ORIGINAL ARTICLE

Influence of Saliva and Blood Contamination on Microtensile Bond Strength between Fiber Posts and Composite Core Materials

Queenie Guey Zhen Tan[1], Eason Soo[1], Dalia Abdullah[1]

[1] Department of Restorative Dentistry, Faculty of Dentistry, Universiti Kebangsaan Malaysia (UKM), Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.

ABSTRACT

Introduction: This study evaluated the effect of saliva and blood contamination on microtensile bond strength between fibre post and composite core, and methods of fibre post decontamination using materials available in the standard clinical setting. **Materials and Methods:** Fibre posts were divided into three groups: (i) NC – non-contaminated, (ii) S0 – saliva-contaminated and (iii) B0 – blood-contaminated. Composite cores were constructed around the fibre posts and sectioned into sticks for microtensile bond strength testing. For each contaminant [Saliva (S), Blood (B)], the posts were decontaminated by; (i) S1, B1 – washing with water, (ii) S2, B2 – cleaning with 99.8% ethanol, (iii) S3, B3 – 3% sodium hypochlorite, and (iv) S4, B4 – 2% chlorhexidine solutions. **Results:** Pre-test failures were observed in all groups except for NC and S3. Analysis including pre-test failures assigned a value of 0 MPa indicated saliva and blood contamination was significantly associated with reduced microtensile bond strength, regardless of the type of contaminant ($p < .001$). Cleaning saliva-contaminated fibre posts with sodium hypochlorite regained bond strength similar to that of non-contaminated posts ($p = .237$). However, analysis of surviving specimens only demonstrated that blood contamination had a more negative effect than saliva on bond strength ($p = .142$). None of the decontamination methods recovered the bond strength of blood-contaminated fibre posts. **Conclusion:** Contamination of fibre posts affects its bond to the composite core. Sodium hypochlorite may be able to clean saliva-contaminated fibre posts. Care must be taken to avoid contamination during core build-up.

Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP9):124-129. doi:10.47836/mjmhs.21.s9.15

Keywords: Composite Resins, Dental Bonding, Post and Core Technique, Saliva, Blood

Corresponding Author:
Eason Soo, DDS, MDS (Endo), AdvDipEndodont, M Endo RCSEdin, FDS RCSEdin
Email: easonso@ukm.edu.my
Tel : +603 9289 7723

INTRODUCTION

According After endodontic treatment, posts are used in teeth with extensive coronal structure loss for core retention and the subsequent final restoration. With the advent of contemporary adhesive techniques, there has been a rise in preference for fibre posts and composite resin over metal posts and cores (1).

With glass fibre posts, the resin composite of the core bonds to the post by micromechanical interlocking and chemical interactions. Therefore, the composite core should adapt well to the post with good adhesion and minimal voids to produce a stable bond between the structures (2).

During core build-up, contamination of the operating field with saliva and blood is not inconceivable. The adverse effect of contamination on adhesive techniques has been reported (3–5). The prevalence of general practitioners who do not use the dental dam during endodontic treatment has been reported to range from 11% to 90%. The effectiveness of a dental dam in maintaining complete isolation of the operating field relies on a good quality seal. A wide range of armamentariums may be required with teeth that are difficult to isolate, which is often the case with teeth requiring fibre posts due to extensive loss of tooth structure (6).

Most published studies evaluate the effect of contamination between tooth structure, adhesive, and composite resin. However, very few studies investigated the effect of fibre post contamination.

Therefore, the null hypotheses of this study were:

1) Saliva and blood contamination of fibre posts have no effect on the fibre post's microtensile bond strength (μTBS) to the composite core material.
2) Decontamination methods using water spray, alcohol swabs, 3% sodium hypochlorite (NaOCl), and 2% chlorhexidine (CHX) solutions have no effect on the μTBS of blood- and saliva-contaminated fibre posts to composite core material.
3) There are no differences in μTBS between contaminated, decontaminated, and non-contaminated fibre posts to composite core material.

MATERIALS AND METHODS

Samples

A total of 40 fibre posts (TENAX Fibre Trans®, Coltune/ Whaledent AG, Switzerland) were used in this study. Each fibre post produced about 5 to 7 specimens for testing. All fibre posts were cleaned with an alcohol swab prior to preparation. The fibre posts were prepared and assigned to experimental groups according to contaminant and decontamination methods (Fig. 1).

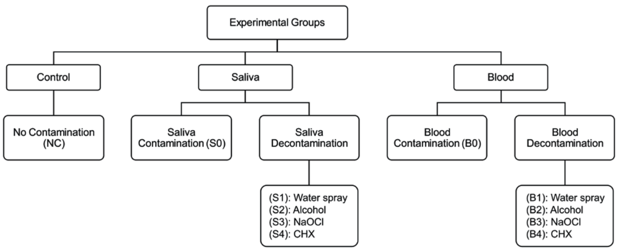

Figure 1: Flowchart illustrating experimental groups and study design. Sample sizes of NC, S0 and B0 are n = 24, and S1/B1, S2/B2, S3/B3, S4/B4 are n = 16.

The composite core material used was a dual-curing glass-reinforced methacrylate-based composite resin (ParaCore® TRANS SLOW Coltune/ Whaledent AG, Switzerland).

Contamination of fibre posts

The minimum sample size was calculated with G*Power version 3.9.1.7 (7), with a large effect size of 0.38, significance level $\alpha = .05$, and 80% power.

The source of saliva was fresh, unstimulated whole saliva collected from a researcher (QTGZ) in the morning at least one hour after eating and drinking (8). Fresh capillary blood (9) was obtained via finger prick with a sterile lancet from the fingertip of the same researcher. Both contaminants were used immediately after collection. Contaminants were rubbed over the surface of the fibre post with a microbrush for 10 seconds, then left undisturbed until visibly dry.

Preparation of composite core build-up was adapted from Goracci et. al (2005) (10) (Fig. 2). The contaminated fibre posts were placed upright on a glass slab and stabilised with wax. Hollow plastic tubes measuring about 14mm in height and 10mm in diameter were used as moulds. The fibre post was positioned in the middle of the mould. Following the manufacturer's recommendations, composite core material was built up around the fibre post in 2 mm increments with the supplied mixing tip and light-cured for 20 seconds from the top with the Bluephase N® MC (Ivoclar Vivadent AG, Liechtenstein). This produced a cylinder of composite core material with a fibre post in the centre.

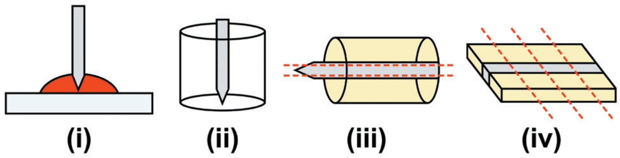

Figure 2: Schematic diagram of composite core build-up and specimen preparation. (i) Fibre post positioned on a glass slab. (ii) Mould positioned around fibre post. (iii) Longitudinal sectioning. (iv) Serial sectioning into sticks.

Specimens were sectioned with the IsoMet® 4000 Linear Precision Saw (Buehler, USA), first longitudinally to produce a rectangular block of 1 mm thickness, then serially into 1 mm slices to form sticks measuring approximately 1 mm x 1mm x 10mm for μTBS testing.

μTBS testing for all specimens was performed within 24 hours of specimen preparation to obtain immediate bond strength (11. 12). The method depicted by Zhang et al. (2020) (13) was adapted for mounting of specimens for testing. Specimen sticks were attached to the jig of a universal testing machine (AG-X, Shimadzu, Japan) with cyanoacrylate glue. The Trapezium X operation software (Shimadzu, Japan) was used for standardising the test method. Tensile load was applied at 0.5mm/ minute until failure. The μTBS was expressed in MPa, obtained by dividing force at failure (N) by bonded area (mm2) (14).

Any occurrence of pre-testing failure (ptf) was recorded. Criteria for a specimen to be classified as a pre-testing failure were failure at the adhesive interface at any point during sectioning or before mounting for μTBS testing, and not attributable to mishandling (15). Mishandled specimens were discarded and replaced with fresh specimens.

Decontamination of fibre posts

The minimum sample size for five groups to be analysed was calculated with a large effect size of 0.4, significance level $\alpha = .05$, and 80% power.

Contaminated fibre posts were prepared as described in the first part of the study and then decontaminated. For groups S1/ B1, decontamination was performed by rinsing the fibre posts with water spray from a three-way syringe for 10 seconds. For groups S2/ B2, S3/ B3, and S4/ B4, cotton pellets soaked to saturation with alcohol (Ethyl alcohol 99.8%(V/V), ChemAR®, Systerm, Malaysia), NaOCl (CanalPro™ NaOCl 3% Coltиne/ Whaledent AG, Switzerland), and CHX (CanalPro™ CHX-Ultra™ Coltиne/ Whaledent AG, Switzerland) respectively were applied to contaminated fibre post surface with rubbing action for 10 seconds.

Decontaminated specimens were prepared for µTBS testing and analysed as previously described.

Verification of failure pathway

Specimens were visually examined without magnification (16) and under adequate lighting. Verification of failure pathway was performed by examination of specimens with a light microscope (Nikon, Japan) (10) at 40x magnification (17). Specimens with failure pathways not involving the bonded interface were discarded and replaced with fresh specimens.

Statistical analysis

Data was tabulated and analysed with IBM® Statistical Package for Social Sciences (SPSS®) Version 26. An alpha level of $p = .05$ was used for all statistical tests. Effect of contamination and decontamination on frequency of ptf was analysed with chi-square test of independence by converting data into Pass/ Fail metrics. Specimens that fulfilled the criteria to be classified as ptf were categorised as "Fail." Surviving specimens were classified as "Pass." Numerical analysis of µTBS was further conducted through two separate approaches: with and without ptf. When ptf were included, they were assigned a value of 0 MPa, and analysed with Kruskal-Wallis test, followed by post hoc pairwise comparison with Bonferroni correction. Mean µTBS of surviving specimens were compared with one-way ANOVA (Analysis of Variance) if data met requirements of normality and homoscedasticity, followed by Tukey's test when difference between the groups was significant, $p < 0.05$.

RESULTS

A considerable number of ptf were observed in all groups except for NC and S3 (Table I). All specimens failed correctly at the adhesive interface and thus were included for data analysis. Results are tabulated as in Table II. Comparison between bond strengths analysed with and without ptf is illustrated in Fig. 3.

Table I: Number and frequency expressed in % of ptf and surviving specimens in each test group.

Observation	Group[1]										
	NC	S0	S1	S2	S3	S4	B0	B1	B2	B3	B4
ptf[2]	-	17	10	13	-	6	15	7	12	9	8
%		70.8	62.5	81.3		37.5	62.5	43.8	75	56.3	50
Surviving	24	7	6	3	16	10	9	9	4	7	8
%	100	29.2	37.5	18.8	100	62.5	37.5	56.3	25	43.8	50
Total	24	24	16	16	16	16	24	16	16	16	16

[1] NC representing the non-contaminated, and S0 and B0 representing the saliva and blood-contaminated groups respectively. For each contaminant [Saliva (S), Blood (B)], (S1, B1), (S2, B2), (S3, B3), (S4, B4) representing washing with water, ethanol, sodium hypochlorite and chlorhexidine solutions decontamination groups respectively.
[2] ptf = pre-testing failures

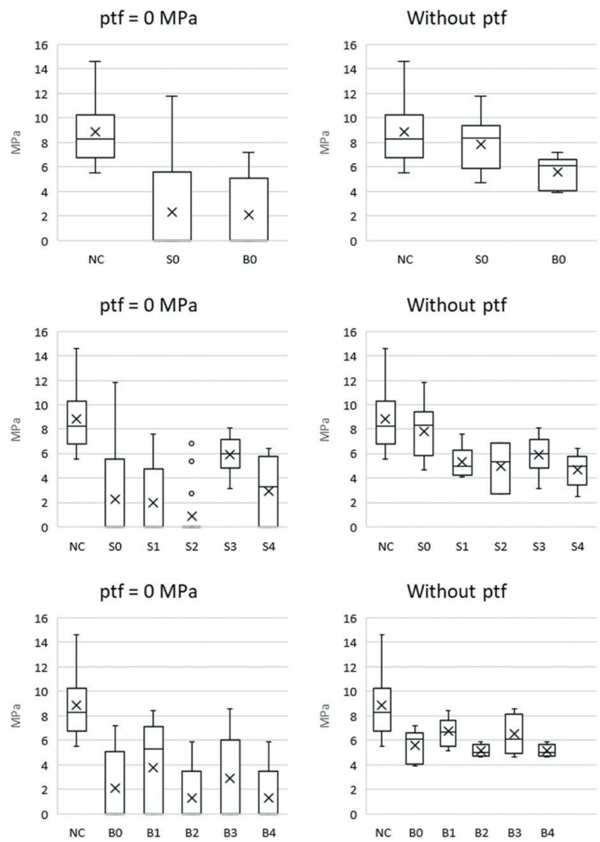

Figure 3: Microtensile bond strength of fibre post and composite core, expressed in MPa. Mean values are represented by cross marks. Each pair of box plots shows the data distribution for saliva and blood contamination (top), saliva decontamination (middle), and blood decontamination (bottom). Box plots on the left represent all specimens, including ptf assigned value of 0 MPa. Box plots on the right represent surviving specimens, excluding ptf.

Frequency of pre-testing failure

Presence of saliva and blood contamination was found to be strongly associated with ptf, X2 (2, N = 72) = 29.14, $p < .001$, V = .64. Comparison between groups S0 and B0 found that the type of contaminant, whether saliva or blood, had no significant relationship with occurrence of ptf, X2 (1, N = 48) = .38, p = .54. Saliva decontamination with NaOCl did not produce any ptf. When compared to the saliva contamination and among the other saliva decontamination groups, this finding was statistically significant with a high strength of association, X2 (4, N = 88) = 28.29, p < .001, V = .567. Ptf were observed in all blood decontamination groups. None of the decontamination methods could produce any significant change in frequency of pre-testing failure compared to the blood-contaminated group, X2 (4, N = 88) = 3.87, p = .424.

Effect of contamination

When ptf were included, the analysis indicated that both saliva and blood contamination resulted in significantly lower μTBS compared to the non-contaminated control group, p < .001. There was no significant difference between the saliva and blood contamination groups. Conversely, omitting ptf (Table II) found that mean μTBS produced by blood contamination was significantly lower than both non-contaminated (p = .002) and saliva-contaminated (p = .142) groups.

Table II: Mean and standard deviation of μTBS of fiber post to composite core of surviving specimens expressed in MPa.

Observation	Group[1]										
	NC	S0	S1	S2	S3	S4	B0	B1	B2	B3	B4
Mean μTBS[2]	8.87	7.81	5.31	4.99	5.96	4.71	5.55	6.71	5.12	6.53	6.85
SD[3]	2.54	2.46	1.27	2.07	1.40	1.38	1.29	1.15	0.52	1.58	1.67

[1] NC representing the non-contaminated, and S0 and B0 representing the saliva and blood-contaminated groups respectively. For each contaminant [Saliva (S), Blood (B)], (S1, B1), (S2, B2), (S3, B3), (S4, B4) representing washing with water, ethanol, sodium hypochlorite and chlorhexidine solutions decontamination groups respectively.
[2] Mean μTBS = Mean microtensile bond strength expressed in MPa
[3] SD = Standard deviation

Saliva decontamination

Analysis with the inclusion of ptf found that decontamination with NaOCl significantly increased the μTBS compared to the saliva-contaminated group, p < .001. Additionally, compared to the non-contaminated fibre posts, the mean rank μTBS values between groups NC and S3 were statistically similar, p = .237. However, when ptf were excluded (Table II), analysis demonstrated that none of the decontamination methods could significantly increase μTBS compared to the saliva-contaminated fibre groups nor regain μTBS statistically similar to that of non-contaminated fibre posts.

Blood decontamination

Analysis with and without ptf produced similar findings (Table II). None of the decontamination methods could increase nor recover the μTBS of blood-contaminated fibre posts to the composite core.

DISCUSSION

The present study was carried out to evaluate the effect of saliva and blood contamination on the μTBS of fibre post to composite core material, and the effectiveness of cleaning contaminated fibre posts using materials commonly used in the clinical setting.

Pre-testing failures have been attributed to stresses induced by trimming during specimen preparation (18) and is not seen in specimens with relatively higher μTBS (19), indicating that the bond strength was inadequate to survive stresses from sectioning. In this study, no pre-testing failures were seen in the control group, in concurrence with this statement.

Several approaches to statistical analysis have been described concerning pre-testing failures. Pre-testing failures have been omitted, assigned bond strength of 0 MPa or a value equivalent to the lowest measured bond strength in the experimental group (15, 20). When pre-testing failures were included in this study, saliva and blood contamination were found to reduce bond strength, regardless of the type of contaminant. However, analysis of surviving specimens only indicated that blood contamination had a more deleterious effect on the bond strength. Other studies also reported similar results when comparing saliva and blood as contaminants, where blood had a more adverse effect on bond strength than saliva (21, 22). The difference between the effect of saliva and blood contamination could be explained by the proportion of organic material of the two contaminants. Blood is 55% plasma, of which 90% is water (23), thus leaving behind more residual organic debris compared to saliva that is 99% water (24).

The difference in findings between the inclusion and omission of pre-testing failures in this study contradicts a previous report that found no difference between interpretations between inclusion and exclusion of pre-testing failures (20). Including pre-testing failures at 0 MPa inevitably produced a skewed data distribution that could only be analysed with the less powerful, non-parametric Kruskal-Wallis test. For this reason, surviving specimens were separately analysed with one-way ANOVA (19, 20), however results of which are at risk of Type II error from the substantial loss of sample size due to pre-testing failures, and therefore may not accurately represent μTBS values after contamination and decontamination (25).

The considerable number of pre-testing failures is a known drawback of the μTBS test (19). A possible contributing factor is the study design in which the bond strength is deliberately lowered by contamination. An alternative test is the thin slice push-out test (2); however, it incorporates frictional forces unlike μTBS testing which specifically evaluates the interfacial bond strength (26).

The inclusion of pre-testing failures found that only NaOCl had some capacity for cleaning saliva-contaminated fibre posts. This may be due to its ability to dissolve organic matter (27). The failure of 99.8% ethanol in decontaminating the fibre post might be explained by its composition. 99.8% ethanol has a higher concentration of alcohol with lower water content (28, 29); while this would be ideal for disinfectant action, it may be less effective in removing organic matter. CHX was also unable to decontaminate the fibre posts, which may be attributed to its inability to dissolve organic matter (27, 30).

None of the tested decontamination methods could clean blood-contaminated fibre posts effectively. In the same way that blood contamination could have a more negative effect on the μTBS, cleaning of blood-contaminated fibre posts may require more vigorous decontaminant action than the methods tested in this study. During decontamination, the author observed that in most blood-contaminated specimens, the fibre posts remained stained with blood. While this served as a significant visual cue, it was not present with saliva, which may cause the clinician to be unaware of saliva contamination.

Contradictory to this study, when the posts were cleaned immediately, Imai et. al (2022) (26) reported recovery of bond strength between fibre posts contaminated with artificial saliva and composite core. The conflicting findings may also be attributed to the use of push-out testing to evaluate bond strength at 24 hours. This could suggest that contaminants should not be left to dry on the fibre post surface. Instead, the organic debris can first be rehydrated by copious washing with water spray. NaOCl could then be applied in rubbing motion to make use of its tissue dissolution properties.

CONCLUSION

This study found that saliva and blood contamination of fibre posts affect the μTBS of the fibre post to the composite core material. Decontamination with 3% NaOCl solution may be able to recover the lost μTBS of saliva-contaminated fibre posts to composite core material. None of the tested decontamination methods were able to clean blood-contaminated fibre posts.

ACKNOWLEDGEMENTS

This study was supported by the Universiti Kebangsaan Malaysia Faculty Research Grant (Grant No: DD-2022-003).

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