

Storage Media: A Neglected Variable for *in vitro* Studies

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ABSTRACT

Introduction: Various storage media have been used in orthodontic shear bond strength studies, however, the effect of the storage media on human enamel has not been studied.

Aim: The purpose of this *in vitro* study was to determine the effect of four commonly used storage media on the shear bond strength values of orthodontic brackets bonded on extracted human teeth.

Materials and methods: A total of 60 freshly extracted noncarious premolars teeth were randomly divided into four groups. Group 1 was stored in distilled water, samples of group 2 were stored in 10% formalin, group 3 were preserved in 70% ethanol and samples of group 4 were stored in isotonic saline solution. After a storage period of 30 days, the teeth were rinsed and brackets bonded with Transbond XT and the specimens were evaluated for shear bond strength.

Results: The 10% formalin sample had statistically significant greater bond strength than the other test groups. The 70% ethanol group had statistically lowest bond strength compared to other groups. The distilled water and isotonic saline solution group showed bond strength which was comparable.

Conclusion: Storage media have an effect on bond strength results. Distilled water and isotonic saline storage produced comparable bond strength. Formalin and ethanol storage produced extreme variation in SBS values.

Keywords: Storage media, Human enamel, Shear bond strength (SBS).

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INTRODUCTION

Technological advancements in the field of material sciences necessitate various laboratory testing procedures before a material is cleared and introduced commercially into clinical practice. The clinician is faced with an array of orthodontic bonding adhesives flooded in the market. The researcher provides valuable input into the range of bond strengths of the materials available to the clinician. Most of the orthodontic adhesives are tested for bond strength and biocompatibility *in vitro*, as there are ethical and clinical issues involved with testing these materials *in vivo*. These difficulties in research have led to the *in vitro* testing procedures on extracted human/bovine teeth.

The extracted teeth are a potential source for cross-contamination to laboratory equipments and personnel, hence

they need to be decontaminated before storage. Numerous solutions for storage and disinfection/sterilization methods to treat extracted teeth are in practice. It is vital to remember that the reliability and success of bond strength studies and bonding procedures depend both on the integrity of enamel/dentin as well as the bonding material used. The storage media usually provide adequate disinfection/sterilization of the teeth, however the focus of concern is on the ability of these media to conserve the integrity of surfaces to be bonded, i.e. the enamel and/or dentin.

Hence, the question arises, if these storage media have an adulterating effect on research studies assessing bond strength. Therefore, it was decided to conduct this research on the effects of storage media, which may be considered as a neglected variable in most of the *in vitro* bond strength studies.

AIM

The purpose of this study was to determine the effect of four commonly used storage media (distilled water, 10% formalin, 70% ethanol and isotonic saline solution) on the shear bond strength values of orthodontic brackets bonded on extracted human teeth.

MATERIALS AND METHODS

Sixty freshly extracted noncarious premolars obtained from therapeutic orthodontic extractions were used in the study. The teeth were debrided by rinsing under running water followed

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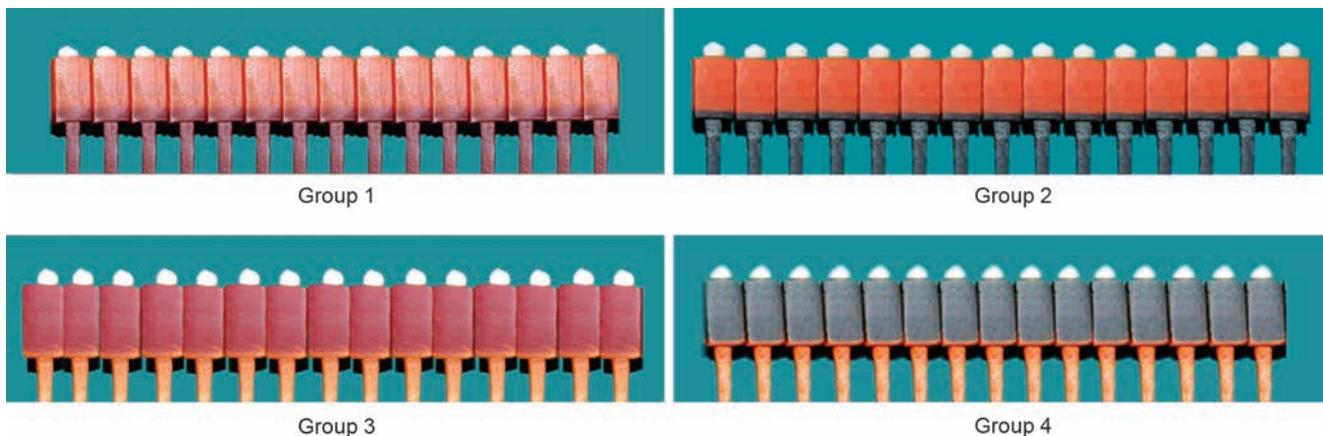


Fig. 1: Samples mounted on color-coded acrylic testing cylinders

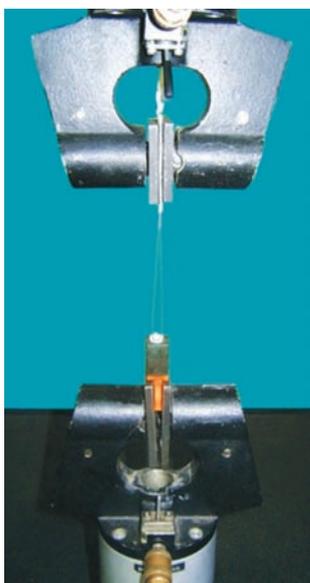


Fig. 2: Testing of SBS on an instron testing machine

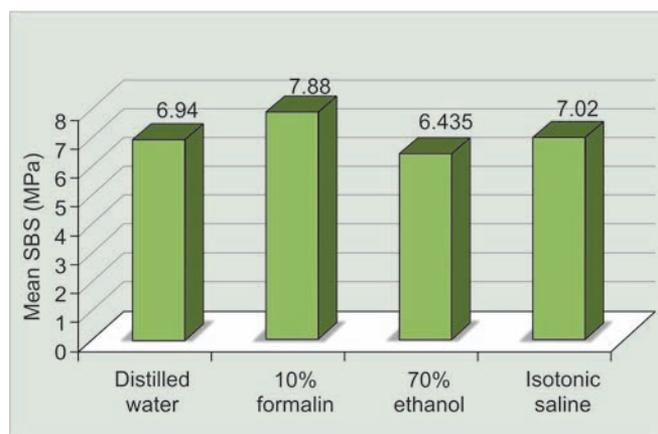


Fig. 3: Comparison of mean SBS values

by prophylaxis using EMS™ Piezon systems and polished with pumice rubber cups. The teeth were randomly divided into four groups with 15 samples each. Group 1 was stored in distilled water. Samples of group 2 were stored in 10% formalin. Group 3 were preserved in 70% ethanol and samples of group 4 were stored in isotonic saline solution. The teeth were stored in the media for a period of 30 days prior to testing. They were then removed from their respective storage media and rinsed in distilled water and mounted in color-coded acrylic testing cylinders for identification (Fig. 1).

The samples were bonded with brackets from 3M Unitek (Gemini series) with Transbond XT (3M Unitek) as per the manufacturer’s instructions, after acid etching for 30 seconds with 37% orthophosphoric acid. The adhesive was cured with a halogen curing light (Blue Luxcer™, M-855) for a total of 40 seconds, with 20 seconds on the mesial and 20 seconds on the distal surfaces. All the samples were dipped in distilled water at room temperature for a period of 24 hours prior to testing. Subsequently, the samples were subjected to testing for shear

bond strength (SBS) on an Instron universal testing machine at a cross-head speed of 1 mm per minute (Fig. 2).

RESULTS

The data collected was tabulated and subjected to one-way ANOVA for intergroup variability assessment. Samples stored in distilled water showed the maximum SBS of 7.4 MPa and minimum of 6.5 MPa with a mean of 6.94 MPa. For the samples stored in 10 % formalin, the SBS ranged from 7.6 to 8.2 MPa with a mean of 7.88 MPa. The ethanol group showed a maximum SBS value of 6.9 MPa and a minimum of 6.2 MPa with a mean of 6.43 MPa. The sample stored in isotonic saline solution exhibited SBS values ranging from 6.5 to 7.4 MPa with a mean of 7.02 MPa (Table 1 and Fig. 3).

Comparison of the mean SBS values revealed that the formalin group exhibited the highest values followed by both distilled water and isotonic saline solution showing comparable values and the ethanol group showing the lowest SBS values (Fig. 3).

Table 1: SBS values

Sample no.	Distilled water (MPa)	10% formalin (MPa)	70% ethanol (MPa)	Isotonic saline (MPa)
1	7.2	8.0	6.2	6.9
2	7.4	7.7	6.5	6.9
3	6.9	7.9	6.5	7.1
4	7.1	7.8	6.3	7.0
5	6.8	7.7	6.3	6.5
6	6.5	8.2	6.5	7.2
7	7.0	7.9	6.4	7.0
8	6.9	7.9	6.5	7.4
9	6.8	8.1	6.9	7.2
10	6.9	7.6	0.0	6.9
11	6.9	7.7	6.4	7.0
12	7.0	7.9	6.5	6.9
13	6.8	7.8	6.2	7.1
14	7.1	8.1	6.3	7.0
15	6.9	7.9	6.6	7.2

DISCUSSION

Research in the field of orthodontic material science focuses to a large extent on the bond strengths of adhesives. The bond strength values of orthodontic adhesives stand on a vivid platform which is remarkably different from the bond strength requirements of material used in other fields. In orthodontics, one would hardly appreciate the two extremes of the spectrum but would rather concentrate at the more desirable optimum range between 6 and 8 MPa. The success of *in vitro* bond strength testing has so far completely relied upon the close simulation of extracted teeth to near natural conditions. Bovine teeth have been used in most studies as the radiodensity of human teeth is a close match to that of the bovine teeth. The need for imperative sterilization and disinfection protocols of these extracted teeth are indeed unequivocal. However, stress has never been laid on the implications of the storage conditions on the structure of enamel per se, which is usually presumed to be left intact.

Literature evidences a few studies addressed to the effects of storage media on dentine by Kimura et al.¹ Retief et al.² and Goodis et al.³ However, the focus of concern in orthodontics is enamel, to which most of the orthodontic attachments are bonded. Due to the hardness, high inorganic and low organic content, it has often been thought that the effect of storage media on enamel would be minimal. A thorough literature review revealed a singular study conducted to assess the effect of storage media on bovine enamel by Jaffer et al.⁴ However, the lacuna of information still existed in relation to the effects of storage media on human enamel. This study was aimed to provide insights into the effect of commonly used storage media on SBS of brackets bonded to human enamel. This is the first reported study of its kind on human enamel. The storage media selected for the study were those most commonly used by researchers—distilled water, 10% formalin, 70% ethanol and isotonic saline solution.

The results of the present study indicate variations in the bond strengths of teeth stored in different media. The 70% ethanol group showed mean shear bond strength of 6.43 MPa, which was the lowest compared to other groups over a period

of one month storage. This is similar to the observations of Retief et al.² when testing the effect of ethanol on dentin bonding and Jaffer et al.⁴ on bovine enamel. A plausible explanation for this effect could be due to the established desiccating action of ethanol. The mean SBS of samples stored in isotonic saline solution and distilled water were comparable at 7.02 MPa and 6.94 MPa respectively. These findings are in consonance with the results of Jaffer et al.⁴

The 10% formalin group showed the highest SBS with a mean value of 7.88 MPa, which was statistically significant and similar to the results of Jaffer et al.⁴ Formalin has primarily been used for fixing tissues for microscopy and histology, and for embalming and temporarily preserving human and animal remains. Formaldehyde fixes tissues and cells by crosslinking primary amino-group with nearby nitrogen atoms in proteins and DNA through $-CH_2-$ linkage while simultaneously acting as a disinfectant of repute. This property of formaldehyde has made it the most commonly used media of choice for disinfecting and storing extracted teeth prior to testing. However, this storage media seems to alter the structure of bonding surfaces which no longer simulates the natural *in vivo* conditions, thereby resulting in erroneous bond strength values.

Cogent evidence of similar adulterating effect of formalin has also been shown on bone specimen studies in medical field. Wilke et al.⁵ and Burkhart et al.⁶ stated that formalin storage altered biomechanical properties of bone. The specimens stored in formalin showed higher stiffness values of about 40% and decreased range of motion by 80% as compared to fresh samples. The study concluded that formalin stored bone specimens were not representative of *in vivo* conditions. The effect on stored extracted teeth could be further elucidated by the physical property of high dielectric constant of formalin and the chemical property of stabilizing collagen by fixing and crosslinking proteins, which prevents the network of decalcified collagen fibrils from collapsing, thus resulting in higher bond strength values.

Shear bond strength tests though used widely *in vitro* have been criticized for their lack of standardization and consistency. Similarly, studies on effect of storage media are also replete with lacuna due to the lack of protocol standardization and presence of numerous variables which can affect the outcome. To achieve highly conclusive results, studies should be conducted with longer storage time on larger sample size with variation in storage duration and test specimen incorporating young and old enamel. The disinfecting storage media, like Chloramine T, 0-1% Thymol, sodium hypochlorite and 2% glutaraldehyde need to be evaluated as they would fulfill the additional need for disinfecting the extracted teeth which will not be provided by isotonic saline or distilled water. Sterilization modalities like autoclaving, gamma radiation and cryotechnology would be worth studying supported by SEM and Cone form microscopic studies to ascertain the changes in the structure of enamel caused by the storage media.

CONCLUSION

As storage media have an effect on bond strength, the proper selection of the storage media is imperative for realistic and

translative research of *in vitro* bond strength testing. The recommendations from this research study based on the four media indicate that 70% ethanol should be avoided as a storage media due to its desiccating effect on enamel. SBS values of 10% formalin group were found to be at the upper extreme of the optimum range after one month storage, hence the results from SBS studies may be unreliable. Isotonic saline and distilled water can be safely recommended as optimum media for storage of extracted teeth prior to SBS testing for short term as the efficacy as disinfectants are questionable .

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