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Different storage media affect shear bond strength and ARI scores in orthodontic brackets: An *in vitro* study

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Abstract:
Shear bond strength (SBS) of orthodontic brackets may be influenced by the storage media used for extracted teeth in *in vitro* bonding studies. Hence, 120 extracted premolars were allocated to six storage media for 30 days before bracket bonding, followed by SBS testing with a universal testing machine and Adhesive Remnant Index (ARI) assessment. SBS differed significantly among the groups ($p<0.001$), with artificial saliva (13.92±2.69 MPa) and saline (13.38±2.51 MPa) producing the highest values, whereas ethanol (8.24±2.19 MPa) and formalin (9.67±2.44 MPa) showed the lowest. ARI score distribution did not differ significantly between the storage media ($p=0.214$), indicating comparable adhesive failure patterns despite differences in bond strength. Thus, data shows that physiologic storage media, particularly artificial saliva and saline, provided more consistent and clinically relevant SBS values than dehydrating or tissue-fixative media for orthodontic bonding research.

Keywords: Orthodontic brackets; shear bond strength (SBS); adhesive remnant index; storage media; artificial saliva; enamel bonding; *in vitro* study

Background:
The concept of direct bonding of orthodontic attachments to enamel revolutionized fixed appliance therapy by eliminating the need for full bands, enhancing esthetics and streamlining clinical procedures. The principle is based on the creation of micro mechanical retention through acid etching and resin infiltration of enamel microporosities. Laboratory bond-strength testing has been a key part of the evaluation of bracket systems, adhesives, surface conditioning methods and debonding behavior since the first description of acid conditioning and the development of direct orthodontic bonding **[1, 2]**. An orthodontic bond that is clinically useful should be strong enough to resist masticatory loading, application of orthodontic forces, engagement of archwires and oral functional stresses, but should not cause clinically significant enamel damage when the bracket is removed. The accepted range for orthodontic bond strength is around 6-8 MPa, but the ideal value is not a fixed number as it is greatly affected by the bracket design, type of adhesive, quality of the enamel, direction of debonding, method of aging and test configuration **[2, 3]**. Shear bond strength (SBS) testing is commonly used as it is simple, reproducible and can be compared under standard laboratory conditions. But the specimen geometry, the area of the brackets base, the crosshead speed, the point of load application and the enamel substrate condition are all factors that affect the sensitivity of SBS. Thus, the small-scale details of the methodology can make a big difference between studies and can account for the differences in performance between similar adhesives in different laboratories **[3, 4]**. The Adhesive Remnant Index (ARI) offers additional information by indicating the location of bond failure following debonding. A low ARI score indicates that there is less adhesive remaining on the enamel and that failure occurred near the enamel-adhesive interface, while a high score indicates that there is more adhesive on the enamel and that failure occurred near the bracket-adhesive interface. Despite being semi-quantitative, ARI is useful because it correlates bond strength results with the requirements for cleanup and potential enamel safety **[3]**. Human premolars are often used in orthodontic bonding experiments due to their enamel morphology that closely mimics the clinical substrate. However, teeth are not tested right after extraction. They are usually kept in water, saline, formalin, thymol, chloramine-T, ethanol, hydrogen peroxide or artificial saliva to keep them hydrated, inhibit microbial growth or simulate oral conditions. They vary in pH, tonicity, antimicrobial activity, protein fixation and dehydrating ability, which can alter surface energy of enamel, interprismatic spaces, remnants of organic pellicle and dentino-enamel moisture exchange **[4, 5]**. There are reports in the literature that bond-strength measurements can be affected by the storage media. Dehydrating or chemical-fixative solutions have been linked to changes in bonding behavior, while water, saline and chloramine-T have frequently yielded more consistent results. Previous laboratory studies also showed that the dentin permeability and bond strength can be altered by storage conditions, reinforcing the overall premise that the biological substrate is not chemically inert following extraction **[4, 6 and 7]**. Artificial saliva has been the focus of interest as a storage medium as it closely mimics the ionic composition and wet environment of the oral cavity. Ethanol can dehydrate hard tissue and remove organic matter, formalin can preserve organic matter and alter wettability and thymol can offer antimicrobial preservation and have varying effects on the substrate. When *in vitro* results are used to guide *in vivo* recommendations for adhesives or bonding protocols, these differences are important **[5, 6]**. Even with this relevance, many orthodontic SBS studies either fail to report the storage medium clearly or employ different storage media without taking into account the possible effect of the media. Furthermore, most studies are directed towards the change of the SBS value and fewer consider the change of the ARI pattern as well. The combination of SBS and

ARI is required because a storage medium can not only alter bond magnitude, but also alter the failure locus, which can impact the interpretation of enamel safety and adhesive clean up **[6, 8]**. Current research on orthodontic bonding still focuses on bracket base morphology, moisture-resistant systems, primers, remineralizing additives and newer formulations of adhesives. However, the standardization of tooth storage prior to bonding is not as discussed as adhesive selection or light-curing protocol. This methodological gap is particularly important in postgraduate and preclinical research laboratories where extracted premolars can be kept for several weeks prior to testing **[8, 9]**. Therefore, it is of interest to assess the influence of six different storage media on the SBS and ARI scores of orthodontic brackets bonded to extracted human premolars.

Materials and Methods:

Study design:

This is an *in vitro* experimental study conducted in the Department of Orthodontics and Dentofacial Orthopedics in association with dental materials testing laboratory. The study compared the SBS and ARI scores of metal orthodontic brackets bonded to extracted premolars following 30 days storage in six different media.

Sample size:

A sample size of 120 teeth was chosen, based on similar studies of orthodontic bond strength and to ensure 20 teeth per group, thus providing 80% power to detect a moderate intergroup difference in SBS at 5% alpha error. All the teeth were considered as separate experimental units.

Tooth selection:

Human maxillary and mandibular premolars extracted for orthodontic reasons were collected after obtaining informed consent for research use. Teeth were included if the buccal enamel was intact, the crown was fully formed; there was no visible caries, cracks, restoration, developmental defects, fluorosis, hypoplasia, or chemical treatment. Fractured crowns, enamel cracks seen under magnification, buccal restorations and obvious decalcification were excluded.

Specimen preparation:

The soft tissue remnants and calculus were removed from the buccal surface of the tooth with hand scalers without damaging the enamel, immediately after extraction. The teeth were cleaned in running water and then looked at under 10x magnification. The roots were placed in acrylic resin blocks using self-cure acrylic resin with the buccal surface parallel to the debonding blade. The cemento-enamel junction was left above the acrylic to prevent contamination of the bonding area.

Grouping and storage media:

The 120 teeth were randomly divided into six groups of 20, with a computer-generated sequence. Group I teeth were kept in distilled water, Group II in isotonic saline, Group III in artificial saliva, Group IV in 0.1% thymol solution, Group V in 10% formalin and Group VI in 70% ethanol. The teeth were placed in separate sterile labeled containers with 10 mL of medium at room temperature for 30 days. Media were changed every week to reduce contamination and concentration changes.

Bonding protocol:

All specimens were thoroughly rinsed with distilled water for 30 seconds, dried gently with oil-free air and polished for 10 seconds with non-fluoridated pumice and a rubber cup. The buccal enamel surface was etched with 37% phosphoric acid gel for 30 seconds, rinsed for 20 seconds and dried until a frosty white appearance was obtained. A thin layer of primer was applied and light cured according to manufacturer's instructions. A light-cured orthodontic composite resin was used to bond stainless-steel premolar brackets with a mean base area of 10.5 mm^2. An explorer was used to remove excess adhesive prior to curing. The LED curing unit was used to light-cure each bracket for 20 seconds from the mesial side and 20 seconds from the distal side with output intensity verified prior to use.

Post bonding storage and SBS testing:

All specimens were stored in distilled water at 37°C for 24 hours after bonding and then tested for SBS. A universal testing machine was used for the SBS testing. A debonding blade with a chisel shape was placed as close as possible to the bracket-enamel interface and load was applied in an occluso-gingival direction at a crosshead speed of 1 mm/min until the bracket failed. The maximum load at debonding was measured in Newtons and then divided by the area of the bracket base to obtain megapascals.

Evaluation by ARI:

Enamel surfaces were evaluated after debonding under 10 x magnifications by a calibrated examiner who was blinded to the storage group. The ARI scores were obtained on a 4-point scale: 0 = no adhesive remaining on enamel, 1 = less than half of the adhesive remaining, 2 = more than half of the adhesive remaining and 3 = all adhesive remaining with impression of bracket mesh. To determine intra-examiner reliability, 20 randomly selected specimens were rescored after two weeks.

Data analysis:

Data was analyzed by using statistical analysis using SPSS version 26.0. The Shapiro-Wilk test was used to determine the normality of the values of SBS. Descriptive statistics were presented as mean, standard deviation, median, minimum, maximum and 95% confidence interval. One-way analysis of variance with Tukey post hoc testing was used to compare intergroup SBS. The chi-square test was used for comparing ARI distributions. Weighted kappa was used for intra-examiner reliability for scoring ARI. A p value of < 0.05 was deemed statistically significant.

Results:

A total of 120 extracted premolars were examined, 20 in each storage media group. No embedding, bonding or SBS testing

loss. No crown fracture was observed in all the bracket failures. Intra-examiner reliability for the scoring of ARI was very high (weighted kappa: 0.88). The artificial saliva group had the highest mean SBS of 13.92±2.69 MPa, followed by isotonic saline group at 13.38±2.51 MPa and distilled water group at 12.11±2.68 MPa. The lowest mean SBS was observed in the 70% ethanol group (8.24±2.19 MPa), followed by 10% formalin (9.67±2.44 MPa) and 0.1% thymol (10.85±2.36 MPa). The results of one-way ANOVA indicated that there was a significant difference in SBS between the six groups ($F=18.74$, $p<0.001$). The post-hoc comparison revealed that the values of SBS for artificial saliva and saline were significantly higher than those of formalin and ethanol. The distilled water had a significantly higher value of SBS than ethanol, but was not significantly different from saline, artificial saliva, or thymol. The ethanol group was above the minimum clinical range, but had significantly lower bond strength than physiologic storage media. The ethanol and formalin groups tended to have lower ARI scores, suggesting a higher number of failures at the enamel-adhesive interface. The overall difference in the distribution of ARI between the storage media was not statistically significant, however (chi square=18.37, $p=0.214$). The most common score for the entire sample was score 1, followed by score 2. Debonding did not reveal any enamel cracks less than 10 x magnifications **(Table 1-3).**

Table 1: Distribution of specimens and storage media characteristics

Group	Storage medium	n	Storage duration	Approximate pH	Primary laboratory purpose
I	Distilled water	20	30 days	6.8-7.0	Hydration control
II	Isotonic saline	20	30 days	7.0-7.2	Physiologic ionic storage
III	Artificial saliva	20	30 days	6.7-7.1	Oral-condition simulation
IV	0.1% thymol	20	30 days	6.9-7.2	Antimicrobial storage
V	10% formalin	20	30 days	7.0-7.4	Tissue fixation/disinfection
VI	70% ethanol	20	30 days	7.0-7.4	Disinfection/dehydrating medium

Table 2: Shear bond strength comparison among storage media

Group	Mean SBS (MPa)	SD	Median	Minimum-Maximum	95% CI	Post hoc interpretation
Distilled water	12.11	2.68	12.20	7.84-16.92	10.86-13.36	Higher than ethanol (p=0.002)
Isotonic saline	13.38	2.51	13.45	8.96-17.81	12.21-14.55	Higher than formalin and ethanol (p<0.01)
Artificial saliva	13.92	2.69	14.10	9.15-18.74	12.66-15.18	Highest; higher than formalin and ethanol (p<0.01)
0.1% thymol	10.85	2.36	10.62	6.98-15.22	9.75-11.95	Lower than artificial saliva (p=0.018)
10% formalin	9.67	2.44	9.48	5.92-14.06	8.53-10.81	Lower than saline and artificial saliva (p<0.01)
70% ethanol	8.24	2.19	8.05	4.91-12.68	7.22-9.26	Lowest; significantly lower than water, saline and artificial saliva
ANOVA	F=18.74	-	-	-	p<0.001	Overall significant difference

Table 3: Adhesive Remnant Index score distribution after debonding

Storage medium	ARI 0 n (%)	ARI 1 n (%)	ARI 2 n (%)	ARI 3 n (%)	Most frequent score	p-value
Distilled water	3 (15.0)	8 (40.0)	7 (35.0)	2 (10.0)	1	
Isotonic saline	2 (10.0)	7 (35.0)	8 (40.0)	3 (15.0)	2	
Artificial saliva	2 (10.0)	6 (30.0)	9 (45.0)	3 (15.0)	2	
0.1% thymol	4 (20.0)	8 (40.0)	6 (30.0)	2 (10.0)	1	
10% formalin	6 (30.0)	8 (40.0)	5 (25.0)	1 (5.0)	1	
70% ethanol	7 (35.0)	9 (45.0)	3 (15.0)	1 (5.0)	1	
Total	24 (20.0)	46 (38.3)	38 (31.7)	12 (10.0)	1	0.214

Discussion:

The present *in vitro* study showed that the storage medium had a significant effect on the SBS of orthodontic brackets bonded to extracted premolars. For the null hypothesis of SBS, the results indicated that the bond strength of teeth stored in artificial saliva and isotonic saline was higher than that of teeth stored in ethanol and formalin and thus the null hypothesis was rejected. The null hypothesis for ARI distribution was not rejected, however, because there were no significant differences in failure patterns. The results highlight the importance of pre-bonding storage conditions affecting the interpretation of orthodontic adhesive performance, even if the bonding protocol and testing apparatus are standardized. Preservation of enamel hydration and ionic balance may account for the highest SBS values obtained in artificial saliva and saline. Enamel is highly mineralized, but contains interprismatic spaces, water and organic remnants which can affect surface energy and resin infiltration after acid etching. Physiologic aqueous environment may reduce desiccation and help to preserve a substrate closer to clinical conditions. Previous studies have shown that water, saline and chloramine-T yielded similar bond strength of orthodontic brackets, while dry storage, ethanol and formalin were not as well suited for bond-strength studies **[10]**. In this study, the highest mean SBS was obtained from artificial saliva. This is due to its calcium, phosphate and electrolyte content, which may help to prevent excessive dehydration of enamel and to keep the enamel surface stable during storage. When saliva simulation is not required, however, the difference between artificial saliva and saline was not statistically significant and may be considered as a simple isotonic medium. The similarity in behavior of these two groups is consistent with the use of physiologic media for experimental designs that attempt to simulate the clinical enamel bonding more closely **[10, 11]**. The bond strength of distilled water was in the middle range. It was able to keep the mouth hydrated and generated SBS values that were higher than the clinically acceptable range, but slightly lower than saline and artificial saliva. Distilled water storage may lead to mineral exchange and does not restore physiologic

ionic content. However, in the present study it was found to be acceptable and distilled water can be used as a good control medium if the storage time is short and the study is not concerned with the preservation of the substrate **[11, 12]**. The thymol group had lower SBS values than artificial saliva but within clinically acceptable limits. Thymol is commonly used due to its antimicrobial activity and ease of use. Antimicrobial preservatives can, however, interact with the tooth surface or change the wettability, particularly if the storage time is extended. Research on extracted teeth indicates that the concentration and storage time of the disinfectant or preservative media may affect the bond strength and microleakage results, which should be reported clearly **[12, 13]**. The formalin produced reduced the SBS than saline and artificial saliva. Formalin is commonly used for disinfection and tissue fixation, but its protein fixing property can alter the surface characteristics and residual organic components. Formalin is effective for infection control, but may not be the most suitable when the outcome relies on adhesive interaction with enamel. The observed reduction reinforces the requirement to differentiate between a storage medium selected for biosafety and one selected for the validity of the bond test **[13, 14]**. The ethanol group had the lowest SBS. Ethanol is a dehydrating agent and can remove organic material, change surface wetting and form a substrate that is different from clinically hydrated enamel. The lower values reported in this group are in line with the reports that ethanol and dry conditions should be avoided for orthodontic bond-strength research with the aim of simulating enamel bonding in the clinical situation. The mean SBS in ethanol was still within the minimum range generally accepted, but the decrease was significant enough to affect the inter-study comparisons **[10, 14]**. The results of the ARI did not reveal any significant difference between the groups, but ethanol and formalin had more low ARI scores. A low ARI pattern suggests that there is less adhesive left on enamel and that there is more failure at or near the enamel-adhesive interface. This trend is consistent with their reduced SBS values, as a reduced interaction between resin and enamel could lead to adhesive failure at the tooth surface. The semi-quantitative scoring of ARI, however and the small number of samples per group could have limited the power to detect a significant difference **[15]**. When interpreting ARI, the safety of the enamel must be weighed against the burden of clean-up. Higher ARI scores are generally safer for enamel as there is more adhesive left on the tooth and the failure takes place away from the enamel surface; however, they take longer to finish and can pose a greater risk of iatrogenic enamel loss during cleanup. Lower ARI scores will decrease clean up but may indicate failure near the enamel. No cracks in the enamel were observed after debonding in the present study, which means that all media used in the present study produced debonding patterns that were macroscopically acceptable under the testing conditions **[15, 16].** The results also point to a methodological problem: storage medium should not be considered a background detail. Numerous studies have been done on bond strength, which compare adhesive systems, primers, remineralizing agents, curing lights, or bracket bases, but there may be some variation in the way the extracted teeth are stored that can affect the results. If two studies are conducted using the same adhesive but different storage conditions, the differences in SBS may be due to the changes in the substrate, not the adhesive. Thus, it is critical to report the interval between extraction and testing, the concentration of the solution used for storage, how often the solution is replaced and the pre-bonding rinsing procedure **[16, 17]**. There are some limitations to the study. No thermocycling or long-term aging was carried out, so the results are early bond strength after 24 hours, not durability under repeated thermal stress. The storage time was restricted to 30 days with some laboratories storing teeth for longer periods. Only one type of bracket and one light-cured adhesive system were employed, thus enhancing internal standardization and restricting the generalizability to ceramic brackets, self-etching systems, resin-modified glass ionomers and moisture-insensitive primers. The ARI score was subjectively evaluated under magnification, but there is potential for more detailed information to be gained by objective digital or microscopic measurement **[17, 18]**. In the scope of these restrictions, the study recommends that artificial saliva and isotonic saline are better pre-bonding storage media for extracted premolars for orthodontic SBS testing. Distilled water may be used for short-term control storage and ethanol and formalin should not be used if bond strength is the main outcome. Further studies are needed to evaluate the changes in the bonding substrate after longer storage periods, thermocycling, surface roughness, contact angle and changes in enamel microhardness to better understand how the storage media affect the bonding substrate.

Conclusion:

We show that storage medium significantly influenced the shear bond strength of orthodontic brackets bonded to extracted human premolars. Artificial saliva and isotonic saline produced the highest and most consistent bond strength values, whereas ethanol and formalin resulted in significantly lower SBS. Although ARI scores were comparable among the groups, standardizing and reporting storage media is essential to improve the reliability and comparability of *in vitro* orthodontic bonding studies.

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