Effect of decontamination materials on bond strength of saliva-contaminated CAD/CAM resin block and dentin

Kei TAKAHASHI1, Tomohiro YOSHIYAMA1, Akihito YOKOYAMA1, Yasushi SHIMADA2 and Masahiro YOSHIYAMA1

1 Department of Operative Dentistry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University
2 Department of Cariology and Operative Dentistry, Graduate school of Medical and Dental Sciences, Tokyo Medical and Dental University

Corresponding author, Kei TAKAHASHI; E-mail: takaha-k@cc.okayama-u.ac.jp

The aim of this study was to evaluate the effect of decontamination agents against saliva contamination on the bonding of computer-aided design/computer-aided manufacturing (CAD/CAM) resin block. Three commercially available decontamination agents were used in this study. The samples were subjected to microtensile bond strength test. A saliva protein staining test was performed by the pigment binding method to investigate the effect of removing saliva protein. All the decontamination agents could significantly restore the bond strength from the saliva contamination, and KATANA Cleaner showed no significant difference with the control. From the results of the saliva protein staining test, KATANA Cleaner showed higher removal effect of saliva protein for CAD/CAM resin block surface than the other materials due to the surface active effect of MDP salt. It was suggested that cleaners containing MDP salt were more effective in removing artificial saliva contamination than cleaners containing other ingredients for CAD/CAM resin blocks.

Keywords: Saliva contamination, Decontaminant, Microtensile bond strength, CAD/CAM resin block, Dentin

INTRODUCTION

The introduction of computer-aided design/computer-aided manufacturing (CAD/CAM) technology into dentistry is expected to make dentistry safer, more efficient, and more standardized, as well as to move away from metal restorations, and is attracting attention as a new restorative procedure to replace conventional metal restorations4-7. Aesthetic requirements in dentistry are increasing year by year, and beautiful white teeth, which were previously required only for anterior teeth, are now required for molars as well8. As a result, CAD/CAM resin block was developed that can be used for molars that are subjected to strong occlusal pressure by filling the restorative material with a high density of period-free filler9,10. In recent years, advances in dental CAD/CAM technology have made it possible to manufacture high-quality aesthetic restorative crowns in a short time and at low cost10-15. In response to the rising price of dental metals and the increasing aesthetic demands of patients, a CAD/CAM resin block was introduced for bicuspids. However, there have been cases of CAD/CAM resin block detachment in clinical practice, and countermeasures are desired16-20. There are various factors that cause CAD/CAM resin block delamination. Contamination of the bonding surface by saliva, blood, exudate, exhaled air, temporary bonding material, temporary sealing material, etc. are considered as the factors inhibit bonding in clinical practice15,18. In conventional adhesive systems, saliva and blood contamination can significantly reduce the bond strength to enamel and dentin12,19. It has also been reported that saliva and blood contamination of dentin surfaces, restorations, and the inner surface of crown prostheses significantly reduces the bond strength of recent adhesive systems16,17. Salivary contamination of CAD/CAM resin block can also be observed in the abutment teeth and fossa cavity, saliva decontaminating agents have been developed and used in clinical practice18,19. Despite the saliva decontaminants have been used in clinical practice, some decontaminating agents are contraindicated for use in the oral cavity, or some products that can be used in the oral cavity are less effective in removing saliva contamination16,18,20. The new decontamination material (KATANA Cleaner) can be used in the oral cavity and is expected to have a decontamination effect by MDP salt20. In this study, we focused on both the inner surface of CAD/CAM resin blocks and dentin, the surface to be bonded, and compared the changes in bond strength of various decontaminants after artificial saliva contamination. The effect of various decontamination agents with different ingredients on the removal of artificial saliva contamination was evaluated.

MATERIALS AND METHODS

This experiment was conducted in compliance with the ethical principles of the Declaration of Helsinki (Recommendations for Physicians Engaged in Biological Research Involving Human Subjects) and with the permission of the Ethics Committee of Okayama University (Approval No. 189). Forty eight healthy human molars were used, which had been extracted and kept refrigerated at 4°C.

Materials used in the experiment

Table 1 shows the composition of the various decontaminating materials used in this experiment, the adhesive resin cement (PANAVIA V5, Kuraray Noritake Dental, Tokyo, Japan), and the artificial saliva.
Table 1  Materials used in this study with their application procedure

| Product                      | Manufacturer          | Composition                                                                 |
|------------------------------|-----------------------|-----------------------------------------------------------------------------|
| KATANA Cleaner (Lot: T181116) | Kuraray Noritake Dental | Water, 10-Methacryloyloxydecyl dihydrogen phosphate (MDP), Triethanolamine, Polyethylene glycol, Stabilizer, Dyes |
| Ivoclean (Lot: X28703)       | Ivoclar Vivadent      | Zirconium oxide, Water, Polyethylene glycol, Sodium hydroxide, Pigments, additives |
| Multi Etchant (Lot: 01091825) | YAMAKIN               | Purified water, Phosphoric acid monomer, Thickening agents, Dye              |
| PANAVIA V5 Paste (Lot: AB0089)| Kuraray Noritake Dental | Past A: Bis-GMA, TEGDMA, Hydrophobic aromaticdimethacrylate, Hydrophilic aliphatic dimethacrylate, Initiators, Accelerators, Silanated barium glass filler, Silanated, fluoroalminosilicate glass filler, Colloidal silica  
Past B: Bis-GMA, Hydrophobic aromatic dimethacrylate, Hydrophilic aliphatic dimethacrylate, Silanated barium glass filler, Silanated alminium oxide filler, Accelerators, Di-Camphorquinone, Pigments |
| Tooth Primer (Lot: AG0057)   |                       | MDP, HEMA, Hydrophilic aliphatic dimethacrylate, Accelerators, Water          |
| Clearfil Ceramic Primer Plus (Lot: AA0039) | | 3-trimethoxysilypropyl methacrylate, MDP, Ethanol |
| [Artificial saliva]          |                       | CaCl2, KH2PO4, NaOAc, NaN3, Casein, H2O                                     |
| KATANA AVENCIA Block Universal (Lot: 000752) | Kuraray Noritake Dental | Mixed filler with colloidal silica (Ø40 nm) and aluminum (Ø20 nm) oxide, Cured resins consisting of methacrylate monomer (Copolymer of Urethane dimethacrylate and other methacrylate monomers), Pigments |

containing (CaCl2, KH2PO4, NaOAc, NaN3, Casein, H2O) used as the contaminant23.

Preparation of saliva-contaminated specimens
In this study, experiments were conducted on the assumption that a CAD/CAM resin block would be used for restorative treatment on the dentin surface after formation. For this purpose, the CAD/CAM resin block and the dentin surfaces were contaminated with immersion in artificial saliva for 60 s, and the microtensile bond strengths were measured and compared. CAD/CAM resin blocks (KATANA AVENCIA Block Universal, Kuraray Noritake Dental) was bonded to the dentin surface of a crown that had been polished with #600-grit silicon carbide paper using PANAVIA V5. The KATANA AVENCIA Block was cut to a thickness of 6 mm, and the flat surface was polished with #400-grit silicon carbide paper to form the standard roughness of adherend surface. After immersion in artificial saliva for 1 min, rinsing with water for 30 s, drying for 10 s, and treatment with each decontamination material as directed by the manufacturer, resin blocks were treated with silane coupling using primer (Clearfil Ceramic Primer Plus, Kuraray Noritake Dental). The resin cement surface was treated with primer (PANAVIA V5 Tooth Primer, Kuraray Noritake Dental). The adhesive resin cement PANAVIA V5 was used as the luting cement, and was bonded by light irradiation from four directions for 10 s each using an LED light curing machine (PENCURE, J. Morita, Kyoto, Japan) with a mean output of 1,000 mW/cm2. Saliva decontaminants were cleaner (Ivoclean, Ivoclar Vivadent, Schaan, Liechtenstein), (Multi Etchant, YAMAKIN, Kochi, Japan), and (KATANA Cleaner, Kuraray Noritake Dental).

The experimental groups were as follows (Figs. 1 and 2):

1. No saliva contamination on both resin block and dentin surface was used as control group.
2. Group with only resin block surface contaminated with saliva and no dentin surface contaminated with saliva.
3. Group treated with Ivoclean (Leave for 20 s after application, then rinse and dry) after saliva contamination of resin block surface only and no dentin surface contaminated with saliva.
4. Group treated with Multi Etchant (Rub for 20 s, leave for 10 s, rinse and dry) after saliva contamination of resin block surface only and no dentin surface contaminated with saliva.
5. Group treated with KATANA Cleaner (Rinse and dry after rubbing for 10 s) after saliva contamination of resin block surface only and no dentin surface contaminated with saliva.
6. Group with only dentin surface contaminated with saliva and no resin block surface contaminated with saliva.
Measurement of microtensile bond strength

The specimens stored in 37°C distilled water 24 h were cut into continuous sections of approximately 1.0 mm thickness perpendicular to the adhesive interface using a hard tissue precision low speed saw (Isomet low speed saw, Buehler, Evanstone, IL, USA). Each section was trimmed into a dumbbell shape under water injection using an air turbine equipped with a fine diamond point (K2ff, GC, Tokyo, Japan). The obtained sections were fixed to a Bencor Multi-T testing device (Danville Engineering, San Ramon, CA, USA) using cyanoacrylate adhesive (Zapit, DVA, Anaheim, CA, USA), and then tested on a small tabletop tester (EZ Test, Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min to measure the microtensile bond strength. The number of specimens in each group was 10. The statistically analyzed each CAD/CAM resin block contaminated group and dentin contaminated group using one-way analysis of variance (ANOVA) and Tukey’s test. The statistical analysis program was conducted using IBM SPSS Statistics version 21 (IBM, Chicago, IL, USA).

Analysis of adhesive failure mode

The fracture surface of the specimens after the microtensile adhesion test was observed using a digital microscope (VH-5500, KEYENCE, Osaka, Japan) to determine the mode of adhesive failure (magnification ×200). The failure modes were classified into interfacial failure, cohesive failure, and mixed failure according to the percentage of adhesive resin cement remaining in the fracture surface. The results of the mode of failure were analyzed using the Kruskal-Wallis test with statistical significance defined as \( p < 0.05 \).

Evaluation of salivary protein removal ability of each decontamination material

A 0.25% Coomassie Brilliant Blue (CBB) solution was prepared by adding 45% methanol 10% acetic acid for CBB stain. Then, five samples of CAD/CAM resin blocks polished with #1000 abrasive paper were prepared, immersed in artificial saliva for 1 min, and cleaned with one of the decontaminant agents used in this study according to the manufacturer’s instructions. The samples were then immersed in 0.25% CBB solution for 12 h, rinsed and dried. Colorimetric measurements of the CAD/CAM resin blocks were carried out using a spectrophotometer (SE6000, Yonezawa Denshoku Industries, Tokyo, Japan) within a range of 10 mm in diameter. The color difference between the samples and the control was calculated based on the colorimetric data, and the salivary protein removal ability of each decontaminant was evaluated.

RESULTS

The results of the microtensile bond test for the experimental groups of CAD/CAM resin blocks, and dentin surfaces are shown in Figs. 3 and 4, respectively. The bond strength of both CAD/CAM resin block and dentin was significantly decreased by saliva contamination \(( p < 0.001)\). The bond strength of contaminated CAD/CAM resin blocks were recovered by the saliva removal methods (Ivoclean, Multi Etchant, KATANA Cleaner) after saliva contamination. KATANA Cleaner showed the highest recovery of bond strength the among the decontaminant materials to the same level of bond strength as the control group \(( p = 0.880)\). After the saliva contamination, the bond strength of dentin was recovered by each saliva removal method (Multi Etchant, KATANA Cleaner). The bond strength recovery for KATANA Cleaner showed the same level of bond strength as the control group \(( p = 0.953)\).

The results of mode of failure analysis after the microtensile bond test are shown in Table 2 for the CAD/CAM resin blocks \(( p = 0.070)\) and in Table 3 for the dentin \(( p = 0.077)\). Mixing failure of dentin and cement was observed only in the control group. In the other
DISCUSSION

In the indirect method of adhesive restoration, it has been reported that when the completed restoration is tried in the oral cavity, the adhesive bond to the resin cement is degraded due to contamination by adhesion inhibitors such as saliva, gingival sulcus exudate, or blood\(^{10,11,15}\). When the inner surface of the restoration is contaminated with saliva, non-covalent adsorption of salivary proteins occurs on the surface, forming an organic coating that cannot be completely removed by water rinsing\(^{26}\). It
Fig. 5 Evaluation of removal ability of saliva protein on the decontamination materials. Values with the same alphabets indicate no significant difference ($p > 0.05$)

Fig. 6 Photographs of representative specimens after protein staining test.

has also been reported that contamination by salivary proteins cannot be completely removed by washing with 35–45% phosphoric acid etching27,28.

Ivoclean can be used for many materials such as glass-ceramics, composite resins for indirect restorations, zirconium oxide, aluminum oxide, precious metals, and base metals18,29. However, due to its strong alkalinity (pH 13.5), it is contraindicated for use in the oral cavity and cannot be applied to dentin surfaces after cavity formation or direct method composite resin restorations. The sodium hydroxide contained in Ivoclean is highly alkaline, extremely detergent, and has the ability to dissolve proteins. It is known to impart a negative charge to the surface of the adherend, making it easier to pull off dirt using electrical repulsive force. It also acts as a surfactant.

Multi Etchant has a pH of 1.6, which is mildly more acidic than the 35–45% aqueous phosphoric acid solution normally used as an etching agent suppression over etching. Therefore, they can be used for dentin in the oral cavity. By adopting adhesive phosphoric acid monomer (11-methacryloyl oxytetraethylene glycol dihydrogen phosphate, M-TEG-P) instead of phosphoric acid as the Multi Etchant, a mild etching effect can be expected and over-demineralization can be suppressed even when used on dentin. Consequently, KATANA Cleaner has a priming effect and it can be used for many materials such as zirconia, titanium, silver alloy, resin block, and dentin in oral cavity.

The new decontaminant KATANA Cleaner is weakly acidic at pH 4.5 and can be used in the oral cavity. KATANA Cleaner can be used for a wide range of applications, including natural teeth (enamel, dentin), dental ceramics (zirconia, dental porcelain, lithium nisilicate), resin materials (CAD/CAM resin crowns, composite resins), dental posts (glass fiber posts, metal posts), and dental metals (precious metal alloys, non-precious metal alloys).

The results of the protein staining test using the dye-binding method showed that the new decontaminant KATANA Cleaner had a significant protein removal effect on the CAD/CAM resin blocks compared with Ivoclean and Multi Etchant.

Higher bond test results of KATANA Cleaner in this study is probably due to the excellent saliva protein removal effect for both CAD/CAM resin block and dentin.

Although Ivoclean was reported to be more effective in removing salvia contamination for zirconia30,31, KATANA Cleaner was significantly more effective in removing contamination for CAD/CAM resin blocks based on the results of CBB staining. It has been reported that Multi Etchant have a lower saliva decontamination effect on zirconia compared to Ivoclean32, but in this experiment, the saliva decontamination effect on CAD/CAM resin blocks was equivalent to that of Ivoclean. In addition, the bond strength to dentin was restored to the same level as that of KATANA Cleaner by increasing the treatment time to 30 s, as recommended by the manufacturer.

In general, surfactants, which are the main components of detergents, have a chemical structure characterized by the combination of a hydrophilic group and a hydrophobic group in a single molecule. In water, the hydrophobic groups of the molecule aggregate to form an aggregate (micelle) with the hydrophilic groups facing outward. It is known that the inner hydrophobic groups of these micelles have the property of absorbing contaminants. Since MDP is presumed that the MDP salt contained in KATANA Cleaner also forms micelles in water, thereby exerting its cleaning action. Therefore, it is considered that KATANA Cleaner has a higher salivary protein removal effect than other materials because MDP, which is normally insoluble in water, is dissolved in water by adding a basic component to MDP to form MDP salt, and it exerts a high surfactant effect using water as a solvent. Compared to other acidic monomer salts, the MDP salt showed a significant decrease in surface tension, suggesting that it has a penetrating effect on organic substances, which is a characteristic of surfactants33. Furthermore, the MDP in cleaner can be considered to improve the chemical bonding to dentin and the interactions between dentin and resin cement34. Therefore, it is suggested that the
surfactant effect of MDP salt contained in KATANA Cleaner on saliva contamination of CAD/CAM resin blocks is more effective in removing saliva contamination than the cleaning effect of sodium hydroxide contained in Ivoclean or the cleaning effect of adhesive phosphate monomer contained in Multi Etchant.

However, many CAD/CAM resin blocks have been developed so far, and the properties of each block vary. Kameyama et al. compared and investigated the physical properties of eight types of CAD/CAM resin blocks currently available on the market, and reported that there was a significant difference in inorganic filler content among all resin blocks. Therefore, it is necessary to examine whether similar results can be obtained for other CAD/CAM resin blocks. In this study, the bond strength was measured after 24 h, and further studies are needed to evaluate the long-term prognosis.

CONCLUSION

The effect of artificial saliva decontamination on CAD/CAM resin blocks varied depending on the product. Once the CAD/CAM resin blocks were contaminated with artificial saliva, no cleaner could completely remove the attached salivary proteins. It was suggested that cleaners containing MDP salt were more effective in removing attached salivary proteins. It was suggested that cleaners containing MDP salt were more effective in removing artificial saliva contamination than cleaners containing other ingredients for CAD/CAM resin blocks.

The bond strength to dentin contaminated with artificial saliva was improved by using each cleaner. There was no significant difference between Multi Etchant and KATANA Cleaner.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding the products herein investigated.

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