfaces were prepared by cutting the mid-coronal portion of the crown segments using a low-speed diamond saw (Isomet 1000, Buehler; Lake Bluff, IL, USA) and polishing with 600-grit silicon carbide paper under running water.[20] The bonding procedures used in this study are shown in Table 2.

Two teeth per group were used. One control and four experimental groups were used as follows: a) dentin surfaces treated with 10-3 solution (Green Conditioner, Sun Medical; Moriyama, Japan), followed by rinsing with water, drying by air blowing for 3 s, and subsequently bonding an acrylic rod with hand-mixed 4META/MMA-TBB resin (Super-Bond C&B, Sun Medical) (control); b1: dentin surfaces treated with 10-3 solution, followed by rinsing with water and application of CPN (100%) as a primer solution for 60 s, rinsing with water for 20 s, drying by air blowing for 3 s, then bonding an acrylic rod with Super-Bond C&B (Etch-CPN [100]); b2: same as b1, but with 10% CPN (Etch-CPN [10]), c1: dentin surfaces treated with CPN (100% or 10%) for 60 s, rinsed with water for 20 s, followed by application of 10-3 etching solution, rinsing with water, drying by air blowing for 3 s, then bonding an acrylic rod with Super-Bond C&B (CPN-Etch [100]). c2: same as c1 but with 10% CPN (CPN-Etch[10]).

Microtensile Bond Strength

After storage in 37°C water for 24 h, the bonded specimens were sectioned into beams (cross-sectional area: 1 mm^2) using an Isomet diamond saw. Fifteen beams from 2 teeth were prepared per group. Microtensile bond strength testing was carried out using a portable testing machine (EZ-test, Shimadzu; Kyoto, Japan) at a crosshead speed of 1 mm/min until failure occurred.[15] The microtensile bond strength was expressed in MPa, dividing the force (N) at failure by the bonded area (mm^2). The data were analyzed using the Games-Howell method ($p < 0.05$; $n = 15$).

SEM Observation of the Resin/Dentin Interface

To observe the morphology of the resin/dentin interface, the dentin surfaces from a further 5 teeth were prepared and bonded as in the microtensile bond strength experiment. The resin-bonded teeth were sectioned perpendicular to the adhesive interface using an Isomet

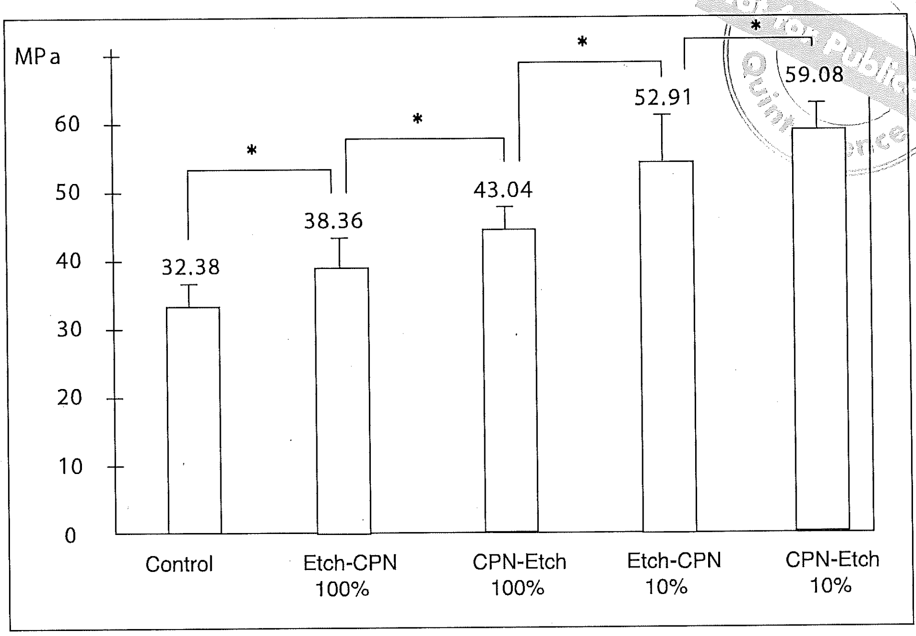


Fig 1 Microtensile bond strength. The asterisk indicates the no significant difference. Numbers indicate mean values. n = 15.

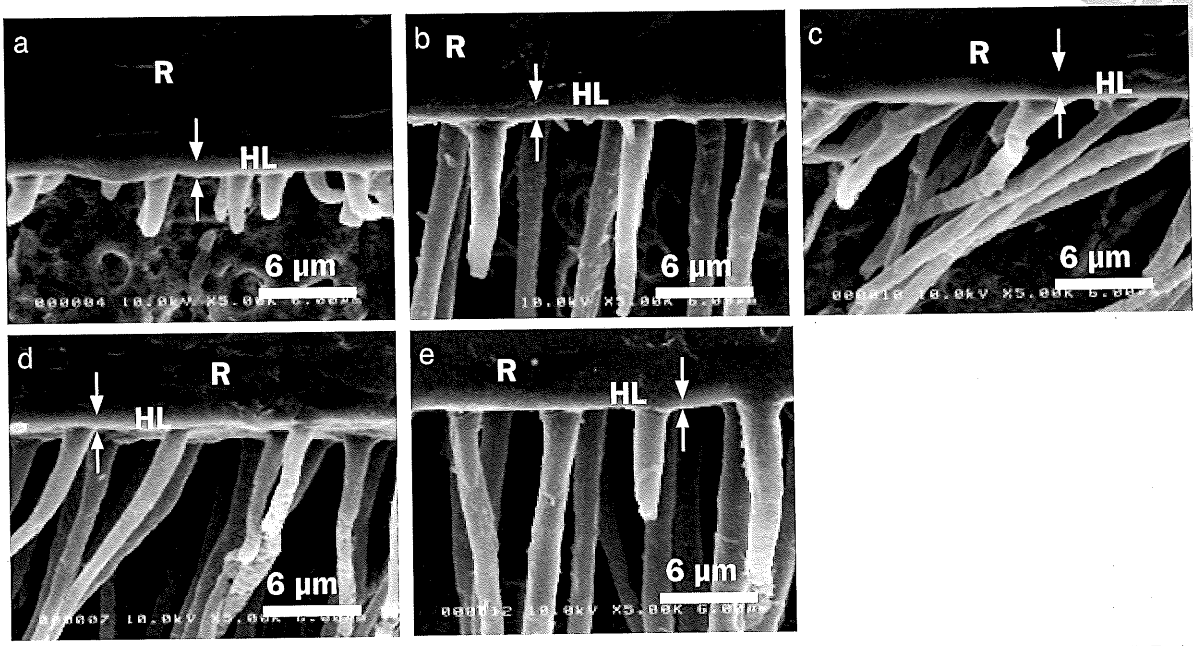
Table 2 Bonding procedures of this study

Group	Procedure
a: control	10-3 solution (5 s) →rinsed with water (3 s) → acrylic rod was bonded with hand-mixed Super-Bond C&B
b1: Etch-CPN (100)	10-3 solution (5 s) → rinsed with water (3 s) → 100% CPN (60 s) → rinsed with water (20 s) → acrylic rod was bonded with hand-mixed Super-Bond C&B
b2: Etch-CPN (10)	Same as b1 but with 10% CPN.
c1: CPN-Etch (100)	100% CPN (60 s) → rinsed with water (20 s) → 10-3 solution (5 s) → rinsed with water (3 s) → acrylic rod was bonded with hand-mixed Super-Bond C&B
c2: CPN-Etch (10)	Same as c2 but with 10% CPN

saw to obtain slabs of 2 mm thickness. Two specimens per group were prepared. The cut surfaces were sequentially polished with 600-, 800-, and 1000-grit silicon carbide paper under running water. These were subsequently polished with 6-μm, 3-μm and 1-μm diamond paste (DP-Paste, Struers; Tokyo, Japan) in turn and cleaned with an ultrasonic device between each paste polishing. The samples were then immersed in 1 mol/l hydrochloric acid for 30 s and 5% sodium hypochlorite for 5 min and rinsed with water. When dry, these were sputter coated with platinum-palladium for 150 s. The resin/dentin interface was observed using a scanning electron microscope (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 an Isomet saw, and the dentin surfaces were polished with 600-grit silicon carbide paper under running water. One specimen per group was prepared. The surface treatment was the same as previously described. After surface treatment, the samples were air dried at room temperature. The surface composition was determined by x-ray photoelectron spectroscopy (XPS) (JEOL JPC – 9010; Tokyo, Japan). XPS provides the composition and chemical state of the material surface. The measurements were carried out in 10^{-6} Pa. In this experiment, a Mg Kα (voltage: 10.0 kV, current 10.0 mA) was employed as the x-ray source. The binding energy was calibrated by the C 1s peak, which was set at 284.5 eV.



Figs 2a to 2e SEM images of resin/dentin interface of a) control, b) Etch-CPN (100%), c) of CPN-Etch (100%), d) Etch-CPN (10%), e) CPN-Etch (10%). Magnification 5000X, bar 6 µm, R = acrylic rod; HL = hybrid layer.

RESULTS

Microtensile Bond Strength

The results of the microtensile bond strength study are shown in Fig 1.

The Etch-CPN (10%) and the CPN-Etch (10%) showed significantly higher bond strengths compared to the control and the Etch-CPN (100%). The CPN-Etch (100%) and the Etch-CPN (10%) showed no significant differences between each other. The CPN-Etch (10%) showed significantly higher bond strengths compared to the CPN-Etch (100%). When using 10% CPN, the highest bond strength was obtained. 10% CPN application made the bond strength approximately double that of the control.

SEM Observation of the Resin/Dentin Interface

The SEM images of the resin/dentin interface are shown in Fig 2.

In the control specimens (Fig 2a), the hybrid layer was about 2 to 3 µm in thickness. In Etch-CPN (100%) (Fig 2b) and CPN-Etch (100%) (Fig 2c), the hybrid layer was 2 to 3 µm thick and there were no morphological differences compared to the control at this magnification. When observing resin tags, deeper penetration and a solid image of the resin tags were noted in the Etch-CPN (10%, 100%) (Figs 2b and 2d) compared to the control (Fig 2a). In addition, the hybrid layer in the Etch-CPN (10%) (Fig 2d) and CPN-Etch (10%) (Fig 2e) specimens was 2 to 3 µm in thickness, and there were no morphological differences compared to the control (Fig 2a). With regard to the resin tags, they were longer and appeared as solid resin tags (Figs 2d and 2e) compared to the control (Fig 2a). Generally, the CPN group appeared to create longer and more dense resin tag formation (Figs 2b to 2e) compared to the control (Fig 2a). There were no morphological differences in resin tags between the CPN application groups.

X-ray Photoelectron Spectroscopy Analysis

Figure 3 shows the XPS wide scan analysis of the samples.

Pt 4f 7/2 appeared around 71.2 eV. We could not detect any Pt peak at the control. The intensity ratios of Pt to O and Pt to N are listed in Table 3.

The Etch-CPN (100%) group showed the strongest Pt peaks. CPN-Etch (100%) and Etch-CPN (10%) showed weaker Pt peaks than Etch-CPN (100%), but stronger Pt peaks were shown in these two groups compared to CPN-Etch (10%). The weakest peak was found in the CPN-Etch (10%) group, but the Pt peak was detectable.

DISCUSSION

From the results of the microtensile bond strengths, the use of 100% and 10% CPN increased the bond strength between 4META-MMA/TBB resin and dentin. Interestingly, CPN-Etch (10%) showed the highest bond strength, which means the platinum effect did not disappear after etching. The use of CPN may be effective in establishing the bond strength. Nagano et al[9] showed that the bond strength between dentin and 4META-MMA/TBB resin using the "dip on" technique was doubled by the use of CPN, although there were no statistical differences between using 100% CPN and 10% CPN. In con-

Table 3 **Intensity ratio of XPS peaks**

	Etch-CPN (100%)	CPN-Etch (100%)	Etch-CPN (10%)	CPN-Etch (10%)
Pt/O	1.27	0.356	0.17	0.07
Pt/N	3.96	1.1	1.2	0.15

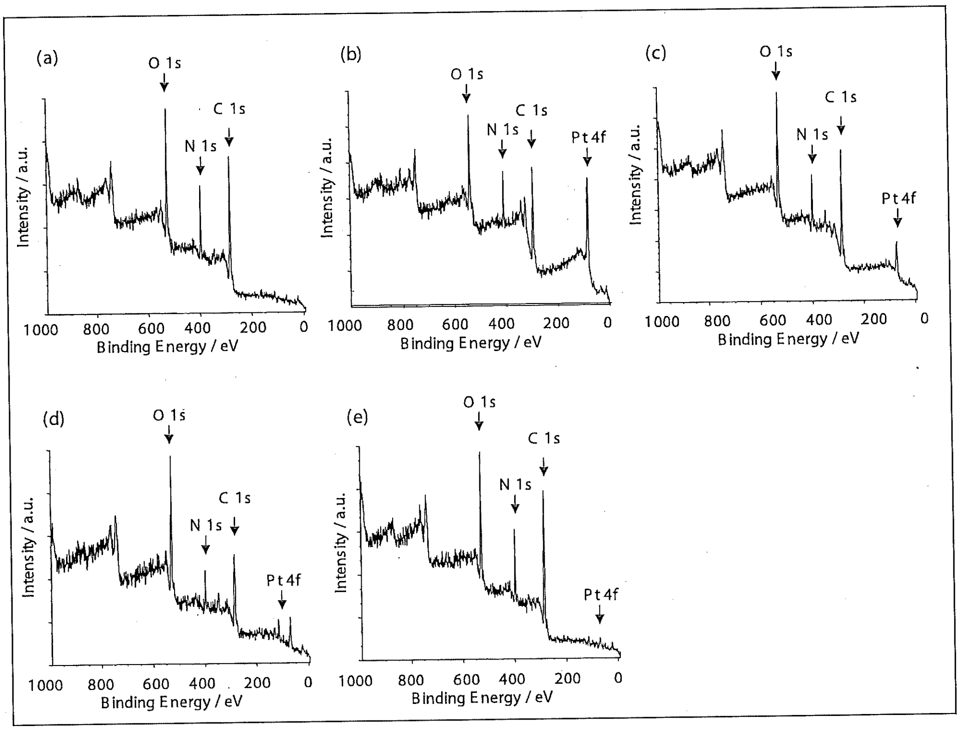


Fig 3 The XPS wide scan of the samples. (a) control, (b) Etch-CPN (100%), (c) CPN-Etch (100%), (d) Etch-CPN (10%), (e) CPN-Etch (10%).

trast, the present report shows a clear difference when using 100% CPN and 10% CPN. This difference may be caused by different methods of application of 4META/MMA-TBB resin. From the SEM observation of the interface, the resin tags appeared to penetrate into deep dentin tubules compared to the control. These findings were observed in CPN application groups. The hybrid layer is important for dentin adhesion in 4META-MMA/TBB resin,[11,18] although there were almost no morphological differences between the control and CPN application groups at lower magnification. Increased bond strength with CPN application may be due to increased conversion of resin in the hybrid layer and/or resin tags. For example, platinum has been used as a catalyst for addition polymerization.[19] Measuring the conversion of the resin within the hybrid layer is very difficult and further study is needed to confirm this hypothesis. Mechanical properties and the density of the resin tags could contribute to an increase in the bond strength.[13] However, resin tags between CPN application groups showed no morphological differences. Hence, the long and solid appearance of the resin tags may be an indirect reflection of the increased mechanical properties of the resin tags when using CPN as a catalyst.

It was difficult to detect the presence of platinum on dentin surfaces using energy dispersive x-ray spectroscopy (Genesis spectrum, EDAX; Mahwah, NJ, USA) in the preliminary study. Therefore, XPS was employed in this study, as it is useful for detecting small amounts of platinum. It is probable that Pt may show some adsorption onto superficial dentin. The existence of Pt was confirmed by XPS analysis. In the CPN application groups, Pt was detected even after 20 s of rinsing with water. In Etch-CPN (100%) and CPN-Etch (100%), a greater amount of Pt was detected than in Etch-CPN (10%) and CPN-Etch (10%). Pt may have some kind of catalytic behavior in situ. The Pt intensity ratio was stronger in group b than in group c (group b: Etch-CPN [100 or 10]; group c: CPN-Etch [100 or 10]), perhaps because in group c, after application of CPN, the specimens were rinsed with water for 20 s, followed by etching, then rinsing with water again.

Presumably there is an optimal concentration of Pt for facilitating robust adhesion between dentin and 4META-MMA/TBB resin. There are some reports about the double etching effect with 10-3 solution and anti-oxidant liquid, eg, ascorbic acid, causing the reduction of the bond strength.[12] Since the CPN did not possess such etching ability, as the pH is 6.5 to 6.7, the 10-3 solution and CPN did not show a double etching effect.

According to the results of this study, the amount of platinum adsorbed on the dentin surface affected the microtensile bond strength. The conversion of resin and infiltration of resin might be increased and consequently yield high bond strength when applying CPN. When using the combination of CPN and Clearfil Photo Bond (a bis-GMA-based adhesive; Kuraray), CPN application increased the bond strength, but the mechanism of increased bond performance was still unclear.[3] To analyze conversion of resin, evaluation with Raman spectroscopy would be effective. To analyze infiltration of resin, TEM observation would be helpful. An adequate concentration of CPN for obtaining high bond strength may exist. Therefore, the null hypothesis that there is no difference in bond strength for concentration of CPN was rejected. Further study is needed to find out the optimum concentration and application time of CPN to find the best way to use it in daily dental practice. The mechanism of bonding, long-term durability,[16,21] and biological effects of CPN require further investigation.

CONCLUSION

The results of this study showed that high bond strengths may be obatined when treating dentin surfaces with CPN in a 4-META/MMA-TBB-based bonding system.

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Clinical relevance: The use of Colloidal Platinum Nanoparticles as a primer solution may represent a simple method for considerably improving the bond strength of 4META/MMA-TBB resin to dentin.