Original Research

Effect of Storage Media and Sterilization Method on Shear Bond Strength of Composite to Enamel of Extracted Teeth

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Received 10 April 2017 and Accepted 13 June 2017

Abstract

Aim: To evaluate the effect of storage media and autoclaving on shear bond strength of composite to enamel**. Materials and Methods:** 100 human premolars were randomly divided into ten groups of ten as follows: C: freshly extracted teeth. A: Autoclaved freshly extracted teeth. CH-6: 6 month storage in 0.5 % chloramine T. CA-6: 6 month storage in 0.5 % chloramine T + autoclaving. T-6: 6 month storage in 0.04 % thymol. TA-6: 6 month storage in 0.4% thymol + autoclaving. Ch-12:12 month storage in 0.5 % chloramine T. CA-12: 12 month storage in 0.5 % chloramine T + autoclaving. T-12: 12 month storage in 0.4% thymol. TA-12:12 month storage in 0.4% thymol + autoclaving. One composite cylinder was bonded on each specimen for evaluation of shear bond strength (SBS) and failure modes. Data were analyzed using three-way ANOVA, Tukey's post hoc and Chi square tests and the level of significance was set at P= 0.05.

Results: Autoclaving and storage media had no significant effect on SBS (P-value = 0.818 for storage media and P-value = 0.221 for autoclaving). However, storage duration significantly changed SBS (P-value = 0.00). There were no correlations among the variables (P-value > 0.05). Storage media and duration had no significant effect on failure modes (P-value > 0.05), but autoclaving significantly increased cohesive failure of enamel (P-value =0.039). **Conclusion:** Storage of teeth in chloramine T and thymol had no significant effect on bond strength to enamel. Although autoclaving of specimens may not alter bond strength, it can significantly increase the cohesive failure of enamel.

Keywords: Autoclaving, bond strength, chloramine T, extracted teeth, Storage media, thymol

Boruziniat A, Danaifard N, Gifani M, Hakimi N. Effect of Storage Media and Sterilization Method on Shear Bond Strength of Composite to Enamel of Extracted Teeth**.** J Dent Mater Tech 2017; 6 (3): 96-102.

Introduction

For educational use and research purposes, dental materials are tested on collected teeth under appropriate ethical guidelines. The extracted teeth are of a high value for pre-clinically practice for the students for on various practical or technical skills(1). Furthermore, these human teeth are frequently utilized in researches such as *in-vitro* bonding to dentin or enamel before preforming clinical trials (2). One important issue in clinical dental practice is bonding to tooth structure. New adhesive systems are being introduced by the manufacturers who claim an improvement in their compositions and bonding to tooth structure; However, these claims need to be substantiated by scientists and researchers via *in-vitro* screening and testing of such systems before their *in vivo* applications(3).

It should be considered that the teeth that have been inadvertently left for a short time and dried are not suitable for use in research and are often discarded(3). Therefore, to achieve reliable results, storage of teeth according to ISO guidelines of adhesion of dental materials to tooth structure (1991) is very important (4). Disinfection of the extracted teeth is also important since they can be a potential source of blood-borne pathogens as mentioned by the Occupational Safety and Health Administration (OSHA)(5). The United States Center for Disease Control and Prevention (CDC), has developed a guideline for sterilization of extracted teeth used for research and educational purposes(6). According to this guideline, storage in 10% formalin for two weeks and autoclaving at 121°C/20 psi for 40 min should be used for the teeth with and without amalgam restorations respectively(7). A major advantage of the extracted teeth utilized for educational and research purposes is that cutting condition is the same as clinical situations, so the sterilization procedure used for these teeth should not ideally change these properties. On the other hand, the validity of the results of *in-vitro* researches may be reduced by the possible changes induced by storage media or sterilization methods and thus these researches don't reflect a real clinical situation (7, 8).

To date several solutions such as thymol, ethanol, formalin, chloramine-T, aqua chloramine, sodium hypochlorite and glutaraldehyde have been suggested as storage media in the literature(9). Although storage of teeth in some of these media doesn't affect the bond strength of composite to tooth structure, these are not efficient sterilants (2). Moreover, some storage media like formaldehyde may be hazardous to health due to irritation of upper respiratory track or carcinogenic effects(10, 11) or may affect bond strength, microleakage or dentin permeability(2, 12-16).

Because of the antibacterial properties, thymol has been commonly applied as a storage solution in adhesion studies (16, 17). Various concentrations of thymol (0.02% up to 0.1%) and durations (from 24h to 6 months) have been used in different studies (1, 9, 16-18). Nevertheless, there is controversy in literature regarding the effect of thymol on bond strength and structure of teeth (4, 17).

Chloramine T is another storage media that has been used by several investigators for disinfection of teeth (3, 17). Although it is a close analogue to sodium hypochlorite, it has no effect on collagen(3). The usual concentration of Chloramine T is 1%, however the duration varies in different studies, from one week to 7 months (4, 6, 19).

Autoclaving at a typical temperature of 121°C for at least 15 min with high-pressure steam is the gold standard method of sterilization that can completely inactivate bacteria (1). However this procedure may affect the mechanical properties of teeth, causing them to become brittle and less fracture resistant during experiments (1). Pashley et al. (20) and DeWald et al. (2) concluded that autoclaving had no effect on bond strength. However, Lee et al. (6) and Carvalho et al. (21) demonstrated the negative effect of autoclaving on bond strength.

Questions have been raised regarding how post extraction changes within enamel may influence adhesion in *in-vitro* enamel bonding studies(16). The aim of this study was to investigate the effect of different storage conditions for human extracted teeth on bond strength of composite to enamel. The first null hypothesis was that the types of storage media and storage duration have no effect on bond strength of composite to enamel. The second hypothesis was that autoclaving of extracted teeth has no effect on bond strength.

Methods and Materials

One hundred non-carious premolars that had been extracted for orthodontic reasons were used in the present study. All teeth were debrided and divided into 10 groups of 10 according to the sterilizing method as follows:

Control group (C): freshly extracted teeth were used without storage or autoclave.

Autoclave group (A): the fresh teeth were autoclaved at 121^0C/20 psi for 40 minutes.

CH-6 and CH-12 groups: the teeth were stored in o.5% chloramine for 6 and 12 months respectively.

CA-6 and CA-12 groups: the storage procedures were the same as the former groups, but after storage, the samples were autoclaved at 121^0C/ 20 psi for 40 minutes.

T-6 and T-12 groups: the samples were stored in 0.04% thymol for 6 and 12 months respectively.

TA-6 and TA-12 groups: the storage procedures were the same as the former groups, but after storage, the samples were autoclaved at 121 ^{0}C/20 psi for 40 minutes.

Bonding procedure

The labial surfaces of all teeth were ground flat using wet 600- grit silicon carbide paper (Garmisch. Germany). The plastic tube (4×2 mm) was placed on the enamel surface. After that, the enamel surface was etched using 37% phosphoric acid for 20 seconds and then rinsed for 10 seconds. The adhesive (Single Bond, 3M ESPE; St. Paul, MN, USA) was applied and polymerized according to the manufacturer's instructions. Composite resin (Z250, shade A1; Single Bond, 3M ESPE; St. Paul, MN) was placed incrementally and polymerized directly for 40 seconds using a light-curing unit (Blue Phase C8, IvoclarVivadent, Schaan, Lichtenstien) with intensity of 800 mw/cm^2. The samples were then stored in distilled water at 37℃ for 24 hours.

Bond Strength Test

The shear bond strength test was performed by the universal testing machine (Zwick/Z250, Type KAP-Z, Zwick Roell Group; Ulm, Germany) at a crosshead speed of 1 mm/minutes. The failure modes were observed at 40X magnification using a stereomicroscope (LEO, 1450 UP, Zeiss; Oberkochen, Germany) and classified as adhesive, cohesive in enamel, cohesive in composite` and mixed.

Statistical analysis

The data were collected in SPSS software (version 11.5, SPSS Inc. Chicago, IL). Normal distribution of the data was confirmed using Kolmogorov-Smirnov test and data were analyzed using three-way ANOVA and Tukey's post-hoc and chi-square tests with a pre-set significance level of 0.05

Results

The mean SBS values and standard deviation are shown in Table1. Three-way ANOVA indicated that:

1. The storage media (Chloramine, Thymol or without storage) and autoclaving have no significant effect on SBS. (P-value = 0.818 for storage media and P-value =0.221 for autoclaving) .
2. Storage duration significantly affects SBS (P-value = 0.005). Post-hoc Tukey's test showed that SBS of 6-month storage groups were significantly higher than 12-month storage groups (P-value = 0.014). However, there was no significant difference between the storage groups (6-month and 12-month) and the control group (fresh teeth).
3. No relationship was found among the three variables. (Table 2, P-value > 0.05)

Different failure modes observed in each group are shown in Table 3 and figure 1. According to the Chi square test, autoclaving significantly affects the failure modes (P-value =0.039); however, other factors (storage media or storage durations) do not have significant effects (P-value = 0.209 and P-value = 0.096 respectively).

Table 1. The mean ± SD values of bond strength (MPa) of experimental groups

time / group	0	6 month	12 month
C	16.97±4.80	*	*
A	15.98±2.48	*	*
CH	*	18.81±4.94	13.70±3.42
CA	*	17.32±5.56	14.54±2.49
T	*	20.75±7.70	15.87±5.10
TA	*	16.73±5.25	13.97±6.70

C: Freshly extracted teeth. A: Freshly extracted teeth +autoclave. CH-6: 6-month chloramine storage. CA-6: 6-month chloramine+ autoclave. T-6: 6-month thymol storage. TA-6: 6-month thymol+ autoclave. CH-12: 12-month chloramine storage;
CA-12: 12-month chloramine+ autoclave; T-12:12-month thymol storage; TA-12: 12-month thymol+ autoclave

Table 2. Tests of Between-Subjects Effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	357.168[a]	9	39.685	1.409	0.20
Intercept	26043.864	1	26043.864	924.433	<0.001
solution	1.499	1	1.499	0.053	0.8
time	233.506	1	233.506	8.288	0.005
autoclave	42.837	1	42.837	1.521	0.22
solution * time	3.236	1	3.236	0.115	0.73
solution * autoclave	14.649	1	14.649	0.520	0.48
time * autoclave	8.528	1	8.528	0.303	0.58
solution * time * autoclave	3.414	1	3.414	0.121	0.73
Error	2535.551	90	28.173		
Total	30500.757	100			
Corrected Total	2892.719	99			

a. R Squared = .123 (Adjusted R Squared = .036)

Table 3. Distribution of different failure modes among experimental groups.

Groups	Enamel Cohesive	Composite cohesive	Mixed	Adhesive	Total
C	3	2	1	4	10
A	3	2	1	4	10
CH-6	0	1	2	7	10
CA-6	6	0	2	2	10
T-6	3	2	0	5	10
TA-6	6	0	0	4	10
CH-12	1	0	1	8	10
CA-12	2	0	2	6	10
T-12	1	1	2	6	10
TA-12	3	0	1	6	10

C: Freshly extracted teeth; A: Freshly extracted teeth +autoclave; CH-6: 6-month chloramine storage; CA-6: 6-month chloramine+ autoclave; T-6: 6-month thymol storage; TA-6: 6-month thymol+ autoclave; CH-12: 12-month chloramine storage;
CA-12: 12-month chloramine+ autoclave; T-12:12-month thymol storage; TA-12: 12-month thymol+ autoclave

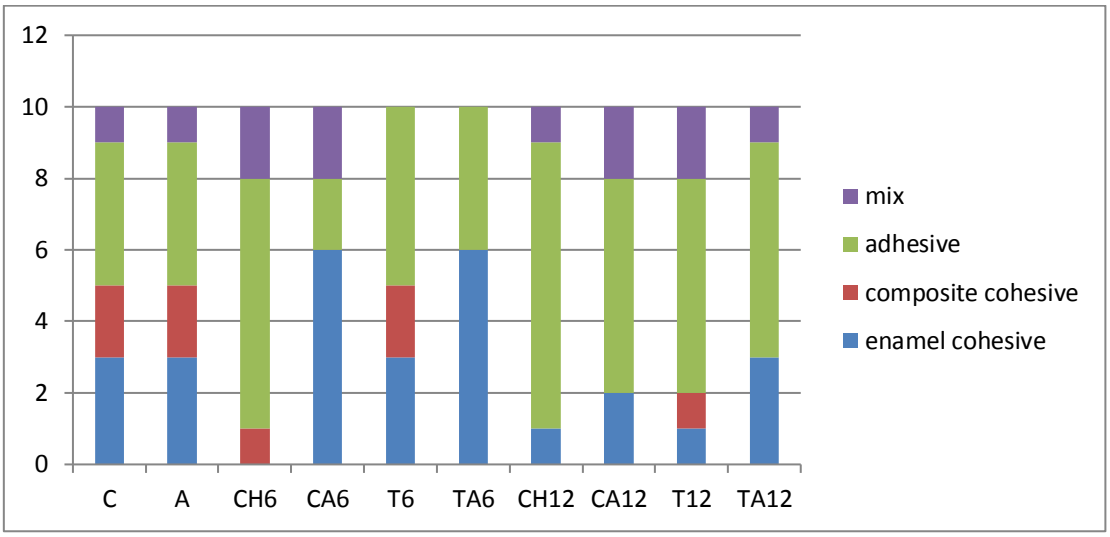

Figure1. Distribution (in percentage) of failure modes of each group

Discussion

The results of the current study show that the first hypothesis was true and the storage media had no effect on SBS. Regarding the second hypothesis, autoclaving just affected the type of bond failure.

According to US OSHA, the teeth used for researches or educational purposes are a potential source of blood borne pathogens(9, 18). Different storage media such as sodium hypochlorite, glutaraldehyde, chloramine, thymol, formalin, normal saline or phosphate buffered saline(4, 9) and sterilization methods ,autoclave, dry heat, gamma radiation, ethylene oxide, ozone gas and freezing, (22) have been suggested by researchers for disinfection or sterilization of extracted teeth. Chloramine T and thymol are common storage media used in different studies for this purpose. Various researches have demonstrated that these two storage media have minimal effect on teeth structure (16, 22, 23).

Results of the current study indicated that thymol had no significant effect on shear bond strength of composite to enamel. In line with the results of the current study, Ziskind et al. (24) reported that storage of teeth in 0.1% thymol had no significant effect on enamel bond strength and permeability. Nevertheless, other investigators have reported that thymol can reduce bond strength and structure of enamel (4, 16). Thymol has a mild acidity, therefore may affect the mineral content of tooth structure in long term storage(9). Also storage of teeth in chloramine media had no significant effect on SBS of composite to enamel. Other studies found same results using similar concentrations of chloramine(3, 19). This solution has some basic properties which don't cause change in tooth structure(17).

The storage process may be affected by other factors such as the storage duration ranging from a few hours to several years and the frequency of refreshing the solution. However, it is not clear how enamel and dentin are influenced by the storage solutions (6, 9). In the current study, two storage durations were evaluated. No significant difference was found between SBS of the control group and 6-month or 12-month storage groups, though Bond strength of 12-month storage group was significantly lower than 6-month group.

Sterilization of specimens with autoclave had no significant effect on bond strength to enamel, but this method had a significant effect on the failure mode. Enamel cohesive failure was the most observed failure mode in specimens that were autoclaved. It should be considered that the purpose of bond strength test is to assess bond efficacy, so cohesive failure may interrupt with this aim. Although autoclaving of teeth had no significant effect on bond strength values, it may change the cohesive strength of tooth structure by increasing enamel cracks due to its high temperature. In another study, the negative effect of autoclave on shear bond strength of dentin was found(25)while other studies demonstrated no change in tooth structure when teeth were autoclaved(2, 20). Another important point is that autoclaving alongside different storage medium may have different results on SBS of specimens. Lee et al. (6) concluded that sterilization with autoclave may reduce bond strength to dentin when the teeth were initially stored in distilled water or formalin.

Since mercury vapor may be released in the air by autoclaving and the residual mercury can further lead to environmental contamination, autoclaving should not be done for the extracted teeth with amalgam restorations. Furthermore, teeth with amalgam restorations may undergo fractures caused by thermal cycling since their coefficients of expansion are different (5).

It should be noted that almost all storage media such as chloramine or thymol can be used just as disinfectant agents. Therefore, if there is a need for sterilization of teeth, other methods such as autoclave, dry heat, gamma radiation and freezing should be considered (3, 6, 22). It was shown by White et al. (26) that the structure and function of dentin are not altered by teeth sterilization through gamma radiation, which unfortunately requires expensive equipment.

In the current study, SBS was used for evaluation of bond strength. An *in-vitro* Shear Bond Strength (SBS) Test is commonly employed by investigators for a quantitative analysis and ranking of bonding performance of adhesive resins on the surface of enamel and dentin(6). An SBS specimen can be pre-stressed only by mold removal before testing, which is a significant advantage over the Tensile Bond Strength (TBS) Method. SBS methods are especially useful tests for those substrates that are susceptible to crack propagation during sample preparation such as glass ionomers or enamel(27).

With the limitation of this study, only the effect of storage media and autoclave on bond strength to enamel and mode of failure was evaluated. The effect of these factors on bond strength to dentin is also required. Further research regarding the effect of storage media on mechanical properties of tooth structure such as micro-hardness is suggested.

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