

# Effect of Different Storage Solutions and Autoclaving on Shear Bond Strength of Composite to Dentin

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## ABSTRACT

**Purpose:** Extracted teeth contain various types of micro-organisms which possess the risk of cross infection also the procedure of disinfection can be harmful to the structure of in-vitro tooth samples. To investigate the effect of storage solutions and autoclaving on the shear bond strength of composite resins to dentin.

**Materials and Methods:** Sixty human molar teeth were randomly allocated into different groups according to storage solution, and autoclaving: (G1) fresh teeth, (G2) fresh teeth autoclaved at 121°C, 15 psi pressure; (G3) storage in Chloramin-6 months; (G4) G3+autoclave; (G5) storage in Thymol-6 months; (G6) G5+autoclave. The shear bond strength of composite to dentin was assessed by Instron machine. Kruskal-Wallis and Mann-Whitney test were performed using SPSS software (version 11.5) for analysis.

**Results:** The results showed that the storage of teeth in Chloramine solution significantly increase the bond strength ( $P$ -value $<0.05$ ). However, storing in Thymol had no significant effect on bond strength ( $P$ -value $>0.05$ ). Autoclaving process did not significantly affect the shear bond strength in comparison to control and even after solution storage (Thymol/Chloramine) ( $P$ -value $>0.05$ ).

**Conclusion:** For more than 6-month storage Thymol is a better solution to store the teeth in comparison to Chloramine. Autoclaving recommended before in-vitro studies for sterilizing extracted teeth.

**Key message:** 6 months storing teeth in Thymol had no significant effect on bond strength of composite to dentin. According to the results, Thymol is a better solution to store the teeth in comparison to Chloramin. Autoclaving also did not affect the bond strength significantly.

**Key words:** Extracted teeth, Storage solution, Sterilization method, Bond strength

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## INTRODUCTION

To accomplish any investigation on bonding agents such as bond strength test are difficult in clinical studies, therefore researchers tend to use extracted teeth for *in-vitro* studies.

The risk of cross infection when working on teeth, especially recently extracted teeth, is similar to clinical conditions. The American Occupational Health and Safety Authority has stated in its instruction that human extracted teeth should be considered as sources of pathogens with blood origin, therefore, to carry out *in-vitro* studies, the risk of cross infection to those involved should be thought-out [1].

Dental specimen storage conditions, disinfection and sterilization methods, and the duration of dental specimen storage affect the mechanical and chemical properties of the extracted teeth and interfere with the result of *in-vitro* researches [2]. Therefore it is important to disinfect or sterilize the teeth before any laboratory application.

In most studies in the field of extracted teeth environments and tooth disinfection, Chloramine solution is used in two concentrations of 0.5% and 1% [3-9]. Also, in many studies the concentration effects of 0.01% to 1% Thymol (which has antibacterial properties) on the teeth has been investigated [6-14].

Putting the teeth in a temperature of 121°C and a pressure of 15 Pa for 40 minutes can sterilize the teeth [15,16]. Some investigations recommended the autoclaving process before *in-vitro* studies [4,9,17-19].

The aim of this study was to assess the effect of different dental storage solution and autoclaving and period of tooth storage on the shear bond strength of composite resin to dentin.

### MATERIALS AND METHODS

In this study we used 60 human molar teeth. After extraction, all the teeth were first debrided and cleaned, and then the occlusal surfaces of all the teeth were completely flattened using a 3000 rpm diamond disc trimmer (MESTRA-spain) until they reached a uniform flat dentin. Twenty teeth were placed in 0.5% Chloramine solution (Merck, Germany) (all the teeth were immersed

in the solution completely). Twenty teeth were placed in 0.04% Thymol solution (Merck, Germany). The storage solutions were replaced every week to expose the extracted teeth to fresh solution.

In order to prepare a 0.5% w-v (weight-volume) Chloramine solution, due to the presence of crystallized water in its salt structure, the amount of 0.63 g of solid Chloramine was dissolved in distilled water and its volume brought to  $10^{-4} \text{ m}^3$ . In order to prepare a solution of 0.04% p' w-v of Thymol, 4 mg of solid Thymol was dissolved in 96% ethanol the volume was brought to  $10^{-4} \text{ m}^3$ .

**Table 1: Description of study groups**

Study Group Name	Description	Number
Fresh teeth	Immediately after the debridement (fresh)(control group)	10
Fresh teeth with autoclaving	The teeth were autoclaved for 40 minutes at 121°C and the pressure of 15 psi.	10
6-month Chloramine without autoclaving	The teeth were stored in Chloramine solution of 0.5% for 6 months.	10
6-month Chloramine with autoclaving	The teeth were stored in Chloramine solution of 0.5% for 6 months then autoclaved at 121°C and the pressure of 15 psi for 40 minutes.	10
6-month Thymol without autoclaving	The teeth were stored in Chloramine solution of 0.5% for 12 months.	10
6-month Thymol with autoclaving	The teeth were stored in Chloramine solution of 0.5% for 12 months then autoclaved at 121°C and the pressure of 15 psi for 40 minutes.	10

Six different processes were done before the teeth were bonded to composite and each process was performed on ten teeth (Table 1). As it is described in the Table 1, half of the samples after storing in the solutions but before composite bonding process were Autoclaved.

For composite bonding process, first we mounted all the teeth from one millimeter above Cementoenamel Junction inside acrylic blocks. Then we flattened them with 1200 grit silicon carbide plates (Garmic, Germany). After that we placed a plastic gasket with the internal diameter of 2.4 mm and height of 3 mm on the prepared occlusal surface of each tooth and fixed it with the glue wax. We acidified the dentin surface with 37% phosphoric acid for 15 seconds and washed for 10 seconds and air dried for 10 seconds. We placed single-bond adhesive (3M ESPE, U.S.A) on the specimens according to the manufacturer's instruction and then light cured with intensity of 500 cm/2 mw for 20 seconds. The first layer of composite (Z250, 3M ESPE, U.S.A) with 1 mm thickness was placed inside the gasket

and cured for 40 seconds. The next composite layer with the thickness of 1.5 mm was placed and exposed to light for 40 seconds. Finally, the plastic gasket was removed.

All the prepared samples were placed in the incubator for the maximum of 2 weeks and tested in terms of shear bond strength using instron machine (Zwick, Germany) at a speed of 1 mm/min. Then, by dividing the recorded numbers by the surface area of the samples, the shear bond strength of composite to dentin was obtained. The Data were analyzed using SPSS software version 11.5 with Kruskal-Wallis and Mann-Whitney test. Level of significance was considered lower than 0.05.

### RESULT

According to K-S Test, distributions of variables were not normal. Kruskal-Wallis test showed that the shear bond was different among the experimental groups (P-value=0.02) (Table 2).

**Table 2: The mean, standard deviation and of shear bond strength in experimental groups and p-values vs fresh group**

Group	Mean ± SD shear bond strength (MPa)	p-value	
Fresh teeth	without autoclaving (control)	3.40 ± 0.66	-
	With autoclaving	4.29 ± 1.45	0.19
6-month Chloramine	without autoclaving	4.82 ± 1.03	0.01
	With autoclaving	5.20 ± 1.27	0.03

6-month Thymol	without autoclaving	4.05 ± 1.08	0.1
	With autoclaving	4.34 ± 1.44	0.2

Considering significant differences between the groups, Mann-Whitney test was used for Pairwise comparisons. There was a significant difference in shear bond strength of 6-month Chloramine groups (with or without autoclaving) in comparing to fresh group (P-value=0.01, 0.03).

Analyzing the effect of solution on shear bond strength, it was found that there were significant differences among the groups. (P-value=0.005) Shear bond strength Chloramine group was different with the fresh group (P-value=0.02) and Thymol group significantly (P-value=0.03).

Analyzing the effect of autoclaving on shear bond strength, showed that:

1. Shear bond strength in the fresh group had no significant difference with the autoclaved-fresh group (P-value=0.19).
2. Shear bond strength in 6-month Thymol group had no significant difference with the autoclaved-6-month Thymol group (P-value=0.69).
3. Shear bond strength in the 6-month Chloramine group had no significant difference with the autoclaved-6-month Chloramine group (P-value=0.32).

Interference between the type of solutions and use of autoclave on shear bond strength were not significant (P-value>0.05). Figure 1 shows the effect of autoclaving in different solutions on samples.

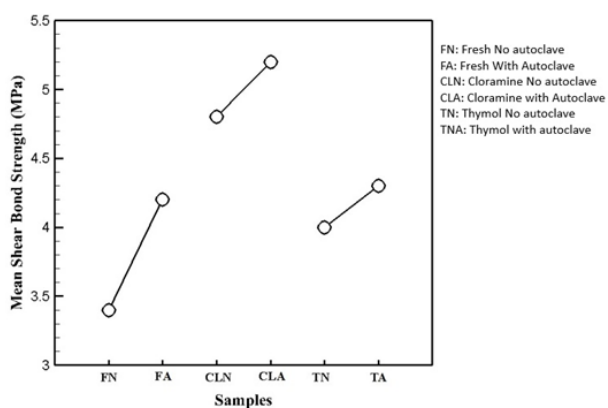


Figure 1: Effect of autoclaving in the solutions

## DISCUSSION

The changes over time in bond strength can be due to the impact of storage solution on the pores and mineral content of dentin. Solderholm and Rueggeberg in two separate review literature studies concluded that it is better to use dental samples for laboratory studies of adhesive systems that are extracted for a maximum of 6 months [20,21].

Given the fact that Thymol is a poor acid, it is possible to change the dentin mineral content over time [22]. Secilmis et al. found that storing teeth in Thymol solution 0.1% had significant impact on calcium, sodium, potassium and phosphorus of dentin [23]. In present study and the investigation of Humel et al. the storage of the teeth in Thymol for 6 months had no significant effect on the shear bond strength of the dentin to composite [9]. Similar to the present study Thymol has no effect on enamel permeability and bond strength and microleakage [7,14]. However, Tosun et al. and Titley et al. observed the storage of teeth for 2 months in Thymol can reduce the shear bond strength of composite to teeth significantly [11,13]. Zheng et al. stated that storage in Thymol 0.02% for maximum 90 days had lower micro tensile bond strength of composite to dentin than the control group [6].

Considering the very poor alkaline ability of Chloramine solution, studies observed no negative impact of Chloramine usage as the storage solution on bond strength and microleakage in their studies [3,4,6,7]. However, our results showed that the storage of teeth in Chloramine solution of 0.5% increased the bond strength of composite to dentin after 6-month storage significantly. Humel et al. found similar results in their studies regarding the usage of Chloramine solution with the same concentration and same duration for sample storage [9].

In this study, it was observed that autoclaving did not significantly affect the shear bond strength of the experimental groups. Similar to the current study, Dewald et al. and also Pashley et al. did not observe negative effect in using autoclave on tooth structure [17,18]. Attam et al. also reported that autoclave does not cause any significant change in the class V leakage repair [19]. Carvalho et al. observed that autoclaving made no significant changes to the morphology of the dentinal tubules or to the chemical composition of dentin [24].

Western et al. concluded that autoclaving was 100% efficient and a reliable method for sterilizing teeth that used for preclinical conservative exercises [25]. Dominici et al. showed that autoclaving sterilized the dental samples effectively. It should be noted that only usage of autoclaving or gamma radiation for a long time can sterilize teeth [15].

## CONCLUSION

Results of this study showed that the storage of the teeth in Thymol had no significant effect on the shear bond strength of composite to dentin. However, storage in Chloramine significantly increases the shear bond strength. For more than 6-month storage, Thymol is a better solution for storing the teeth in comparison to Chloramine. As autoclaving process does not effect on

samples even after a period of solution storages, sterilizing the extracted teeth before *in-vitro* studies is recommended.

#### CONFLICT OF INTEREST

The authors declared no potential conflicts of interests.

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