Impact of Storage Period on the Shear Bond Strength of Various Universal Dental Adhesives

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Impact of Storage Period on the Shear Bond Strength of Various Universal Dental Adhesives

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ABSTRACT

Impact of Storage Period on the Shear Bond Strength of Various Universal Dental Adhesives

The aim of this study was to evaluate the effects of varying storage durations, including both within and up to the expiry date, on the shear bond strengths (SBS) of various universal adhesives in clinic environments and in acclimatization chamber simulations.

The investigation was conducted in three phases. Part A evaluated the SBS of eleven universal adhesives immediately after opening and after 6-month clinic storage. In Part B, the SBS of three selected adhesives —Single Bond Universal (SBU), All Bond Universal (ABU), and K-Bond Universal (KBU)— were assessed after one year in a real clinic environment compared to a simulated environment using an acclimatization chamber. Part C extended this simulation to two years, assessing the SBS of the same adhesives both as-received and after simulated storage for one and two years. Adhesives were applied to the prepared dentin surfaces of extracted bovine teeth, embedded in acrylic resin. SBS testing was performed, and the failure modes were analyzed using a dental operating microscope and scanning electron microscopy (SEM). Statistical analysis involved two-way ANOVA for Parts A and B, with multiple comparisons in Part C using Tukey's post hoc tests. For all analyses, a significance level of 95% ($p < 0.05$) was utilized.

Part A demonstrated no significant difference in SBS between adhesives used immediately and six months post-openin. In Part B, the adhesives stored for one year in real and simulated conditions showed no significant difference in SBS. However, the results of Part C indicated a significant reduction in SBS for ABU and KBU after one and two years of simulated storage, while SBU showed stability after one year but a significant decline after two years.

Within the scope of this study, the following conclusion can be drawn:

1. Various universal adhesives showed varying shear bond strengths to dentin; some of the adhesive systems indicated significantly higher shear bond strength. They all remained stable after six months of clinic storage.

2. There were no significant differences in the SBS between one-year real-time storage and simulated conditions.

3. Accelerated aging simulating two years of clinic storage resulted in a significant reduction in the SBS of universal adhesives; extended storage time after the opening of adhesive bottles may result in reduced bonding performance, emphasizing the importance of adhering to expiration dates for universal adhesives.

Key words : universal adhesive, storage duration, shear bond strength, accelerated aging, simulation, acclimatization chamber, shelf life, expiration date

Impact of Storage Period on the Shear Bond Strength of Various Universal Dental Adhesives

1. Introduction

Dental adhesives play a crucial role in modern dentistry, enabling the durable bonding of composite materials to tooth structures, essential for restorative procedures. These adhesives ensure the longevity and effectiveness of dental restorations, influencing both aesthetic outcomes and functional longevity. The advancement in adhesive technologies has significantly enhanced treatment outcomes, making adhesive dentistry a cornerstone of dental practice today (Cuevas-Suárez et al., 2019; Hardan, L et al, 2021).

Nowadays, the advent of universal adhesives allowed for adhesive system that is less technique sensitive and quicker in application, along with a broadened range of usage (Hardan, L et al, 2021; Geng Vivanco, R et al., 2020). Universal adhesives are designed to perform effectively across a variety of substrates and under different clinical conditions. These adhesives simplify the clinical workflow by being compatible with multiple etching modes and possess enhanced chemical formulations that often include methacrylates and advanced photo-initiators to ensure robust bonding (Iliev et al., 2021; Van Landuyt, K. L., 2007).

Nevertheless, the performance of universal adhesives may be significantly affected by their shelf-life, as it is known to decline as they approach or exceed their expiry dates. These adhesives are particularly susceptible to changes in their properties due to the complex nature of their formulation, which includes solvents, monomers, and other reactive components that are sensitive to environmental conditions and storage durations. Factors such as evaporation of volatile components and hydrolysis of ester bonds in the methacrylic groups may alter the adhesive's viscosity and bonding capability, thus compromising the bond strength and longevity of dental restorations (Iliev et al., 2021; Van Landuyt, K. L., 2007; Van Landuyt, K. L., 2009; Salz, U. et al., 2010; Pongprueksa et al., 2014).

In order to rapidly obtain data regarding the aging behaviour of polymeric materials, accelerated aging (ACC) is often used in industry as well as in research. ACC methods are critical for predicting long-term performance and compliance with regulatory standards, particularly for medical and dental devices and materials such as universal adhesives. The use of acclimatization chambers to simulate extended storage times under controlled conditions allows for the assessment of material stability and degradation over what would equate to years in real-time conditions. ACC techniques allow for studies to provide valuable insights into how adhesives would perform when subjected to extended storage periods, offering a predictive measure of their shelf life and stability under various temperature and humidity conditions. The process adheres to established standards such as ASTM F1980, which provides a scientific basis for accelerated aging by incorporating controlled variations in temperature and humidity to simulate natural aging within a significantly reduced timeframe. (ASTM International, 2021; Krug, N. et al., 2023).

Shear bond strength (SBS) testing is a pivotal method for evaluating the bonding performance of dental adhesives to dentin. The shear bond strength test in accordance with ISO 29022, is widely recognized as a standard testing method for SBS since it is employed for the regulatory certification of dental materials (ISO 29022, 2013; Hu, M. et al., 2016; Schröter, F. J et al., 2023). It offers a direct measure of an adhesive's capacity to withstand forces parallel to the bond interface, mimicking the mechanical stresses encountered in clinical use. As this test provides quantitative data on the adhesive strength of dental materials to tooth structures, it allows for prediction of their clinical performance and durability. The choice of SBS testing for this study was guided by its widespread acceptance for providing reliable and reproducible data, allowing for comparative analysis across different adhesive formulations and storage conditions. SBS tests are particularly valued for their ability to highlight performance under simulated clinical conditions, providing a robust indicator of an adhesive's clinical reliability. (Barkmeier, W. W et al, 1992; Hegde, M. N et al., 2008; Gupta, S et al., 2015; Placido, E et al., 2007).

Thus, the purpose of this study was to evaluate the effects of storage durations on the shear bond strengths of various universal adhesives, particularly focusing on their performance within and up to the expiry dates, under both real-time storage and simulated storage using accelerated aging. The null hypotheses tested were the following: 1) the shear bond strength of various universal adhesives would be stable after six months of clinic storage, 2) there would be no difference between the shear bond strength of universal adhesives stored in clinic for one year and stored in an acclimatization chamber simulating one year of storage, 3) there would be no difference between the shear bond strength of universal adhesives used immediately as-received, stored in an acclimatization chamber simulating one year of storage and stored in the same acclimatization chamber simulating two years of storage.

2. Materials & Methods

2.1. Part A: as-received vs 6-month clinic storage

To evaluate the shear bond strength of various universal adhesives to dentin in their as-received condition (immediately after opening) and after clinic storage for six months, 11 commercially available universal adhesives were selected for evaluation: All Bond

Universal™ (ABU, 6mL, Bisco Inc., Schaumburg, IL, USA), ClearFil S3 Bond Universal® (CBU, 5mL, Kuraray Noritake Dental Inc., Tokyo, Japan), Dia-X Bond Universal™ (DBU, 5mL, Dia-Dent Group International, Chino, CA, USA), GLUMA® Bond Universal (GBU, 4mL, Kulzer GmbH, Hanau, Germany), Hi-Bond Universal™ (HBU, 5mL, Mediclus Co., Ltd., Seoul, South Korea), K-Bond Universal™ (KBU, 5mL, Spident Co., LTD, Incheon, South Korea), OptiBond™ Universal (PBU, 5mL, Kerr Corporation, Orange, CA, USA), Prime & Bond Universal® (PBU, 4mL, Dentsply Sirona, York, PA, USA), Single Bond™ Universal (SBU, 5mL, 3M ESPE, St. Paul, MN, USA), Tetric® N-Bond Universal (TBU, 6g, Ivoclar Vivadent AG, Schaan, Liechtenstein) and Zipbond Universal™ (ZBU, 5mL, SDI Limited, Bayswater, Victoria, Australia). The manufacturer, composition and application method of the universal adhesives explored in this study are described in Table 1. Each adhesive was utilized in two conditions:

1). **As received**: Utilized immediately upon opening.

2). **6-month clinic storage**: Utilized after opening and storing in a dental clinic environment for six months.

The adhesive bottles were stored in the clinic environment, according to the storage conditions recommended by the manufacturers, as shown in Table 2.

2.1.1 Specimen Preparation

One hundred and ten extracted bovine teeth were selected for uniformity in size and structural integrity. The teeth were cleansed of soft tissue, embedded in self-curing acrylic resin (Trayplast NF, Vertex Dental BV, Zeist, Netherlands) within cylindrical molds (Grinding assembly kit, Ultradent, South Jordan, USA), leaving the buccal surface to be exposed. Once set, a trimming machine (Model Trimmer, Sejong, Korea), under running water, was used to expose a flat dentin surface for universal adhesive application.

The prepared specimens were divided randomly according to the adhesive systems used and their storage conditions (n=5). Eleven adhesives, in as-received condition and in 6-month clinic storage condition, were applied to the prepared dentin surfaces according to the manufacturers' instructions (Table 1). For each group, adhesives were applied immediately after removal from specific storage conditions to prevent further environmental impact on the material properties. A uniform layer of adhesive was applied using a disposable applicator, air dried and light-cured (Bluephase, Ivoclar-Vivadent, Schaan, Liechtenstein) for the recommended time specified by the manufacturers. After the light activation of adhesives, each specimen was positioned in a bonding clamp, and a bonding mold with a diameter of 2.38 mm and a specimen well depth of 2mm (Ultradent, South Jordan, USA) was placed to create resin cylinders with a bonding area of 4.45mm^2 and a height of 2mm. The molds were filled with resin composite (EsFlow®, Spident Co., LTD, Incheon, South Korea) and light-cured for 20 seconds. Excess adhesive around the mold was removed with a blade (Paragon, Swann-Morton Limited, Sheffield, England), and then the molds were removed carefully. The prepared specimens were stored in distilled water for 24 hours at 37◦C.

2.1.2. Shear Bond Strength Testing

The shear bond strength testing was performed in accordance with ISO 29022 (ISO 29022, 2013). The prepared specimens were affixed to the clamp of the Shear Bond Tester (Bisco Inc., Schaumburg, IL, USA). A test cradle and blade were aligned to apply shear force at the composite-dentin interface. The crosshead speed was set at 1.0 mm/min. The force at which failure occurred was recorded (in Newtons), and the SBS was calculated in megapascals (MPa).

2.1.3. Statistical Analysis

All statistical analyses were performed using IBM SPSS 27 software (IBM Corp., New York, NY, USA). Two-way Analysis of variance (ANOVA) was conducted to assess the effect of type of universal adhesives and their storage duration on the shear bond strengths to dentin. For all analyses, a significance level of 95% ($p < 0.05$) was utilized.

Name	Manufacturer	Main Components	Application Method
All Bond Universal™ (ABU)	Bisco Inc., Schaumburg, IL, USA	BisGMA, 2-Hydroxyethyl Methacrylate, 10-Methacryloyloxydecyl Dihydrogen Phosphate, Ethyl 4-dimethylaminobenzoate	Apply with rubbing for 10-15s. Air dry for 10s. Light-cure for 10s.
ClearFil S3 Bond Universal® (CBU)	Kuraray Noritake Dental Inc., Tokyo, Japan	bisphenol A diglycidylmethacrylate, 2-hydroxyethyl methacrylate, ethanol, Sodium fluoride, 10-Methacryloyloxydecyl dihydrogen phosphate, Hydrophilic aliphatic dimethacrylate, Colloidal silica, dl-Camphorquinone, Initiators, Accelerators, Water, Hydrophobic aliphatic methacrylate	Apply with rubbing for 20s. Air dry for 10s. Light-cure for 10s.
Dia-X Bond Universal™ (DBU)	Dia-Dent Group International, Chino, CA, USA	10-Metacryloiloxydexyl dihydrogen phosphate, 2-Hydroxy ethyl methacrylate, Bis (methacryloxyethyl)hydrogen phosphate, 3-(trimethoxysilyl)propyl methacrylate, TMPTMA, BisGMA/TEGDMA Monomer blend, Polyethylene glycol dimethacrylate, Diphenyliodonium, hexaflurophosphate, camphorquinone, ethyl alcohol, tert-butylhydroquinone	Apply with rubbing for 20s. Air dry for 5s. Light-cure for 10s.

GLUMA® Bond Universal (GBU)	Kulzer GmbH, Hanau, Germany	Acetone, 7,7,9(or 7,9,9)-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bismethacrylate, 4-methacryloxyethyltrimellitic acid anhydride,	Apply with rubbing for 20s. Air dry until the adhesive film no longer moves, Light-cure for 10s.
Hi-Bond Universal™ (HBU)	Mediclus Co., Ltd., Seoul, South Korea	10-MDP, BAG, Methacrylate monomers, solvent (ethanol)	Apply with rubbing for 20s. Air dry for 10s. Light-cure for 10s.
K-Bond Universal™ (KBU)	Spident Co., LTD, Incheon, South Korea	Bisphenol A glycerolate dimethacrylate, 2-Hydroxyethylmethacrylate, Camphorquinone, Dimethylaminoethyl acrylate	Apply with rubbing for 20s. Air dry for 5s. Light-cure for 20s.
OptiBond™ Universal (OBU)	Kerr Corporation, Orange, CA, USA	Acetone, 2-hydroxyethyl methacrylate, glycerol dimethacrylate, ethanol, glycerol phosphate dimethacrylate.	Apply with rubbing for 20s. Air dry for 5s. Light-cure for 10s.
Prime & Bond Universal® (PBU)	Dentsply Sirona, York, PA, USA	Acetone, Urethane Dimethacrylate Resin, Dipentaerythritol pentaacrylate phosphate, Polymerizable dimethacrylate resin, Polymerizable trimethacrylate resin.	Apply with rubbing for 20s. Air dry for 5s. Light-cure for 20s.
Single Bond™ Universal (SBU)	3M ESPE, St. Paul, MN, USA	2-Hydroxyethyl methacrylate, Bisphenol A Diglycidyl Ether Dimethacrylate, Decamethylene dimethacrylate, ethanol, Silane	Apply with rubbing for 20s. Air dry for 5s. Light-cure for 10s.

		treated silica, water, 2-propenoic acid, 2-Methyl-, reaction products with 1,10-decanediol and phosphorous oxide, copolymer of acrylic and itaconic acid, dimethylamino ethyl methacrylate, camphorquinone, dimethylaminobenzoate, 2,6-di-tert-butyl-P-cresol.	
Tetric® N-Bond Universal (TBU)	Ivoclar Vivadent AG, Schaan, Liechtenstein	2-hydroxyethyl methacrylate, Bisphenol A Diglycidyl Ether Dimethacrylate, ethanol, 1,10-decandiol dimethacrylate, Methacrylated phosphoric acid ester, camphorquinone, 2-dimethylaminoethyl methacrylate.	Apply with rubbing for 20s. Air dry for 5s. Light-cure for 10s.
Zipbond Universal™ (ZBU)	SDI Limited, Bayswater, Victoria, Australia	Adhesive monomers including MDP, Ethanol Water, Fluoride	Apply with rubbing for 10s. Air dry for 5s. Light-cure for 10s.

Table 1. Main composition and application directions of universal adhesives used in this study.

Name	Storage conditions	Expiration date	Lot number
ABU	2 – 25 °C (36 – 77 °F)	2024-11-30	2200007074
CBU	2 – 25 °C	2024-10-31	610050
DBU	2 – 27 °C (35 – 80 °F)	2024-10-10	DXBU2210111
GBU	Below 25 °C (77 °F).	2024-10-13	M010057
HBU	1 – 25 °C (33.8 – 77 °F)	2024-04-10	HB24T392
KBU	2 – 27 °C	2025-07-14	KBU23002
OBU	dry, cool, well-ventilated area	2024-09-27	9327364
PBU	2 – 24 °C (35 – 75 °F)	2024-02-29	2202000594
SBU	2 – 25 °C (36 – 77 °F)	2024-08	20912A
TBU	2 – 28 °C (36– 82 °F)	2024-08-04	Z04202
ZBU	2 – 25 °C (35 – 77 °F).	2024-07	220729

Table 2. Storage conditions (according to the manufacturers) and expiration dates of universal adhesives used in this study.

2.2. Part B: 1-year clinic storage vs 1-year clinic storage simulation

To evaluate the shear bond strength of universal adhesives stored in clinic environment and in the acclimatization chamber artificially simulating clinic storage, three of the above tested universal adhesives were selected for evaluation: SBU, ABU and KBU.

2.2.1 Simulation of storage duration: accelerated aging

For the simulation of clinic storage duration, accelerated aging was conducted in accordance with the procedures outlined by the ASTM F1980-21 standard. The universal adhesives were stored in an acclimatization chamber (Changshin Lab. Seoul, South Korea) at 55°C for the calculated days, which are the equivalent duration for materials to be kept at an accelerated temperature (55°C) to simulate clinic storage duration at the recommended storing temperature (room temperature, 25°C). The accelerated aging periods were calculated according to ASTM F1980-21, with the equation as follows:

$$AAT\text{(Accelerated Aging Time)} = \frac{RT}{AAF}$$

$$AAF\text{(Accelerated Aging Factor)} = Q_{10}\ (\frac{TAA-TRT}{10})$$

where RT represents the real-time aging period; Q_{10} represents the reaction rate coefficient; TAA the accelerated aging temperature (in the acclimatization chamber); TRT the actual storing temperature (recommended temperature) (ASTM International, 2021).

Based on this method, SBU, ABU and KBU were stored in the acclimatization chamber at 55°C for 46 days, which are the equivalent duration in days for the adhesives to be kept at an accelerated temperature (55°C) to simulate clinic storage duration of one year at the recommended storing temperature (room temperature, 25°C). Each adhesive was divided into the following groups:

1-year clinic storage(-1C): Universal adhesive opened and stored within a dental clinic environment without resealing or further use for one year (SBU-1C ; ABU-1C; KBU-1C)

1-year clinic storage simulation(-1AC): Universal adhesive opened and stored in the acclimatization chamber at 55°C for 46 days to simulate one year of clinic storage (SBU-1AC ; ABU-1AC; KBU-1AC)

2.2.2 Specimen Preparation

Sixty extracted bovine teeth were selected and prepared in the same way as 2.1.1. The prepared specimens were divided randomly according to the adhesive systems used and their storage conditions (n=10). The three selected adhesives, in 1-year clinic storage(-1C) condition and 1-year clinic storage simulation(-1AC) condition, were applied to the prepared dentin surfaces according to manufacturers' instructions (Table 1). The application process of adhesive systems and composite resin was carried out in the same way as 2.1.1.

2.2.3 Shear Bond Strength Testing

The shear bond strength testing was performed in accordance with ISO 29022, in the same way as 2.1.2

2.2.4 Analysis of Fracture Modes

Following the failure from the shear bond testing, the specimens were examined under a dental operating microscope (Carl Zeiss OPMI® pico, Zeiss AG, Germany) at a magnification of 25x to identify the fracture modes between adhesive, cohesive, and mixed failures. Specimens were then prepared for scanning electron microscope (SEM) evaluation of fractured surfaces (JEOL-7800F, JEOL Ltd., Tokyo, Japan).

2.2.5. Statistical Analysis

All statistical analyses were performed using IBM SPSS 27 software (IBM Corp., New York, NY, USA). Two-way Analysis of variance (ANOVA) was conducted to assess the effect of type of universal adhesives and their storage duration on the shear bond strengths to dentin. For all analyses, a significance level of 95% ($p < 0.05$) was utilized.

2.3. Part C: As-received vs 1-year clinic storage simulation vs 2-year clinic storage simulation

To evaluate the shear bond strength of universal adhesives in differing storage duration (i.e. in as-received condition, within and up to the expiry date), accelerated aging in the acclimatization chamber was conducted for the three universal adhesives tested in Part B: SBU, ABU and KBU.

2.3.1 Simulation of storage duration: accelerated aging

For the simulation of clinic storage duration, accelerated aging periods were calculated according to ASTM F1980-21, in the same way as 2.2.1. Each adhesive was divided into the following groups:

As-received(-R): Universal adhesive utilized immediately upon opening (SBU-R, ABU-R; KBU-R)

1-year clinic storage simulation(-1AC): Universal adhesive opened and stored in the acclimatization chamber at 55°C for 46 days to simulate one year of clinic storage (SBU-1AC; ABU-1AC; KBU-1AC)

2-year clinic storage simulation(-2AC): Universal adhesive opened and stored in the acclimatization chamber at 55°C for 92 days to simulate two years of clinic storage (SBU-2AC; ABU-2AC; KBU-2AC)

2.3.2 Specimen Preparation

Ninety extracted bovine teeth were selected and prepared in the same way as 2.1.1. The prepared specimens were divided randomly according to the adhesive systems used and their storage conditions (n=10). The three selected adhesives, in as-received(-R) condition, 1-year clinic storage simulation(-1AC) condition and 2-year clinic storage simulation(-2AC) condition, were applied to the prepared dentin surfaces according to manufacturers' instructions (Table 1). The application process of adhesive systems and composite resin was carried out in the same way as 2.1.1.

2.3.3 Shear Bond Strength Testing

The shear bond strength testing was performed in accordance with ISO 29022, in the same way as 2.1.2

2.3.4 Analysis of Fracture Modes

Following the failure from the shear bond testing, the fracture modes were analyzed using a dental operating microscope and SEM, in the same way as 2.2.4.

2.3.5. Statistical Analysis

All statistical analyses were performed using IBM SPSS 27 software (IBM Corp., New York, NY, USA). Two-way Analysis of variance (ANOVA) was conducted to assess the effect of differing storage duration (i.e. in as-received condition, within and beyond the expiry date) on the shear bond strengths of universal adhesives to dentin, followed by multiple comparisons using Tukey's post hoc tests. For all analyses, a significance level of 95% ($p < 0.05$) was utilized.

3. Results

3.1. Part A: as-received vs 6-month clinic storage

The shear bond strength of eleven universal adhesives immediately after opening and six months after opening were assessed. The results are presented in Table 3 and Figure 1. The results of the two-way ANOVA revealed no significant differences in the SBS of adhesives used immediately after opening and six months after opening for all adhesives tested (p >0.05), as shown in Table 3 and Figure 1. However, it should be noted that some of the adhesive systems (i.e. SBU) indicated significantly higher shear bond strength than other adhesive systems ($p < 0.05$).

Group	Shear bond strength (MPa)	
	As-received	6-month clinic storage
All Bond Universal™ (ABU)	11.5±0.93^{Aab}	11.3±1.05^{Aab}
ClearFil S3 Bond **U**niversal® (CBU)	10.8±1.19^{Abc}	9.8±1.69^{Abc}
Dia-X Bond Universal™ (DBU)	10.3±1.18^{Abc}	8.6±0.51^{Ac}
GLUMA® Bond Universal (GBU)	10.0±1.09^{Abc}	9.2±0.72^{Abc}
Hi-Bond Universal™ (HBU)	10.8±0.70^{Abc}	9.6±0.70^{Abc}
K-Bond Universal™ (KBU)	9.7±0.66^{Abc}	8.8±1.01^{Ac}
OptiBond™ Universal (OBU)	10.1±1.17^{Abc}	9.7±1.27^{Abc}
Prime & Bond Universal® (PBU)	8.8±1.05^{Ac}	9.2±0.9^{Abc}
Single Bond™ Universal (SBU)	13.3±1.26^{Aa}	13.5±1.75^{Aa}
Tetric® N-Bond Universal (TBU)	12.2±1.17^{Aab}	11.7±0.85^{Aab}
Zipbond Universal™ (ZBU)	10.5±1.41^{Abc}	10.3±1.08^{Abc}

Table 3. Shear bond strength (in MPa) of various universal adhesives used immediately after opening (As-received) and six months after opening (6-month clinic storage) [mean (±SD)]. Different capital letters (comparisons in same row) and lowercase letters (comparisons in same column) indicate statistically significant differences ($p < 0.05$).

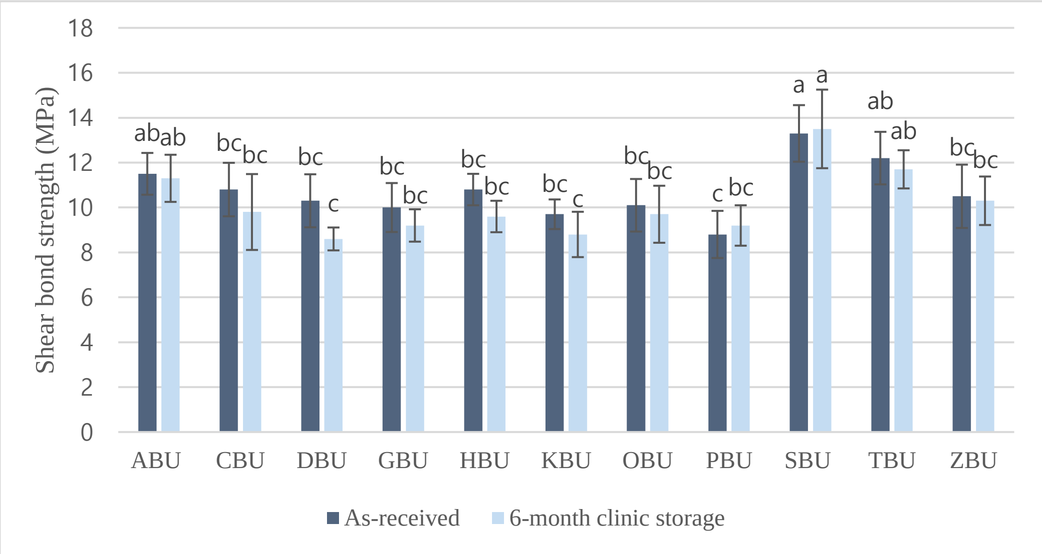

Figure 1. Means and standard deviations of the shear bond strength (in MPa) of various universal adhesives used immediately after opening (As-received) and six months after opening (6-month clinic storage). Similar lowercase letters (comparisons between adhesive type) indicate no significant differences ($p > 0.05$). For all adhesives tested, the differences between As-received group and 6-month clinic storage group were not statistically significant ($p > 0.05$)

3.2. Part B: 1-year clinic storage vs 1-year clinic storage simulation

To compare the effect of real-time clinic storage and clinic storage simulation on the shear bond strengths of universal adhesives, the SBS of SBU, ABU and KBU stored in clinic environment for one year (SBU-1C; ABU-1C; KBU-1C) and in the acclimatization chamber artificially simulating one year of clinic storage (SBU-1AC; ABU-1AC; KBU-1AC) were evaluated. The results are shown in Table 4 and Figure 2. Two-way ANOVA indicated no significant differences between the real-time clinic storage and clinic storage simulation on the shear bond strengths of all adhesives tested (p >0.05). However, similar to the results in Part A, some of the adhesive systems (i.e. SBU) indicated significantly higher shear bond strength than other adhesive systems. ($p < 0.05$).

Group	Shear bond strength (MPa)	
	1C	1AC
Single Bond™ Universal (SBU)	11.2±2.66 ^{Aa}	10.9±1.44^{Aa}
All Bond Universal™ (ABU)	10.0±1.60 ^{Aa}	8.4±2.68^{Ab}
K-Bond Universal™ (KBU)	7.5±1.03b ^{Ab}	6.1±0.78^{Ac}

Table 4. Shear bond strength (in MPa) of universal adhesives stored in clinic environment for one year (SBU-1C; ABU-1C; KBU-1C) and in the acclimatization chamber artificially simulating one year of clinic storage (SBU-1AC; ABU-1AC; KBU-1AC) [mean (±SD)]. Different capital letters (comparisons in same row) and lowercase letters (comparisons in same column) indicate statistically significant differences ($p < 0.05$).

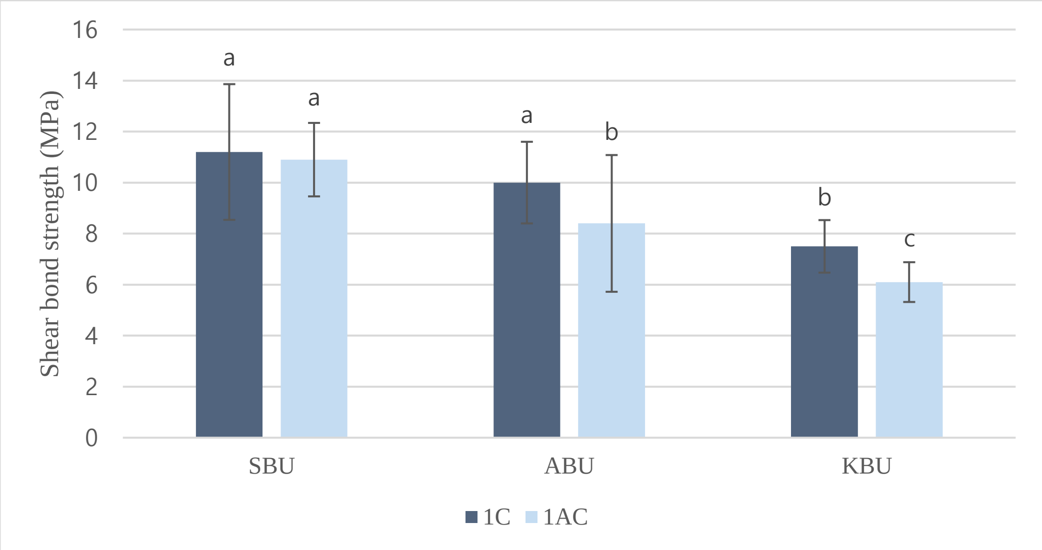

Figure 2. Means and standard deviations of the shear bond strength (in MPa) of universal adhesives stored in clinic environment for one year (SBU-1C; ABU-1C; KBU-1C) and in the acclimatization chamber artificially simulating one year of clinic storage (SBU-1AC; ABU-1AC; KBU-1AC). Similar lowercase letters (comparisons between adhesive type) indicate no significant differences (p >0.05). For all adhesives tested, the differences between -1C group and -1AC group were not statistically significant (p >0.05)

3.3. Part C: As-received vs 1-year clinic storage simulation vs 2-year clinic storage simulation

To assess the effect of differing storage duration (i.e. in as-received condition, within and up to the expiry date) on the shear bond strengths of universal adhesives, the SBS of SBU, ABU and KBU used immediately as-received (SBU-R; ABU-R; KBU-R), stored in the acclimatization chamber artificially simulating one year of clinic storage (SBU-1AC; ABU-1AC; KBU-1AC) and two years of clinic storage (SBU-2AC; ABU-2AC; KBU-2AC) were evaluated. The results of Two-way ANOVA and Tukey's post hoc tests are shown in Table 5, Table 6 and Figure 3. When SBU was used immediately as-received (SBU-R), it showed the highest mean SBS (13.0±1.52). The SBS of SBU remained stable after one-year clinic storage simulation (SBU-1AC) (p >0.05), whereas two-year clinic storage simulation (SBU-2AC) resulted in significant reduction ($p < 0.05$). On the other hand, for ABU and KBU, the SBS decreased significantly after both one-year clinic storage simulation (ABU-1AC; KBU-1AC) and two-year clinic storage simulation (ABU-2AC; KBU-2AC) ($p < 0.05$). Moreover, similar to the results in Part A and Part B, some of the adhesive systems (i.e. SBU) indicated significantly higher shear bond strength than other adhesive systems within the same storage conditions ($p < 0.05$).

Group	Shear bond strength (MPa)		
	R	1AC	2AC
SBU	13.0 ± 1.52^{Aa}	10.9 ± 1.44^{ABa}	9.3 ± 1.72^{Ba}
ABU	11.7 ± 2.75^{Aab}	8.4 ± 2.68^{Bb}	7.1 ± 1.51^{Bb}
KBU	9.4 ± 1.80^{Ab}	6.1 ± 0.78^{Bc}	4.6 ± 1.25^{Bc}

Table 5. Shear bond strength (in MPa) of universal adhesives used immediately as-received (SBU-R; ABU-R; KBU-R), stored in the acclimatization chamber artificially simulating one year of clinic storage (SBU-1AC; ABU-1AC; KBU-1AC) and two years of clinic storage (SBU-2AC; ABU-2AC; KBU-2AC) [mean (±SD)]. Different capital letters (comparisons in same row) and lowercase letters (comparisons in same column) indicate statistically significant differences ($p < 0.05$).

Variable	Interventional Group	Interventional Group	Probability value (*p*)
Shear bond strength of SBU	SBU-R	SBU-1AC	0.019
		SBU-2AC	< 0.001*
	SBU-1AC	SBU-2AC	0.057
Shear bond strength of ABU	ABU-R	ABU-1AC	0.014*
		ABU-2AC	< 0.001*
	ABU-1AC	ABU-2AC	0.436
Shear bond strength of KBU	KBU-R	KBU-1AC	< 0.001*
		KBU-2AC	< 0.001*
	KBU-1AC	KBU-2AC	0.051

Table 6. Tukey's post hoc test for Table 5. * indicates a statistically significant difference ($p < 0.05$).

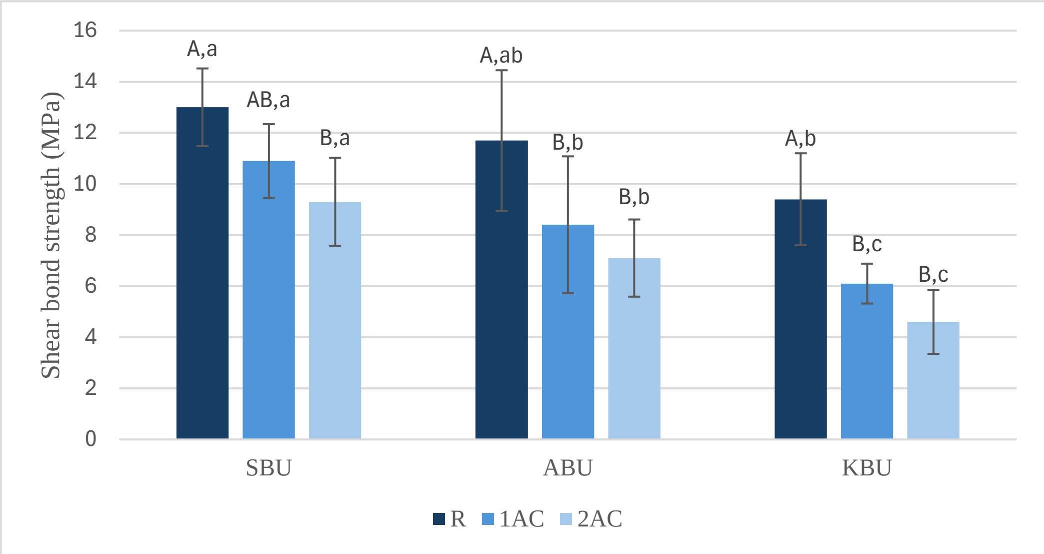

Figure 3. Means and standard deviations of the shear bond strength (in MPa) of universal adhesives used immediately as-received (SBU-R; ABU-R; KBU-R), stored in the acclimatization chamber artificially simulating one year of clinic storage (SBU-1AC; ABU-1AC; KBU-1AC) and two years of clinic storage (SBU-2AC; ABU-2AC; KBU-2AC). Different capital letters (comparisons between -R group, -1AC group and -2AC group) and lowercase letters (comparisons between adhesive type) indicate statistically significant differences ($p < 0.05$).

3.4. Analysis of failure mode

After the shear bond strength testing, the fractured surfaces were examined under a dental operating microscope to classify the failure mode as adhesive, cohesive, and mixed failures. The distribution of failure mode is presented in Figure 4-6. Adhesive failure mode was predominant in all groups tested. The representative SEM images obtained are shown in Figure 7.

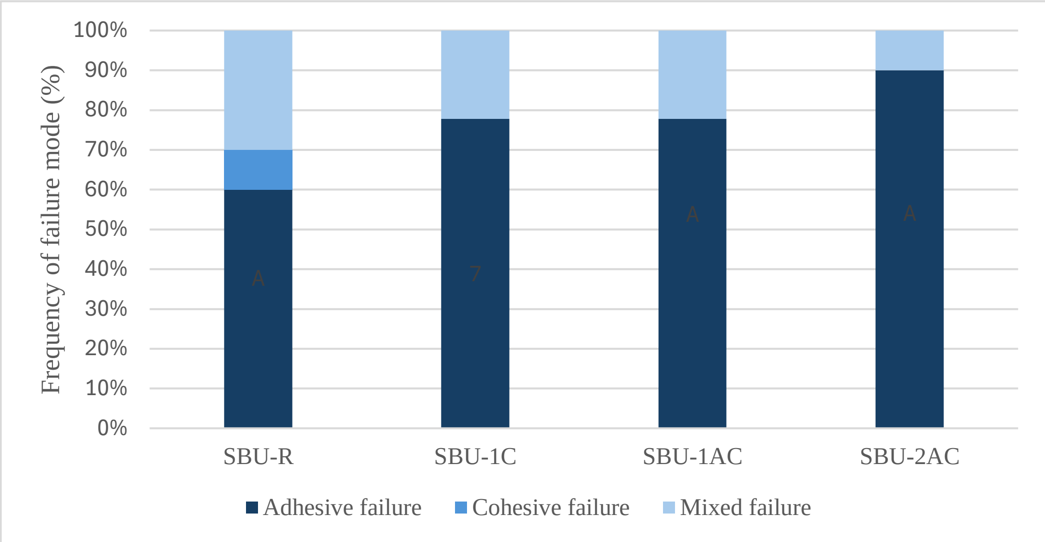

Figure 4. Distribution of failure mode of SBU evaluated after the SBS test.

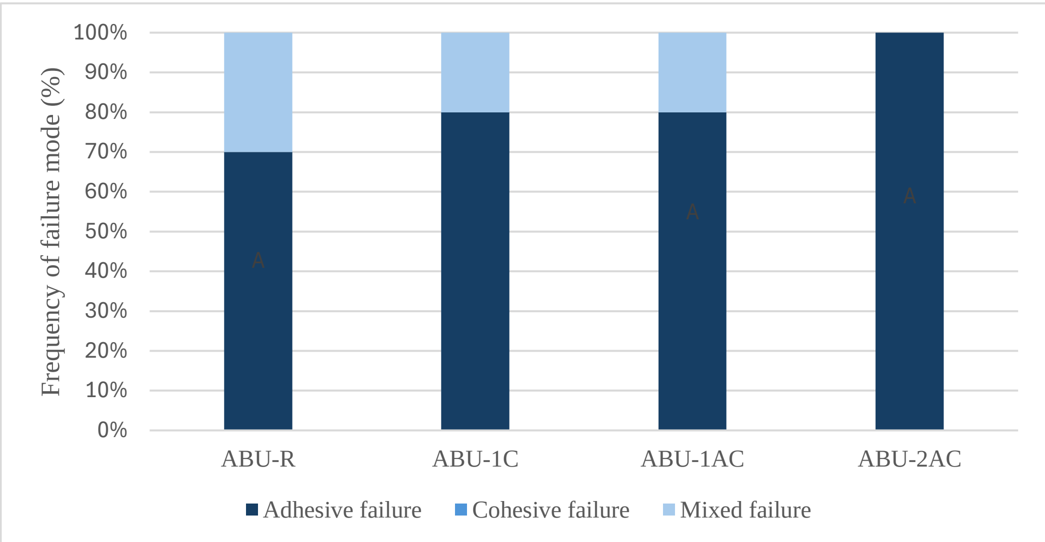

Figure 5. Distribution of failure mode of ABU evaluated after the SBS test.

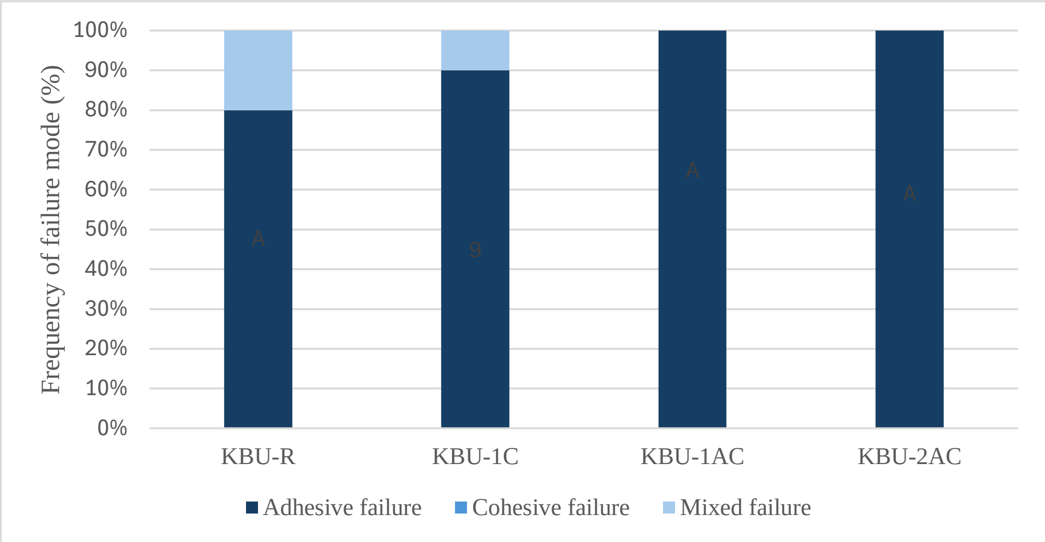

Figure 6. Distribution of failure mode of KBU evaluated after the SBS test.

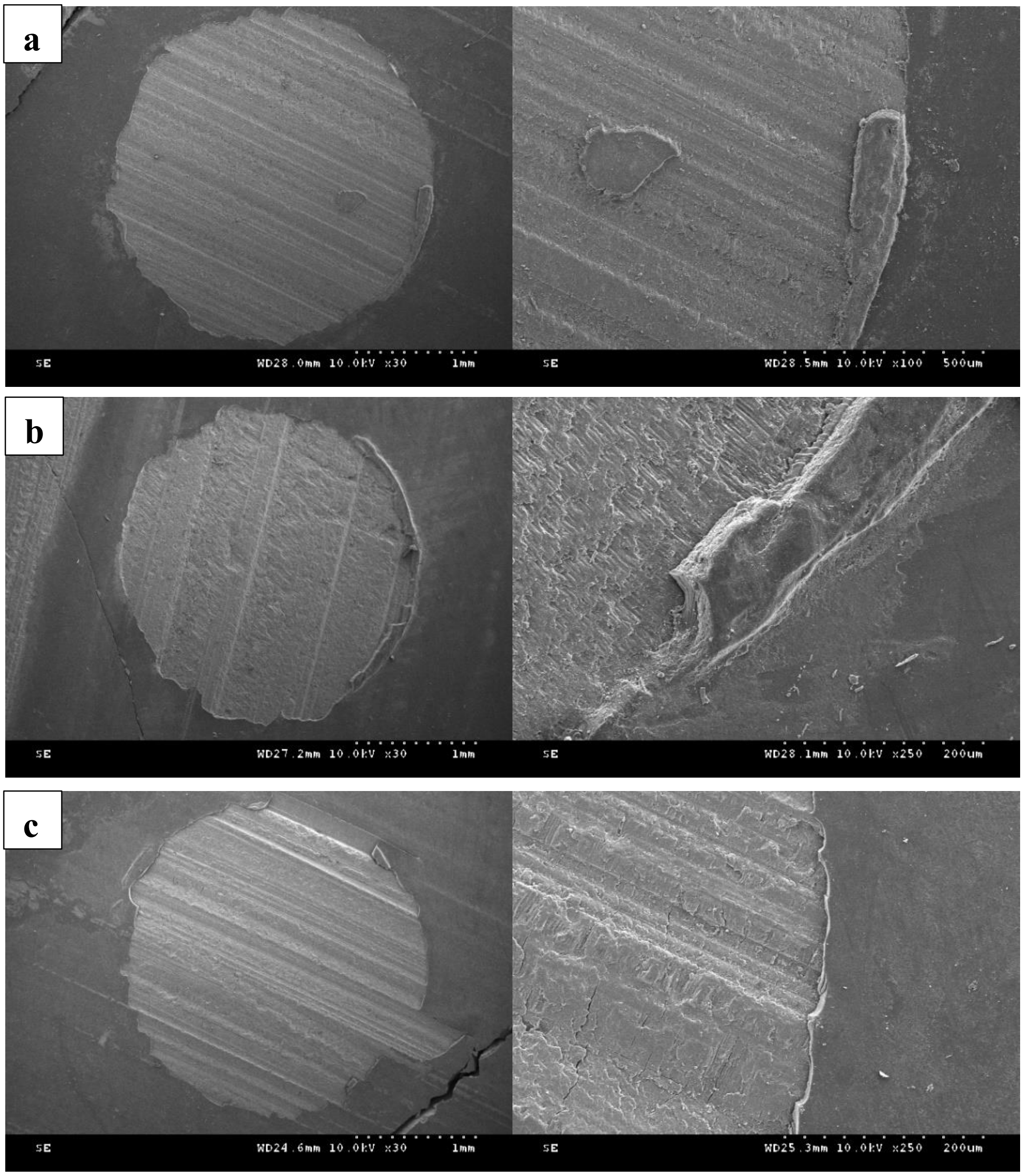

Figure 7. Representative SEM images of fractured surfaces. (a) Mixed failure observed in group SBU-R. Images were taken at x30 and x100 magnification. (b) Mixed failure in group ABU-R. Images were taken at x30 and x 250 magnification. (c) Adhesive failure in Group KBU-R. Images were taken at x30 and x 250 magnification.

4. Discussion

The results of this study indicate significant variability in adhesive performance based on storage durations, which aligns with previous studies suggesting that the chemical stability of adhesives deteriorates over time due to factors like solvent evaporation and hydrolysis of monomers, affecting their bonding efficacy (Cuevas-Suárez et al., 2019; Iliev et al., 2021; Salz, U. et al., 2010; Pongprueksa et al., 2014; Aida, M et al., 2009).

In this study, three null hypotheses were tested. The findings in Part A led to the acceptance of the first null hypothesis that the shear bond strength of various universal adhesives would be stable after six months of clinic storage. There were no significant changes in the SBS after 6 months of clinic storage for all universal adhesives that were explored in this study.

The second hypothesis that there would be no difference between the shear bond strength of universal adhesives stored in clinic for one year and stored in an acclimatization chamber simulating one year of storage, can also be accepted. The results of Part B demonstrated that the clinic storage simulation of universal adhesives using accelerated aging resulted in the shear bond strengths that were not significantly different from those of the real-time clinic storage.

Accelerated aging is commonly employed by both manufactures and researchers to gather data on the aging behavior of polymeric materials. These ACC techniques are essential for forecasting the long-term performance, especially for medical and dental devices and materials like universal adhesives. The result of Part B implies that ACC method performed with the use of acclimatization chambers may be considered reliable in providing critical insights into the performance of universal adhesives with different storage duration. This method allows for the assessment of material stability and degradation over what would equate to years in real-time conditions, offering a predictive measure of their shelf life and stability under various temperature and humidity conditions (Krug, N. et al., 2023; Cardoso, S.D. et al, 2014).

The third hypothesis that there would be no difference between the shear bond strength of universal adhesives used immediately as-received, stored in an acclimatization chamber simulating one year of storage and stored in the same acclimatization chamber simulating two years of storage, is partially rejected. While ABU and KBU that underwent accelerated aging simulating one year and two years of clinic storage resulted in a significant reduction of the shear bond strength, SBU demonstrated significant resistance to accelerated aging for one year.

The more pronounced decline in ABU and KBU than SBU may suggest that the compositions and formulation of the latter might offer better stability against the storage time-related chemical changes. According to the manufacturer, Single Bond Universal adhesive system features a distinctive formulation that sets it apart from other adhesives. This adhesive system includes Vitrebond™ copolymer, recognized for enhancing stability and providing increased resistance to moisture degradation (Cardoso, S.D et al, 2014; Fundingsland J.W et al, 1992). The copolymer is known to have stress relaxation capacity at the adhesive interface through its chemical interaction with hydroxyapatite minerals, thereby enhancing the durability of the bond under humid conditions (Cardoso, S.D et al, 2014; Yoshida, Y. et al., 2000; Cardoso, P. E et al., 1999). This formulation might explain the relatively high and stable bond strength values obtained in this study.

All universal adhesives examined in this study indicated a decreasing trend in the SBS over time. As explored in other literature, extended storage duration may lead to evaporation and degradation of solvent-based components in adhesives, which are crucial for maintaining bonding effectiveness (2007; Iliev et al., 2021; Pongprueksa et al., 2014). Although SBU demonstrated significant resistance to accelerated aging for one year, it also shows decreased performance after accelerated aging for two years, indicating a need for careful consideration of storage duration and expiry date to preserve adhesive properties and adequate performance.

In Part B and C of the study, the adhesive bottles were opened and stored in either clinic environment or acclimatization chamber for certain period of time to evaluate the impact of storage duration. To prevent further environmental impact on the material properties, these adhesives were applied immediately after removal from specific storage conditions. In other words, these vials were kept closed for the entire storage period. However, in real-clinic situations, the adhesive bottles are being opened repeatedly throughout the use. The occasional opening of the adhesive bottle may lead to the evaporation of ingredients, including both organic solvents and small quantities of low-molecular-weight monomers. It is likely that the amount of solvent in an adhesive bottle differs from the first to the last application. (Iliev et al., 2021; Pongprueksa et al., 2014). Previous studies reported that ingredient evaporation of adhesive may result in lower bond strengths, thereby reducing its shelf life (Van Landuyt, K. L., 2007; Pongprueksa et al., 2014; Pashley, E. L et al., 1998; Perdigão, J et al., 1999). In this study, the frequent opening of the bottle and thus its resultant evaporation were not simulated. Hence, the evaporative nature of adhesives should be considered as in actual clinical settings, the adhesive bottles are being opened and recapped repeatedly throughout their use, which could affect the bond strength.

On the other hand, high temperatures may affect the properties of adhesives, including evaporation. In general, variations in temperature and humidity lead to substantial alterations in the chemical, physical and mechanical stability and properties of adhesives as previously research shows. For example, initiator molecules may spontaneously react to generate radicals in elevated temperatures. Some studies have revealed impaired performance of adhesives when stored at high temperatures (Iliev et al., 2021; Van Landuyt, K. L., 2007; Yoshida, Y. et al., 2012). The ACC method performed in this study required the adhesive bottles to be stored in the acclimatization chamber at 55°C. It is obvious that such accelerated temperature is beyond the storage conditions recommended by the manufacturers, and does not reflect the actual clinical settings. Nevertheless, this unrealistically high temperature may reflect an adverse environment during the import and export transportation of universal adhesives. (Iliev et al., 2021; Aida, M et al., 2009; Cardoso, S.D et al, 2014).

Ultimately, this study reinforces the critical nature of storage durations in maintaining the effectiveness of dental universal adhesives and supports ongoing recommendations for rigorous storage management in clinical settings. Future research should focus on identifying formulation strategies that enhance the stability of adhesives to extend their functional shelf life without compromising bond strength and durability.

5. Conclusion

Based on the results of and within the limitations of this study, the following conclusion can be drawn:

1. Various universal adhesives showed varying shear bond strengths to dentin; some of the adhesive systems indicated significantly higher shear bond strength. They all remained stable after six months of clinic storage.

2. Clinic storage simulation of universal adhesives using accelerated aging resulted in shear bond strengths that were not significantly different from those of the real-time clinic storage.

3. Accelerated aging simulating two years of clinic storage resulted in a significant reduction in the shear bond strength of universal adhesives; extended storage time after the opening of adhesive bottles may result in reduced bonding performance, emphasizing the importance of adhering to expiration dates for universal adhesives.

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Abstract in Korean

다양한 유니버셜 치과 접착제의 보관 기간이 전단 결합 강도에 미치는 영향

본 연구의 목적은 실제 치과진료 환경과 온장고에서 시뮬레이션된 환경을 포함한 다양한 보관 기간이 다양한 유니버셜 접착제의 전단 결합 강도에 미치는 영향을 평가하는 것이다. 연구는 세 파트로 진행되었다. 파트 A 에서는 개봉 직후와 6 개월동안 치과진료 환경에서 보관 후의 11 종의 유니버셜 접착제의 전단 결합 강도를 평가했다. 파트 B 에서는 선택된 세 종류의 치과 접착제 (Single Bond Universal, All Bond Universal, K-Bond Universal)를 실제 치과진료 환경과 온장고에서 1 년간 보관한 후의 SBS 를 평가했다. 마지막으로 파트 C 에서는 이 시뮬레이션을 2 년까지 확장하여 동일한 접착제들의 전단 결합 강도를 평가했다. 발거된 소의 치아에 치과 접착제를 적용하였고, 전단 결합 강도 시험을 통해 실패 모드를 분석했다. 통계적 분석으로는 이원배치 분산분석과 Tukey 사후 검정이 사용되었다. 모든 분석에서 95%의 유의 수준 ($p < 0.05$)이 사용되었다.

파트 A 에서 개봉 직후와 6 개월 후의 접착제 사용 간에는 전단 결합 강도에 유의미한 차이가 없었다($p < 0.05$). 파트 B 에서는 실제 1 년 보관과 온장고에서 시뮬레이션된 보관 조건 간에 전단 결합 강도에 유의미한 차이가 없었다 ($p < 0.05$). 그러나 파트 C 에서 ABU 와 KBU 는 1 년 및 2 년 온장고 시뮬레이션 보관 후 유의미하게 감소된 전단 결합 강도를 보였다 ($p < 0.05$), 반면 SBU 는 1 년 후에는 안정적이었으나 2 년 후에는 유의미하게 감소했다 ($p < 0.05$).

이 연구의 한계 내에서 다음과 같은 결론을 얻을 수 있다:

1. 다양한 유니버셜 접착제는 상아질에 대한 다양한 전단 결합 강도를 보이며, 일부 접착 시스템은 유의미하게 높은 전단 결합 강도를 나타냈다. 모든 치과 접착제는 6 개월간의 치과진료 환경에서 보관 후에도 안정적이었다.

2. 1 년 실제 보관과 온장고에서 시뮬레이션된 보관 조건 간에는 전단 결합 강도에 유의미한 차이가 없었다.

3. 2 년간의 치과진료 환경 보관을 온장고에서 시뮬레이션한 가속 노화는 유니버셜 접착제의 전단 결합 강도를 유의미하게 감소시켰다. 즉, 접착제 개봉 후의 보관 시간이 길어질수록 접착 성능이 감소될 수 있음을 시사하며 유니버셜 접착제의 유효기간 준수의 중요성을 강조한다.

핵심되는 말 : 치과용 범용 접착제, 보관 기간, 전단 결합 강도, 가속 노화, 시뮬레이션, 온장고, 유통기한, 유효기간