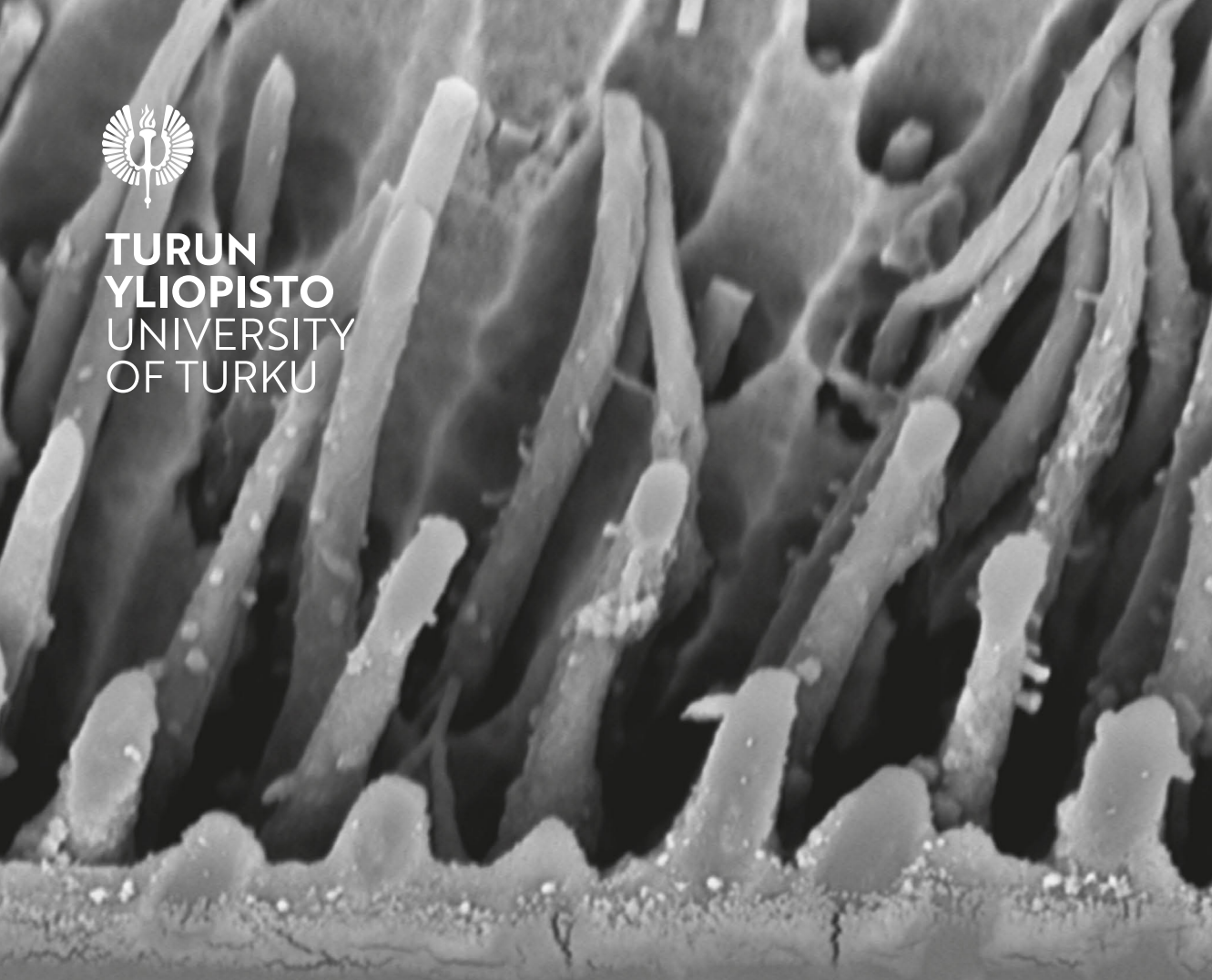

TURUN YLIOPISTO
UNIVERSITY OF TURKU

CLINICAL AND LABORATORY EVALUATION OF DIMETHYL SULFOXIDE DENTIN PRETREATMENTS

Omar Abdelaziz Ismail

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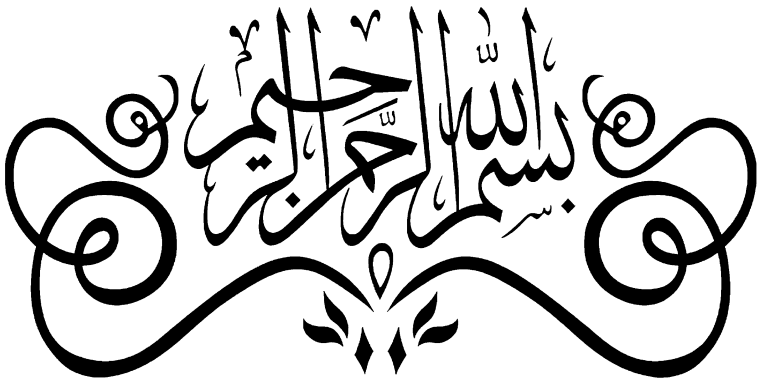

To the soul of my Father,
Mother, Wife and lovely kids
for their unconditional support throughout the journey

UNIVERSITY OF TURKU
Faculty of Medicine
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Department of Cariology and Restorative Dentistry
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ABSTRACT

The durability of the resin-dentin bond is a critical challenge in adhesive dentistry because of the vulnerability of the hybrid layer to hydrolytic and enzymatic degradation. Several laboratory strategies have been proposed to stabilize the adhesive interface. However, many are technique-sensitive and very difficult to implement clinically. Dimethyl sulfoxide (DMSO) has been suggested as a simple adjunct capable of improving resin infiltration and bond stability due to its amphiphilic properties and ability to inhibit dentin proteases.

The aim of this thesis was to evaluate the effect of DMSO dentin pretreatment on the durability of bonding resin to dentin in both in vitro and clinical situations. Laboratory studies evaluated the effect of different DMSO concentrations (5% and 50%) and application times (20 s and 60 s) under dry-bonding conditions. Microtensile bond strength, nanoleakage, and hybrid layer micromorphological characterization were analyzed after short- and long-term storage. Furthermore, two randomized controlled clinical trials (RCTs) were also performed. The first RCT was designed to evaluate DMSO pretreatment in non-carious cervical lesions (NCCLs), while the second one evaluated the same parameters in carious cervical lesions (CCLs). FDI criteria for evaluation of the restoration were assessed in different follow-ups, at baseline, 12, 24, and 36 months.

The in vitro results revealed that both 5% and 50% DMSO pretreatments preserved bond strength over time and reduced nanoleakage compared with the untreated control. Application time was critical, as 60 s of pretreatment produced a more durable adhesive with a thicker, continuous hybrid layer. Clinically, DMSO pretreatment improved restoration survival rate and marginal quality. In NCCLs, pretreatment reduced marginal staining and deterioration over time, while in CCLs, survival was significantly higher (89%) compared with controls (65%) after 36 months.

Within the limitations of this thesis, it could be concluded that DMSO pretreatment improves resin-dentin bond durability and clinical performance. This research represents a promising and clinically feasible strategy for adhesive dentistry.

KEYWORDS: DMSO, Adhesion, Hybrid-layer, RCT, Clinical, Dimethyl-sulfoxide, Dry-bonding, Carious-cervical-lesions.

TURUN YLIOPISTO
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TIIVISTELMÄ

Hampaan dentiinin ja yhdistelmämuovin välisen sidoksen kestävyys on edelleen merkittävä haaste korjaavassa hammashoidossa, sillä hybridikerros on altis hydrolyyttiselle ja entsymaattiselle hajoamiselle. Useita laboratoriomenetelmiä on ehdotettu hybridikerroksen stabiloimiseksi, mutta monet niistä ovat tekniikkaherkkiä ja vaikeasti sovellettavissa kliinisesti. Dimetyylisulfoksidia (DMSO) on esitetty yksinkertaiseksi lisämenetelmäksi sen amfifiilisten ominaisuuksien ja kyvyn estää dentiinin proteaaseja ansiosta, joka voi parantaa sidosaineiden infiltraatiota ja sidoksen pysyvyyttä.

Tämän väitöskirjan tarkoituksena oli tutkia DMSO-esikäsittelyn vaikutusta dentiiniin sidoksen kestävyyteen sekä in vitro -olosuhteissa että kliinisissä tutkimuksissa. Laboratoriokokeissa arvioitiin eri pitoisuuksien (5 % ja 50 %) ja esikäsittelyajan (20 s ja 60 s) merkitystä kuivissa sidostusolosuhteissa. Mikrovetolujuus, nanovuoto ja hybridikerroksen morfologia analysoitiin sekä lyhyen että pitkän säilytyksen jälkeen. Lisäksi toteutettiin kaksi satunnaistettua kontrolloitua kliinistä tutkimusta. Ensimmäisessä arvioitiin DMSO-esikäsittelyä ei-karioitumattomissa hammaskaulan vaurioissa (NCCL) ja toisessa karioituneissa hammaskaulan vaurioissa (CCL). Potilaita seurattiin 12, 24 ja 36 kuukauden ajan Maailman Hammaslääkäriliiton (FDI) kriteerien mukaisesti.

In vitro -tulokset osoittivat, että sekä 5 % että 50 % DMSO-esikäsittely säilyttivät sidoksen lujuuden ja vähensivät nanovuotoa verrattuna käsittelemättömään kuivaan sidostukseen. Esikäsittelyaika oli kriittinen: 60 s esikäsittely tuotti kestävämmän sidoksen ja paksummat, yhtenäiset hybridikerrokset. Kliinisissä tutkimuksissa DMSO paransi restauraatioiden selviytymistä ja sauman laatua. NCCL-vaurioissa DMSO vähensi sauman värjäytymistä ja hajoamista ajan myötä, kun taas CCL-vaurioissa restauraatioiden selviytyminen oli merkittävästi parempi (89 %) verrattuna kontrolliryhmään (65 %) 36 kuukauden jälkeen.

Tutkimuksen rajoituksista huolimatta DMSO-esikäsittely paransi resiini–dentiini-sidoksen kestävyyttä ja kliinistä toimivuutta. DMSO-esikäsittely on lupaava sekä kliinisesti käyttökelpoinen menetelmä korjaavaan hammashoitoon.

AVAINSANAT: Adheesio, hybridikerros, kliininen, dimetyylisulfoksidi, kuivaliimaus, DMSO, RCT, Karies-kohdunkaulan-vauriot.

Table of Contents

Abbreviations

ACP	Amorphous Calcium Phosphate
bis-EMA	Bisphenol A Ethoxylated Dimethacrylate
bis-GMA	Bisphenol A Diglycidyl Methacrylate
CCLs	Carious Cervical Lesions
CHX	Chlorhexidine
DMSO	Dimethyl Sulfoxide
DPI	Diphenyliodonium Hexafluorophosphate
EGCG	Epigallocatechin-3-Gallate
E&R	Etch-and-Rinse
EWBT	Ethanol Wet Bonding Technique
FDI	Federation Dentaire Internationale (World Dental Federation)
HEMA	2-Hydroxyethyl Methacrylate
ICDAS	International Caries Detection and Assessment System
LED	Light-Emitting Diode
MMPs	Matrix Metalloproteinases
NCCLs	Non-Carious Cervical Lesions
PA	Proanthocyanidin
PEGDMA	Polyethylene Glycol Dimethacrylate
RCT	Randomized Controlled Trial
SB	Single Bond 2
SBMP	Scotchbond Multi-Purpose
SE	Self-Etch
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TEGDMA	Triethylene Glycol Dimethacrylate
TWI	Tooth Wear Index
UA	Universal Adhesives
UDMA	Diurethane Dimethacrylate
USPHS	United States Public Health Service
µTBS	Microtensile Bond Strength

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Ismail, O. A., Stape, T. H. S., & Tezvergil-Mutluay, A. (2023). Concentration effect of DMSO-dry bonding on the stability of etch-and-rinse bonds. *Dental Materials*, *39* (12), 1113–1121. https://doi.org/10.1016/j.dental.2023.09.013

II Stape, T. H. S., Ismail, O. A., Capitanio, M., & Tezvergil-Mutluay, A. (2024). Bonding neat hydrophobic-rich resins to etched dentin: A proof of concept. *Dental Materials*, *41*(2), 113–121. https://doi.org/10.1016/j.dental.2024.10.018

III Ismail, O. A., Hassanein, O. E., Hafez, R., Mamdouh, M., Shaalan, O., & Tezvergil-Mutluay, A. (2025). Three years clinical assessment of low concentration of dimethyl sulfoxide primer in non-carious cervical lesions: a randomized control trial. *Journal of Evidence-Based Dental Practice*, *25* (2),102124. https://doi.org/10.1016/j.jebdp.2025.102124

IV Ismail, O. A., Stape, T. H. S., Shaalan, O., Taymour, N., Basha, I. E., Alsamoully, W., & Tezvergil-Mutluay, A. (2026). Clinical evaluation of composite restorations placed on dimethyl sulfoxide-treated cervical carious lesions: a 36-month randomized double-blind controlled trial. *Journal of Dentistry*, *168*, 106587. https://doi.org/10.1016/j.jdent.2026.106587

1 Introduction

Adhesive dentistry is the foundation for recent restorative practices by enabling minimally invasive techniques and long-lasting tooth-colored restorations (Breschi et al., 2025). Recent advances in adhesive dentistry have enabled resin composites to be regarded as the standard of care for restorative procedures (Turkun, 2023). The success of adhesive procedures is determined by the quality and durability of the resin-dentin hybrid layer, which relies on the infiltration of resin monomers into the demineralized collagen network (Tjäderhane, 2015). Bonding to enamel is predictable and durable, but achieving and maintaining a reliable dentin bond remains challenging, due to its complex composition, higher organic content, and different tubular structure (Van Meerbeek et al., 2010).

Etch and rinse ER and self-etch SE adhesive strategies have been developed to improve bonding effectiveness. Conventional three-step etch-and-rinse systems are still considered the gold standard for dentin bonding, but they are highly technique-sensitive (Van Meerbeek et al., 2020). Over-wet dentin dilutes primers. On the other hand, over-dry dentin leads to collagen collapse, which will compromise the resin infiltration and hybrid layer formation (Tay et al., 2002; Van Meerbeek et al., 2020). Self-etch adhesives reduce the sensitivity of the technique. However, clinical studies have shown variable long-term outcomes (Cardoso et al., 2011; Vermelho et al., 2017). Both strategies are susceptible to gradual degradation of the hybrid layer due to hydrolysis of resin polymers and enzymatic breakdown of exposed collagen fibrils. The activity of endogenous proteases, such as matrix metalloproteinases (MMPs) and cysteine cathepsins, leads to nanoleakage, decreased bond strength, and ultimately failure of the restoration (Breschi et al., 2017; Mazzoni et al., 2013).

Several strategies have been implemented to overcome the drawbacks of degradation processes, including chlorhexidine pretreatment to inhibit MMPs (Josic et al., 2021; Pashley et al., 2004), collagen cross-linkers such as proanthocyanidins (Balalaie et al., 2018), ethanol-wet bonding (Kuhn et al., 2015), and alcohol-based optimization (Tezvergil-Mutluay et al., 2011). Despite these methods have shown promise in laboratory studies, their clinical translation has been limited, due to technique sensitivity, inconsistent long-term outcomes and also, degradation of the hybrid layer was not fully prevented (Breschi et al., 2025).

In this framework, dimethyl sulfoxide (DMSO) has been utilized as a promising agent for improving resin-dentin bonding. DMSO is a highly polar aprotic solvent capable of penetrating collagen matrices, displacing water, and enhancing monomer diffusion (Stape et al., 2015; Tjäderhane et al., 2013a). Previous laboratory studies have shown that DMSO pretreatment can preserve dentin collagen structure, reduce nanoleakage, improve immediate bond strength and stabilize it over time (Salim et al., 2018; Zhang et al., 2020; Stape et al., 2021a; Zabeu et al., 2023). Systematic review has also supported its role in improving the durability of adhesive interfaces (Zhang et al., 2022).

More recent evidence suggests that DMSO can improve adhesion to challenging substrates such as eroded or fluorotic dentin (Wendlinger et al., 2023; Zhang et al., 2024). Furthermore, DMSO as a solvent has enabled effective bonding to extensively air-dried demineralized dentin for up to 30 seconds (Stape et al., 2021b). However, some studies report limited or no benefit when DMSO is used with certain universal adhesives (Bayraktar & Harorli, 2024; Mello et al., 2022), highlighting the importance of adhesive formulation and bonding strategy.

Collectively, the literature indicates that DMSO might offer potential to solve two major limitations of adhesive dentistry, moisture sensitivity associated with dentin bonding and enzymatic degradation of the hybrid layer. However, variability in protocols, concentrations, and adhesive types emphasize the need for further investigation. This thesis explores the role of DMSO in resin-dentin bonding by evaluating the concentration effects, application time, bonding strategies, and clinical performance in carious and non-carious lesions. By integrating both in vitro studies and long-term clinical trials, the work aims to provide comprehensive evidence on the potential of DMSO to extend the longevity of adhesive restorations and advance contemporary adhesive dentistry.

2 Review of the Literature

2.1 Microstructure of Enamel and Dentin

Dental enamel and dentin are significantly different in their structure and composition, which diverse bonding effectiveness of the same adhesive to each substrate. Enamel consists of more than 95% inorganic material, most of which are hydroxyapatite crystals arranged into prisms or rods. Its high mineral content and low organic fraction provide an ideal substrate for micromechanical bonding after acid etching that will create predictable and durable adhesion (Han et al., 2021).

Conversely, dentin is a hydrated, collagen-rich tissue with approximately 50% mineral, 30% organic matrix, and 20% water by volume (Agee et al., 2015). The intertubular dentin is primarily collagen, and during dentin bonding its fibrils must be infiltrated by resin monomers to form the hybrid layer (Tjäderhane et al., 2013b). Incomplete resin infiltration leaves collagen fibrils exposed and make them vulnerable to enzymatic degradation, which will compromise the longevity of the bond (Breschi et al., 2008). Moreover, the structural differences between superficial and deep dentin further affect bonding effectiveness. The presence of dentinal tubules that vary in diameter and density with depth leads to increased permeability and complicates adhesive procedures (Pashley et al., 2011). Superficial dentin has fewer and narrower dentinal tubules, so more intertubular dentin is available for bonding. In contrast, deep dentin contains a higher density of wider dentinal tubules, resulting in reduced bonding surface area and increased fluid flow (Pegado et al., 2010). This variation highlights the importance of substrate characteristics in determining different adhesive outcomes.

2.2 Contemporary Dental Adhesives

Since Buonocore's introduction of acid-etching in 1955 (Buonocore, 1955), adhesive dentistry has evolved significantly to improve bond efficiency, strength, and clinical reliability (Van Meerbeek et al., 2020). Current adhesive systems can be broadly divided into etch-and-rinse (E&R), self-etch (SE), and universal adhesives (UA). Each system has distinct mechanisms and clinical implications (Han et al., 2021).

Three-step E&R systems remain the ‘gold standard’ for bonding to dentin, as they separate the etching, priming, and bonding steps (Pashley et al., 2011). Although they provide high immediate bond strength, these systems are highly technique-sensitive and require moist bonding conditions to prevent collagen collapse (Pashley et al., 1999). Two-step E&R systems combine priming and bonding, which reduce complexity but increase solvent dependence (Tjäderhane et al., 2013b).

Self-etch adhesives simplify the protocol by combining etching, priming and bonding. They partially demineralize dentin while preserving residual hydroxyapatite for potential chemical interaction with functional monomers such as 10-MDP (Breschi et al., 2008). Although, they reduce technique sensitivity, their lower acidity may result in less effective enamel etching compared with phosphoric acid (Van Meerbeek et al., 2020).

Universal adhesives represent the latest generation. They are introduced in the market as versatile in either E&R or SE mode. They often contain functional monomers, hydrophilic co-monomers, and solvents to facilitate their infiltration with simplified application. Despite their popularity, long-term studies show variable performance depending on application strategy and substrate conditions (Pashley et al., 2011).

The evolution of adhesive systems reflects a balance between simplification and durability. While simplified adhesives have improved dentists’ convenience, they usually cause greater permeability and water sorption (Abu-Nawareg et al., 2015), that eventually lead to reduced long-term stability (Van Meerbeek et al., 2020). That is why, the choice of adhesive system must consider not only ease of use but also the durability of the hybrid layer over time.

2.3 Solvents in Dental Adhesives

2.3.1 Water

Water is integral to many adhesive systems because it keeps the acid-demineralized dentin matrix expanded (Chiaraputt et al., 2008), preventing collagen collapse and allowing monomers to diffuse into interfibrillar spaces. However, residual water that is not replaced by resin leaves zones of exposed collagen and aqueous channels within the hybrid layer. These pathways are associated with hydrolytic degradation of polymers and enzyme-mediated breakdown of collagen, both of which jeopardize long-term bond stability (Sano et al., 1995; Van Meerbeek et al., 2010). Additionally, residual water interferes with monomer conversion and polymer cross-linking, leading to weaker resin matrices. Because of the hydrophobic resins make an emulsion in association with water, which leads to the presence of many resin chains

alongside the interface in place of a continuous chain of polymerized resin (Tay & Pashley, 2003). This explains why resin-dentin bonds are more vulnerable to aging compared with resin-enamel bonds (Breschi et al., 2018). Reviews of bonding effectiveness further emphasize that the more hydrophilic and permeable an adhesive layer is, the greater its susceptibility to water sorption and nanoleakage over time (Cardoso et al., 2011). In practice, clinicians must balance the need to avoid over drying dentin against the risks of leaving excess water that hinders solvent evaporation and monomer conversion (Armstrong et al., 2017).

2.3.2 Ethanol

Ethanol is used as an alternative solvent to replace water and facilitate infiltration of hydrophobic adhesive monomers. Ethanol is an effective solvent due to its intermediate polarity as it is less polar than water but more polar than acetone (Pashley et al., 2007). This intermediate polarity allows ethanol to act as a transitional solvent between a water-saturated collagen matrix and the hydrophobic components of dental adhesives. Ethanol is full miscible with water, able to displace water from demineralized dentin, and can evaporate rapidly. These properties support collagen stability and enhance resin infiltration (Hosaka et al., 2009). In the ethanol-wet bonding technique, the water within the collagen matrix is progressively substituted by ascending concentrations of ethanol, therefore improve infiltration of hydrophobic dimethacrylates such as bis-GMA (Sadek et al., 2008; Cardoso et al., 2011). In vitro, this approach significantly reduces nanoleakage and enhances hybrid layer stability (Sadek et al., 2010). Nevertheless, ethanol-wet bonding is highly technique sensitive and requires precise solvent exchange steps which limit its clinical feasibility. Systematic reviews have concluded that while ethanol-wet bonding is promising at the bench, its translation into daily practice remains limited (Van Meerbeek et al., 2010; Armstrong et al., 2017). For this reason, its application is mostly restricted to experimental or highly controlled settings.

2.3.3 Dimethyl Sulfoxide (DMSO)

Dimethyl sulfoxide (DMSO) is a highly polar aprotic solvent with both hydrophilic and lipophilic interactions, which make it unique among solvents used in adhesive dentistry. Unlike water or ethanol, DMSO can disrupt hydrogen bonding within collagen fibrils, increase dentin wettability, and promote resin monomer infiltration (Mehtälä et al., 2017).

2.3.3.1 Pharmacological Effects of DMSO

Dimethyl sulfoxide (DMSO) is a bipolar solvent that can dissolve both polar and nonpolar compounds. Its ability to penetrate different biological membranes, make it a valuable pharmaceutical vehicle and drug delivery booster (Jacob & Herschler, 1986). In pharmacology, DMSO has been prescribed as an anti-inflammatory, analgesic, and antioxidant properties. Due to, its ability to scavenge free radicals, modulate inflammatory mediators, and enhance membrane permeability (Tomoe, 2023). However, DMSO is also known to disrupt lipid bilayers and is commonly used in laboratory settings as a membrane-penetrating solvent and, at higher concentrations, as a cell-lysing agent. Therefore, its biological effects are highly dependent on concentration, duration of exposure, and route of administration. So, careful control of these parameters is very important before clinical use. Notably, it remains the only solvent formally approved by FDA for intravesical treatment of interstitial cystitis. The primary mechanisms of action of DMSO for interstitial cystitis are, the anti-inflammatory effect on Hunner lesions and defence against ischemic tissue damage (Tomoe, 2023).

In cell biology, DMSO is very crucial as a cryoprotectant. It commonly used at 5:10% concentrations to prevent intracellular ice crystal formation during freezing of stem cells, embryos, and other sensitive biological tissues (Fahy, 1986; Gurtovenko and Anwar, 2007). It also acts as a free radical absorber that protect cells from oxidative stress (Sanmartín-Suárez et al., 2011).

FDA classifies DMSO as a Class 3 low-toxicity solvent under ICH Q3C/FDA guidelines (FDA. U.S. Department of Health and Human Services Food and Drug Administration, 2017), with residual limits of 0.5% in pharmaceuticals. Low concentrations (<1-2%) are safe in medical and laboratory use but higher systemic doses may cause side effects such as skin erythema, garlic-like breath odor, and gastrointestinal discomfort (Jacob & Herschler, 1986). In vitro, low concentrations are biocompatible, but levels above 10% impair cell viability or differentiation depending on the cell type (Hebling et al., 2015; Salim et al., 2019). To conclude, DMSO is a dual-role compound act as a therapeutic agent and a laboratory solvent with wide biomedical applications. However, it requires careful dosing to avoid toxicity.

2.3.3.2 Effects of DMSO in Dentistry

Research related to the use of dimethyl sulfoxide (DMSO) in the field of dentistry has mainly focused on its application in adhesive restorative procedures. DMSO modifies the resin-dentin interaction by disrupting collagen-water hydrogen bonds, widening interfibrillar spaces, and enhancing dentin wettability (Mehtälä et al., 2017). These changes facilitate deeper penetration of adhesive monomers into the

demineralized dentin matrix which lead to a more uniform and better polymerized hybrid layer (Stape et al., 2015). Laboratory studies further elaborate that this effect is accompanied by reduced nanoleakage and improved long-term bond stability even after aging in artificial saliva at 37 °C for several years (Guo et al., 2017; Salim et al., 2018). Furthermore, DMSO has been shown to inhibit proteolytic activity of endogenous dentinal enzymes such as matrix metalloproteinases (MMPs) and cathepsins that contribute to the preservation of collagen fibrils and hybrid layer integrity (Stape et al., 2018; Zabeu et al., 2022).

A distinctive concept associated with DMSO use is “DMSO dry bonding”. Conventional etch-and-rinse adhesives require a moist dentin surface after etching, as complete air-drying causes collapse of the exposed collagen network and hinders resin infiltration. However, when dentin is treated with DMSO prior to bonding even after air-drying for 30 seconds, it does not cause collapse of the demineralized collagen (Stape et al., 2018). This phenomenon occurs because DMSO penetrates between collagen fibrils, disrupting hydrogen bonds between them and prevents fibril reaggregation after water evaporation. Therefore, the collagen matrix remains expanded and resin monomers can infiltrate effectively despite the absence of water (Stape et al., 2018; Stape et al., 2021b). This ability to maintain hybrid layer openness under dry conditions reduces technique sensitivity related to moisture control.

In addition to the studies on intact dentin, DMSO has been evaluated on substrates that are very challenging for bonding. Research on eroded dentin shows that DMSO pretreatment facilitate the wettability and monomer infiltration of bond, that subsequently leads to improve bond strength and reduce nanoleakage even after aging (Wendlinger et al., 2023, 2025). Correspondingly, bonding to fluorotic dentin a substrate characterized by hyper-mineralized enamel prisms and sclerotic dentin showed better results after DMSO application, due to the formation of more mature hybrid layer (Zhang, et al., 2024). Other dental applications are also emerging. In endodontics, DMSO pretreatment before bio-ceramic sealers has been shown to improve flow and penetration into dentinal tubules, enhancing sealing ability (Salim et al., 2025).

On the other hand, outcomes are not universally positive across all adhesive systems, some universal adhesives show neutral or inconsistent results after DMSO pretreatment (Mello et al., 2022). This research supposed that DMSO is not effective in hindering the degradation with some universal adhesives. However, the aging results were only after six months which may be insufficient to observe degradation. Contrasting evidence has shown that a universal adhesive showed better results regarding bonding to both dry and wet dentin and decreased nanoleakage after DMSO pretreatment (Karadas et al., 2023).

Hence, the current available scientific evidence revealed that DMSO represents a potential adjunct in adhesive dentistry, with strong laboratory support but still requiring optimization of protocols, adjust concentrations and lack of clinical evidence that support this protocol.

2.4 Challenges in Resin-Dentin Bonding

The resin-dentin bond is susceptible to structural and chemical degradation that limits the clinical survival of bonded restorations (Tjäderhane, 2015). Incomplete resin infiltration is one of the most critical problems. Acid etching demineralizes the collagen network, leads to collagen fibrils exposure that must be completely infiltrated by monomers to form a stable hybrid layer. In practice, a discrepancy often exists between the depth of demineralization and the extent of resin tags, leaving exposed collagen fibrils at the base of the hybrid layer (Sano et al., 1995). These fibrils are more susceptible to hydrolysis and enzymatic breakdown (Van Meerbeek et al., 2020).

Another major challenge is the hydrolytic degradation. Water is entrapped inside the hybrid layer blocking resin infiltration and forming hydrophilic regions that absorb additional water over time. It results in the hydrolysis of ester linkages from the resin matrix and creates nanoleakage channels filling these canals, through which fluids and enzymes can penetrate over interface (Tay & Pashley, 2003). This issue is exacerbated in simplified adhesives, which have high contents of hydrophilic monomers providing a semi-permeable membrane (Landuyt et al., 2010).

Matrix metalloproteinases (MMPs) and cysteine cathepsins enzymatically degrade the exposed collagen fibrils, which also contributes to the degradation of the hybrid layer. Those enzymes are activated in acidic and aqueous environment. The unprotected collagen is cleaved little by little with time which leads to failure of hybrid layer scaffold and decrease of bond strength (Tjäderhane et al., 2013a). When collagen fibrils are not fully covered with resin or chemically stabilised, the hybrid layer is fundamentally unstable (Breschi et al., 2025).

Dentin is challenging during bonding due to complex structure and hydration. It has a higher organic and water content than enamel. Moreover, it characterized by tubular heterogeneity and continuous fluid flow, driven by pulpal pressure (approximately 10:20 cm H_2O), which continuously rehydrates the etched surface and can interfere with adhesive infiltration and polymerization (Pashley, 1986). This leads to less durability and longevity compared to enamel bonding (Breschi et al., 2017). Specially deep dentin that showed additional challenges due to increased tubular density and reduced intertubular dentin (Fernandes Pegado et al., 2010). Class II restorations meta-analysis have shown significantly better longevity when margins are located in enamel compared with dentin, highlighting the intrinsic

vulnerability of the resin-dentin interface (Heintze & Rousson, 2012). Also, laboratory research showed that no reductions in enamel bond strength values were observed after 12 months of water storage, regardless of the adhesive evaluated (Loguercio et al., 2008). Unlike dentin bond that showed reduction after water storage in several articles (Tjäderhane et al., 2013a; Van Meerbeek et al., 2020).

Finally, clinical factors such as operator experience, moisture control, adhesive system, and polymerization stresses further compromise the reliability of the bond. These issues explain why resin-dentin bonding remains the weak link in adhesive dentistry and why strategies to improve hybrid layer stability quality a key focus of research (Breschi et al., 2025; Tjäderhanea et al., 2013).

2.4.1 Strategies for hybrid layer stabilization

2.4.1.1 Chlorhexidine (CHX): Inhibition of Host Proteases

Pre-treatment with Chlorhexidine (CHX) has been widely studied as a method to improve the stability of the hybrid layer by suppression of host derived proteases. These enzymes are derived from the dentin matrix, where they are sequestered in an inactive form during development of teeth (Sorsa et al., 2006). They are reactivated when exposed to acidic agents like phosphoric acid used during restorative procedures. These enzymes encompass matrix metalloproteinases (MMP-2, MMP-8, MMP-9, MMP-20) and cysteine cathepsins (cathepsins B and K) which are found in the hybrid layer and play a role, in breaking down exposed collagen fibrils (Tezvergil-Mutluay et al., 2015). The utilization of 0.2-2% CHX after etching was showed promising results regarding the inhibition of MMP activity thereby maintaining resin-dentin bonds in vitro over time (Carrilho et al., 2007; Pashley et al., 2004). Different reviews showed that CHX effectively diminishes activity while maintaining immediate bond strength (Tjäderhane et al., 2013c; Breschi et al., 2018). Clinical findings are variable, some randomized studies involving two-step etch-and-rinse adhesives have shown slightly favorable outcomes, over 18-36 months depending on the assessment methods ‘USPHS & FDI’ (Favetti et al., 2017; Sartori et al., 2013). It should be mentioned that Several cases of CHX allergy have been reported in the dental literature despite rare but could cause anaphylaxis (Donaldson & Goodchild, 2019). Overall, CHX remains a simple, low-cost adjunct with proven biochemical rationale; however, long-term clinical benefit appears adhesive- and protocol-dependent.

2.4.1.2 Collagen Cross-Linkers

In the last decades, numerous crosslinkers have been tested throughout the literature to evaluate their effect on mechanical properties of demineralized dentin and reduce enzymatic degradation. Natural crosslinkers such as proanthocyanidins, quercetin, epigallocatechin-3-gallate (EGCG) and baicalein are plant derived polyphenols that oust water from the fibrillar collagen network and inhibit MMPs and cysteine cathepsins enzymes. Subsequently, hydrolytic and enzymatic degradation of the hybrid layer will be hindered (Fang et al., 2012; Hardan et al., 2022). On the other hand, several synthetic or semisynthetic agents like carbodiimide, glutaraldehyde, riboflavin, EDTA, benzalkonium chloride, and chitosan create additional intramolecular and intermolecular crosslinks within collagen or chelate calcium and zinc ions inhibiting MMPs activity (Liu et al., 2011; Tjäderhane et al., 2013a; Hardan et al., 2022; You et al., 2022). When these crosslinkers are used as dentin pretreatments, often combined with ethanol or water saturated dentin, they have repeatedly shown improved long-term bond strength and reduced interfacial nanoleakage. These actions support their role as a promising adjunct to preserve resin-dentin bond durability (Macedo et al., 2009; You et al., 2022).

Several limitations hinder their transition from laboratory studies to routine clinical practice. Most available evidence originates from in vitro experiments with heterogeneous protocols, and a lack of corresponding clinical trials. A major practical obstacle is that many crosslinkers require long application times to produce meaningful collagen stabilization, which is incompatible with the workflow of conventional adhesive procedures (Liu et al., 2011). Cytotoxicity further restricts the use of potent synthetic agents such as glutaraldehyde, despite its strong collagen-stabilizing capability (Jiang et al., 2022). Proanthocyanidins (PA), naturally occurring polyphenols, can form exogenous cross-links within dentin collagen, increasing stiffness and resistance to enzymatic degradation. In vitro studies show that short PA treatments enhance the mechanical properties of collagen and reduce degradation, with reported improvements in bond strength retention compared with untreated controls (Breschi et al., 2018). Newer phenolic biomodifiers such as curcuminoids have also demonstrated long-term stabilization of resin–dentin bonds (Atay et al., 2022). The principal limitations are potential colour changes, added application time, and variability in formulations and concentrations that affect clinical translation. De Souza evaluated the clinical application of proanthocyanidins as a primer or incorporated into simple adhesives and found that it is negatively affecting the retention of composite resins restorations in non-carious cervical lesions (De Souza et al., 2019a, 2020). Standardized protocols and biocompatibility data are still needed before routine clinical adoption.

2.4.1.3 Biomimetic Remineralization of the Hybrid Layer

Biomimetic remineralization aims to mimic natural dentin mineralization by replenish minerals within the demineralized collagen matrix through ion-rich precursors that nucleate and grow within fibrils (Tjäderhane et al., 2013b). Polyanion-stabilized amorphous calcium phosphate (ACP) nano precursors can be used or related templating systems to restore intrafibrillar, which interrupts water pathways, reduces nanoleakage, and protects collagen from enzyme attack (Breschi et al., 2018). Successful biomimetic strategies should be able to provide a stable source of Ca/PO_4 precursors, incorporate templating analogues to direct intrafibrillar deposition, and be compatible with resin infiltration. Sustained remineralization of hybrid layers has been demonstrated in laboratory models. However, the techniques are complex, time-consuming and incorporation into chairside protocols remains challenging (Breschi et al., 2025). Ongoing work focuses on simplifying delivery systems, combining remineralization with collagen stabilization and MMP inhibition in a single clinically feasible protocol.

2.4.1.4 Ethanol Wet Bonding Technique

The ethanol-wet bonding technique (EWBT) emerged as an alternative to conventional water-wet bonding. It aims to facilitate the infiltration of hydrophobic resin monomers into the etched collagen matrix. Water was substituted with ethanol so collagen fibrils are dehydrated without being collapsed. This created a less hydrophilic substrate that reduces nanoleakage and hydrolytic degradation (Kuhn et al., 2015).

In vitro studies support the potential of EWBT to produce higher bond strengths and more durable hybrid layers. However, clinical outcomes have been less consistent. In a six-month randomized controlled trial, no significant improvement in restoration survival was reported compared to conventional methods (De Souza et al., 2019b), only postoperative sensitivity was reduced in ethanol-treated groups. Similarly, some other clinical studies with short follow-up periods of 6 to 12 months, showed that simplified EWBT protocols showed similar results compared to conventional adhesives (Mortazavi et al., 2012 & Araújo et al., 2013).

To sum up, EWBT represents a promising strategy to solve the problem of the hydrophilicity of conventional adhesive interfaces. Its ability to facilitate the infiltration of hydrophobic resin monomers may translate into improved long-term durability. However, the additional steps and sensitivity of the technique limit its widespread adoption until further robust long-term RCTs confirm its advantages. The technique is laborious and highly sensitive, as collagen must first be maintained in a moist state to prevent the formation of interpeptide hydrogen bonds, followed

by gradual dehydration using sequentially increasing concentrations of ethanol (Pashley et al., 2007).

2.4.1.5 Dry- vs. Wet-Dentin DMSO Bonding

Conventional etch-and-rinse adhesives rely on a 'wet bonding' technique, where collagen fibrils are kept hydrated after acid-etching to prevent collapse and allow resin infiltration. However, wet bonding is highly technique-sensitive, as both over-wet and over-dry conditions compromise adhesion (Pashley et al., 2011). This limitation has prompted research into strategies that expand the acceptable moisture spectrum, among which dimethyl sulfoxide (DMSO) has gained considerable attention.

In the context of wet bonding, (Stape et al., 2015) evaluated the effect of incorporating DMSO into etch-and-rinse adhesive protocols. They demonstrated that DMSO pretreatment improved hybrid layer morphology, increased monomer infiltration, and significantly reduced nanoleakage after two years of water storage. As a result, bond strength was better preserved over time compared to traditional wet bonding, where hydrolytic and enzymatic degradation of the collagen network leads to rapid deterioration (Tjäderhane et al., 2013b).

In contrast, dry bonding traditionally produces poor results because air-drying collapses collagen fibrils and prevents adequate infiltration of adhesives. However, (Stape et al., 2018) introduced the concept of 'DMSO dry bonding,' where dentin pretreated with DMSO resisted collapse even after 30 seconds of air-drying. The mechanism was attributed to DMSO's ability to disrupt hydrogen bonding between collagen and water, preventing reaggregation after water removal. This unique effect not only stabilized the collagen scaffold but also enhanced wettability, thereby facilitating the diffusion of both hydrophilic and hydrophobic monomers.

More recent studies have reinforced the potential of DMSO dry bonding. In Stape investigations, pretreatment with DMSO preserved hybrid layer integrity and bond strength after extended aging periods (Stape, 2021). The authors also emphasized DMSO's inhibitory effect on dentin proteases, which further protected the exposed collagen fibrils from enzymatic degradation. These findings suggest that DMSO dry bonding may provide a clinically feasible solution to reduce the strict moisture sensitivity of etch-and-rinse adhesives.

Stape also highlighted that wet-bonding protocols enhanced with DMSO produced more durable bonds than conventional wet bonding, but the dry-bonding approach was particularly transformative (Stape, 2021). By allowing air-dried dentin to remain receptive to resin infiltration, DMSO expanded the clinical applicability of adhesives to a wider range of moisture conditions. This reduces operator sensitivity and the risk of clinical failure associated with improper moisture control.

The benefits of both wet and dry DMSO bonding can be explained by its amphiphilic molecular structure. DMSO intercalates within the collagen network, replacing bound water and enlarging interfibrillar spaces. This not only facilitates diffusion of resin monomers but also stabilizes collagen fibrils against collapse (Mehtälä et al., 2017). At the same time, DMSO reduces enzymatic activity by partially inactivating MMPs and cathepsins, thereby preventing proteolytic degradation (Zabeu et al., 2022). The combination of physical and biochemical effects makes DMSO uniquely suited for improving hybrid layer durability.

Clinically, these findings are of great relevance. Moisture control is one of the most difficult challenges during restorative procedures, because it is technique sensitive and debatable, how much wet is wet. By broadening the acceptable range of moisture conditions, DMSO reduces the technique sensitivity of bonding protocols and improves the likelihood of achieving long-lasting adhesion. While more randomized clinical trials are needed to validate the long-term performance of DMSO dry bonding, the accumulated evidence suggests that both wet- and dry-bonding techniques benefit substantially from DMSO pretreatment.

2.5 Clinical Evaluation of Dental Adhesives

Laboratory *(in vitro*) tests are essential for mechanical, cytotoxic evaluation and comparison of new materials and protocols. However, they cannot replicate the full clinical challenges that may affect the restoration performance. For instance, patient related factors “age, hygiene, diet, bruxism”, tooth related factors “cavity size, class, occlusion stresses, sub/supragingival margins”, and operator factors “technique, decision-making, experience” (Van Meerbeek et al., 2010).

Randomized controlled trials (RCTs) represent the highest standard for assessing the effectiveness and durability of dental restorations because they minimize bias by random allocation, prospective follow-ups, examiner calibration, and controlled intervention protocols (Grimes et al., 2018). RCTs can allow true causal inferences by balancing confounders between groups. On the other hand, observational and epidemiological studies are relatively limited by selection bias, uncontrolled confounding, non-standardized assessments, and inconsistent follow-up intervals. Nevertheless, they are valuable for calculation of true incidence rates, relative risks and external validity (Ioannidis, 2016; Schwendicke & Opdam, 2018). Case series and case reports provide important early clinical impressions but are descriptive in nature, lack control groups, cannot estimate effect sizes, and are highly susceptible to operator variability and publication bias (Ioannidis, 2016). In contrast, well-designed RCTs ensure transparent reporting of inclusion criteria, standardized clinical protocols, calibration of examiners, and long-term outcome evaluation using validated scoring systems such as the FDI criteria (Hickel et al., 2023). Ultimately,

clinical recommendations based on high-quality RCTs reduce uncertainty and improve the predictability and scientific credibility of adhesive dentistry (Schwendicke & Opdam, 2018).

2.5.1 USPHS Criteria for Evaluation of Dental Restorations

The United States Public Health Service (USPHS; "Ryge") criteria were initially introduced in 1971 (Cvar & Ryge, 1971). Updated in 1980 for clinical evaluation of direct dental restorations (Bayne & Schmalz, 2006). These criteria evaluate aspects using a four-level grading system: Alpha (optimal), Bravo (clinically acceptable variations), Charlie (clinically unacceptable replacement advised for preventive purposes), and Delta (failure, replacement necessary). The criteria includes color match, marginal discoloration, anatomic form, marginal adaptation, caries, fracture/retention, occlusion, and postoperative sensitivity (Bayne & Schmalz, 2006). Despite being widely accepted, it has several limitations, including lower discriminative sensitivity for early changes (ceiling effects at 12:18 months), and lack of uniform definitions across studies, which compromise clinical comparisons (Marquillier et al., 2018).

2.5.2 FDI Criteria for Evaluation of Dental Restorations

The FDI World Dental Federation released a new set of clinical criteria in 2007, intended to tackle issues related to sensitivity and uniformity. It also updated with clinical examples in 2010. The FDI framework classifies properties into functional, biological, and aesthetic outcomes, each score on a five-point scale (1-5): scores 1-3 are clinically acceptable (excellent/good/satisfactory), while scores 4 and 5 indicate unacceptable conditions suggesting repair or replacement, respectively. The criteria are accompanied by web-based calibration (e-calib) and guidance for examiner training (Hickel et al., 2010b).

The application of FDI criteria has grown consistently in trials involving direct restorations, rising from 4.5% of studies in 2010 to 50% in 2016. This increase is due to researchers considering them practical (not optional, with defined domains), relevant (more responsive to early decline), and standardized (enabling easier comparisons across studies) (Marquillier et al., 2018). A 2022 practice-oriented investigation additionally showed the practicality of modified (simplified versus chosen) FDI criteria while cautioning that outcomes cannot be directly equated with those derived from the original criteria (Maillet et al., 2022). In 2023, an expert panel revised and harmonized the eleven most frequently used FDI categories, clarified ambiguities, reordered domains (functional, biological and aesthetic). The update links each score to management strategies, (score 3) no modification needed, (score

4) repair, or (score 5) replacement. The revision also provides detailed recommendations on study design, calibration, follow-up schedules, and reporting. That leads to increase internal validity and comparability across trials (Hickel et al., 2023).

In adhesive dentistry the FDI criteria are typically favoured over USPHS because they offer higher sensitivity for detecting initial alterations in marginal adaptation/staining, material durability and biological results. Additionally, they help minimize ceiling effects observed with USPHS, in short-term studies (Marquillier et al., 2018). Structured domains (functional/biological/aesthetic) aligned to adhesive materials and minimally invasive management (5R: review, refurbishment, reseal, repair, replacement), improving clinical relevance and decision-making (Hickel et al., 2010b; 2023). Moreover, documented rising adoption in clinical research, facilitating meta-analyses and evidence synthesis (Marquillier et al., 2018).

3 Aims

The purpose of this series of studies was to investigate the potential of dimethyl sulfoxide (DMSO) as a dentin pretreatment to enhance the durability and clinical performance of resin-dentin bonding. The research was designed to provide a comprehensive understanding of how DMSO can influence bonding durability, explore its application under different bonding conditions, and validate its effectiveness. This thesis therefore combined in vitro studies and randomized controlled clinical trials to systematically assess how DMSO concentration, application time, and clinical application in different types of cervical lesions influence bonding performance and restoration longevity. The specific aims were:

1. To evaluate the effect of different concentrations of DMSO on the durability of resin-dentin bonds (*Study I*). The hypothesis was that low concentration of DMSO would have the same stabilizing effect on resin-dentin bonding as high DMSO concentration.

2. To investigate the influence of DMSO application time and its role in enabling DMSO-assisted dry bonding (*Study II*). The hypothesis was that 20 and 60 seconds application time would have the same effect on long-term resin-dentin bonding.

3. To evaluate the potentiality of DMSO pretreatment to enhance the bonding of neat hydrophobic resins to demineralized dry dentin (*Study II*). The hypothesis was that DMSO would enhance the infiltration of neat hydrophobic resins to demineralized dry dentin.

4. To assess the clinical effectiveness of DMSO pretreatment in non-carious cervical lesions through a randomized controlled trial (*Study III*). The hypothesis was that DMSO can improve the clinical performance of composite restorations in non-carious cervical lesions.

5. To evaluate the clinical performance of DMSO pretreatment in carious cervical lesions in a randomized controlled trial (*Study IV*). The hypothesis was that DMSO enhance the clinical effectiveness of composite restorations in cervical carious lesions.

4 Materials and Methods

4.1 Part one In-Vitro studies (*Study I & II*)

4.1.1 Tooth Selection and Preparation

Extracted human third molars, confirmed to be free of caries, restorations, and visible cracks, were obtained under institutional ethical approval (University of Oulu, Finland, #23-2003). Following extraction, teeth were stored in 0.5% chloramine at 4 °C for a maximum of three months until use. Each specimen was mounted in acrylic resin blocks, positioning the cemento-enamel junction approximately 2 mm above the surface. Mid-coronal dentin was exposed by sectioning perpendicular to the long axis of the tooth using a water-cooled slow-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA). The absence of residual enamel was confirmed under a stereomicroscope at 40× magnification. To create a standardized smear layer, the dentin surfaces were ground with 320-grit silicon carbide paper (Buehler-Met) under running water for 60 s (Armstrong et al., 2017).

4.1.2 DMSO Solutions and Moisture Control

Dentin was etched with phosphoric acid and rinsed according to each study's protocol, 34% H_3PO_4 applied for 15 s, rinsed for 15 s, then blot-dried in conventional wet bonding control groups. On the other hand, dry-bonding groups in (*Study I&II)* replaced the blot drying step after etch rinsing by air-drying for 30 s before pretreatments. In *study I*, dentin surfaces were pretreated with DMSO ethanolic solutions at two concentrations, 5% and 50% (v/v). Pretreatments were applied by active rubbing with a microbrush for the designated application time, followed by standardized drying per the DMSO-dry bonding protocol where applicable. In *study II*, after etching and 30 s air-drying, dentin surfaces received 50% (v/v) ethanolic DMSO pretreatment, applied actively for either 20 s or 60 s. A fixed volume (50 µL) was dispensed and rubbed with light pressure in circular movements; the surface was then air-dried for 30 s before adhesive application or blot dried in control group.

Table 1. Adhesive Systems and restorative materials' main components according to manufacturers, used through the thesis

Material	Components
Scotchbond Universal Etchant (3M/ESPE, USA)	34% phosphoric acid, fumed silica, polyethylene glycol, aluminium oxide
Single Bond 2 (3M/ESPE; SB)	Ethanol, bis-GMA, silane-treated silica, HEMA, copolymer of acrylic and itaconic acids, GDMA, UDMA, water (<5%), DPI
Scotchbond Multi-Purpose (3M/ESPE; SBMP) (*Study II*)	*Primer*: water (>40%), HEMA, methacrylate copolymer, polyalkenoic acid *Bond:* bis-GMA, HEMA, dimethacrylates, photoinitiators
Nanofilled composite, Filtek Supreme XTE (3M/ESPE) *(Study I, II*)	bis-GMA, UDMA, TEGDMA, and bis-EMA resins non-agglomerated/non-aggregated 20 nm silica filler, 4 to 11 nm zirconia filler, and aggregated zirconia/silica cluster filler
Nanofilled composite, Filtek Z350 XT (3M/ESPE) (*Study III, IV*)	bis-GMA, bis-EMA, UDMA, PEGDMA, TEGDMA Silane treated ceramic fillers, silane treated silica fillers, silane treated zirconia fillers
Dimethyl Sulfoxide (Sigma Aldrich, USA)	5%, 50% DMSO/ Ethanol (*Study I*) 50% DMSO/ Ethanol (*Study II*) 1% DMSO/ H_2O (*Study III*) 10% DMSO/ H_2O (*Study IV*)

Abbreviations: bis-GMA = bis-phenol diglycidylmethacrylate; HEMA = 2-hydroxyethyl methacrylate; GDMA = Glycerol 1,3-dimethacrylate; UDMA = diurethane dimethacrylate; DPI = Diphenyliodonium hexafluorophosphate; bis-EMA= bisphenol A ethoxylated dimethacrylate; PEGDMA=Polyethylene Glycol Dimethacrylate and TEGDMA= Triethylene glycol methacrylate.

4.1.3 Adhesive Systems and Restorative Procedures

Following DMSO pretreatments, etch-and-rinse adhesive systems were applied. In *Study I*, either a simplified two-step system (Adper Single Bond 2, 3M ESPE; SB) or a three-step system (Scotchbond Multi-Purpose, 3M ESPE; SBMP) was used, as outlined in (**Table 1)**. In *Study II*, the three-step SBMP adhesive was employed either as a combined solvated hydrophilic-rich primer with hydrophobic-rich resin (Primer + Bond) or as the hydrophobic-rich resin alone (Bond). In both studies, the adhesive resins were actively rubbed onto the dentin surface under controlled environmental conditions and light-cured (Reis et al., 2007) with an LED curing unit (Elipar DeepCure, 3M ESPE) at an intensity of 1200 mW/cm² for 10 s. Composite build-ups were then placed incrementally. A nanofilled composite (Filtek Supreme XTE, 3M ESPE) was applied in 2 mm layers by a flat instrument carefully in horizontal

direction, increment by increment to avoid gaps, with each increment polymerized for 20 s. The restored specimens were stored in distilled water at 37 °C for 24 h before sectioning.

4.1.4 Sectioning and Specimen Preparation

After restoration, the teeth were sectioned longitudinally in both mesio-distal and bucco-lingual directions across the bonded interface using a water-cooled low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA). This procedure produced resin–dentin sticks (non-trimmed beams) with cross-sectional areas averaging 0.8 mm² (range 0.62-0.89 mm²), with at least 18 beams obtained from each tooth (Armstrong et al., 2017). Specimens were assigned either to immediate testing after 24 h or to long-term storage. In *Study I*, beams were aged for one year, and in *Study II*, for two years, in artificial saliva maintained at 37 °C and buffered at approximately pH 7.4. It was changed biweekly to avoid pH changes. It consisted of 5 mL HEPES, 2.5 mL $CaCl_2 \cdot H_2O$, 0.05 mL $ZnCl_2$ and 0.3 mL NaN_3.

4.1.5 Microtensile Bond Strength (μTBS) Testing

Beams were attached to a microtensile jig with cyanoacrylate adhesive (Loctite 416, Henkel Corp., Dublin, Ireland) stained red and loaded in tension to failure on a universal testing machine (Shimadzu, AGS-X, Maryland, USA). Crosshead speed was maintained at 0.5 mm/min. Bond strength (MPa) was calculated as maximum load (N) divided by the measured cross-sectional area (mm²). Tooth was treated as the statistical unit; individual stick values were averaged per tooth. Pre-test failures were entered as 0 MPa. Failure modes were classified under stereomicroscopy and/or SEM as adhesive, cohesive (in dentin or composite), or mixed (Armstrong et al., 2017).

4.1.6 Interfacial Nanoleakage Evaluation (SEM)

For nanoleakage evaluation, two sticks per tooth (or per experimental group) were selected. Each specimen was coated with nail varnish, leaving approximately 1 mm around the bonded interface exposed. After rehydration in distilled water for 1 h, samples were immersed in 50% (w/v) ammoniacal silver nitrate (pH = 9.5) and kept in the dark for 24 h. They were then rinsed for 120 s and placed in a photo-developing solution (Kodak Professional D-76 developer, Kodak, Rochester, NY, USA) under fluorescent light for 8 h. Following development, the specimens were embedded in epoxy resin, sequentially polished with SiC abrasive papers (600, 1200, and 2000 grit; Carbimet, Buehler Ltd.) and with alumina/diamond suspensions (1.0, 0.25, and

0.05 µm; Buehler Ltd) (Tay et al., 2002). After ultrasonic cleaning and air-drying, samples were mounted on stubs, sputter-coated with carbon, and examined under backscattered-electron SEM (Phenom ProX, Phenom-World, Eindhoven, Netherlands) at 10 kV to assess silver uptake, characterized as reticular or spotted patterns within hybrid layers.

4.1.7 Hybrid Layer Characterization (SEM)

Representative beams (two per tooth) were embedded in epoxy resin, polished as above, and deproteinized to reveal hybrid layer morphology (50% H_3PO_4 for 5 s; 3% NaOCl for 10 min), followed by dehydration in ascending ethanol series (50, 70, 80, 90, 3×100%), hexamethyldisilazane drying, and gold/palladium sputter-coating. SEM micrographs (5000×) were obtained to survey the full interface; three areas between adjacent resin tags were measured for hybrid layer thickness per tooth, using an image software (ImageJ, National Institute of Health, Bethesda, MD, USA).

4.1.8 Statistical Analysis

Normality and homoscedasticity were verified prior to parametric testing, (Shapiro-Wilk; p =0.091) and (Levene Test; p = 0.064) respectively. µTBS values (per-tooth means) were analyzed using factorial ANOVA models reflecting the study factors (DMSO concentration or application time, resin composition, storage period) with Tukey post hoc testing; comparisons to the isolated wet-bonding control used Dunnett's test where applicable. Nanoleakage scores were handled using appropriate non-parametric tests when assumptions were not met. Hybrid layer thickness was analyzed by two-way ANOVA followed by the Tukey test. Statistical significance was set at $\alpha = 0.05$. Calculations were performed by SigmaPlot for Windows, version 14.0 (Alfasoft AB, Sweden).

4.2 Part II Clinical Randomized Controlled Trials (*Study III and Study IV)*

4.2.1 Ethical Approval

Both clinical investigations were conducted in accordance with the Declaration of Helsinki and approved by the appropriate institutional review boards. *Study III* (non-carious cervical lesions, NCCLs) received approval from the Ethics Committee of the Faculty of Dentistry, Cairo University, Egypt and was registered at ClinicalTrials.gov on 22 July 2020, with unique identification number NCT04492306. *Study IV* (carious cervical lesions, CCLs) was approved by the

Ethics Committee of the Faculty of Dentistry, Horus University, Egypt and registered at ClinicalTrials.gov on Sept 10, 2021, under the unique identification number NCT05090085. All participants received detailed information about the study aims and procedures, and written informed consents were obtained before enrollment.

4.2.2 Trial Design and Setting

Both studies were designed as double-blind, parallel-arm randomized controlled clinical trials. Study III was conducted at the outpatient clinics of the Faculty of Dentistry, Cairo University, involving 29 patients with 82 NCCLs, while Study IV took place at Horus University under collaborative supervision with the University of Turku, involving 45 patients presenting with 74 CCLs. Restorations were evaluated at baseline, 12, 24, and 36 months of follow-up. CONSORT flow diagrams outlining the recruitment, allocation, follow-up, and analysis are presented in (**Figure 1,** *Study III*) and (**Figure 2,** *Study IV*).

4.2.3 Sample Size Calculation

Sample size determination in both studies was based on previous clinical trials of cervical restorations (Loguercio et al., 2007), (Koc Vural et al., 2020) with an alpha level of 0.05 and power of 80%. In *Study III*, after 36 months in a previous study (Loguercio et al., 2007), the retention rate of NCCL restorations made with the complete etch adhesive system was 96.7%. Using a two-tailed Z test to determine the difference between two independent proportions power analysis indicated that 33 restorations per group were required; to compensate for potential dropouts, the sample size was raised by 25%, bringing the total number of restorations per group to 41. In *Study IV*, the calculation required 30 restorations per group, which was increased to 37 per group to account for attrition. The final sample size was therefore 82 NCCLs (*Study III*) and 74 CCLs (*Study IV*). For sample size calculations, G-Power version 3.1.9.2 for Windows was used.

4.2.4 Participants – Recruitment, Eligibility, Randomization and Allocation

Patients were recruited from the outpatient dental clinics of Cairo University (*Study III*) and Horus University (*Study IV*). Participants were recruited according to inclusion and exclusion criteria listed in the original study protocols see (**Table 2**). The simplified scoring criteria of the Tooth Wear Index (TWI) (Bardsley, 2008) were applied to evaluate the severity of non-carious cervical lesions (NCCLs) in the

enrolled patients of *Study III*. The simplified scoring criteria of the International Caries Detection and Assessment System (ICDAS) (Banting et al., 2011) were applied to evaluate the severity of carious cervical lesions (CCLs) in the enrolled patients of *Study IV*. Random allocation of lesions was achieved using computer-generated randomization lists (www.random.org) with a 1:1 allocation ratio. Each lesion was randomized independently. Allocation concealment was maintained using sealed opaque envelopes prepared by an assistant not involved in clinical procedures. The operator opened the envelope immediately before treatment. CONSORT flow diagrams present patient enrollment, allocation, and follow-up (**Figure 1,** *Study III*) and (**Figure 2,** *Study IV*).

4.2.5 Blinding and Examiner Calibration

Both studies employed a double-blind design. Participants were blinded to group assignment, and clinical evaluators were unaware of which treatment each restoration received. The operator could not be blinded due to the nature of the interventions. Two independent evaluators, previously trained and calibrated in the use of the FDI criteria, performed all evaluations. Calibration was carried out on a separate set of 20 restorations not included in the study, with evaluations repeated after one week. Inter-examiner agreement was assessed with Cohen's kappa statistics and was found to be >0.89, indicating excellent reliability. Discrepancies were resolved by consensus with a senior supervisor.

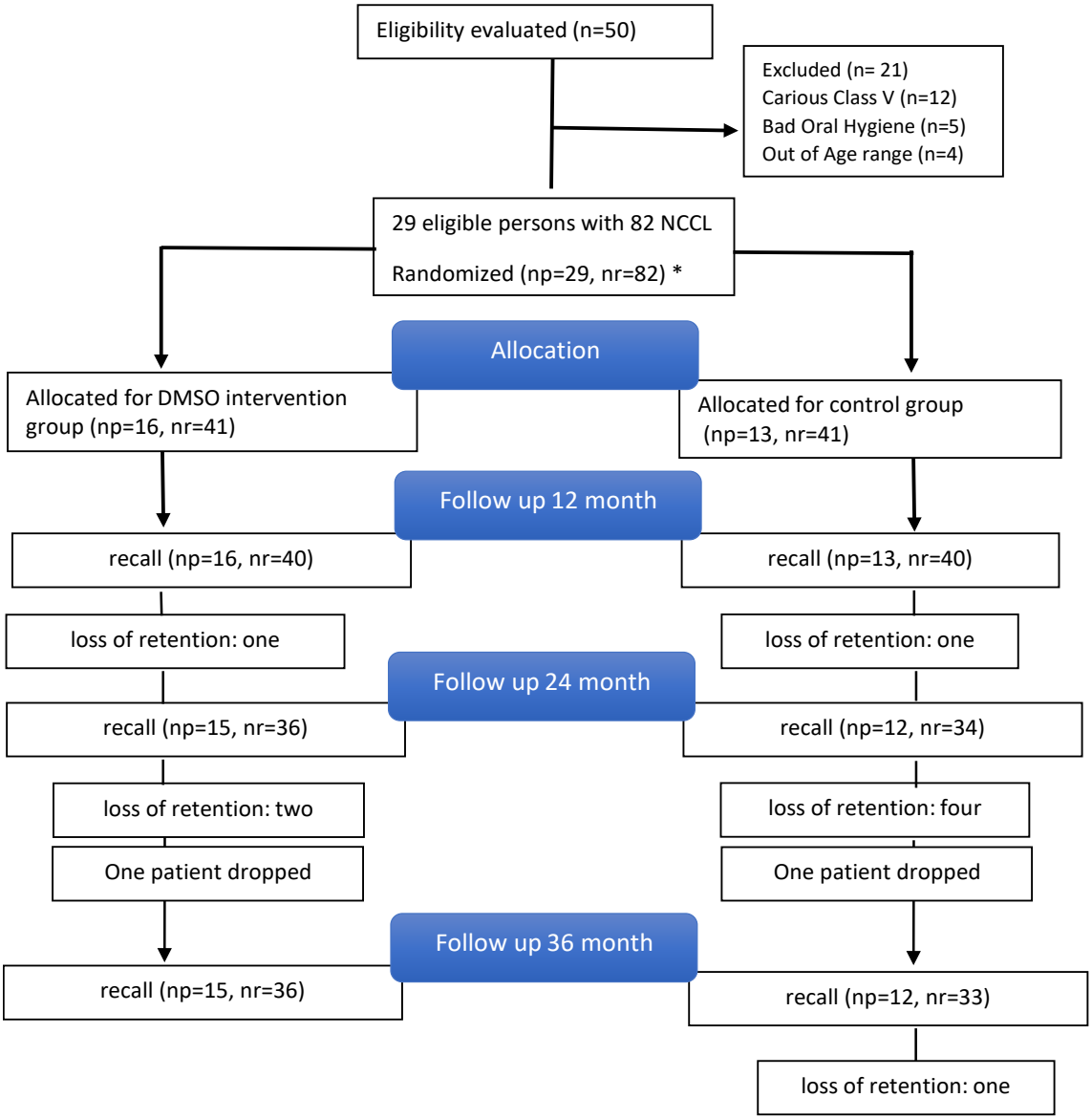


Figure 1. CONSORT flow diagram of Study III, *modified from original publication III*. *np= number of patients, *nr= number of restorations.

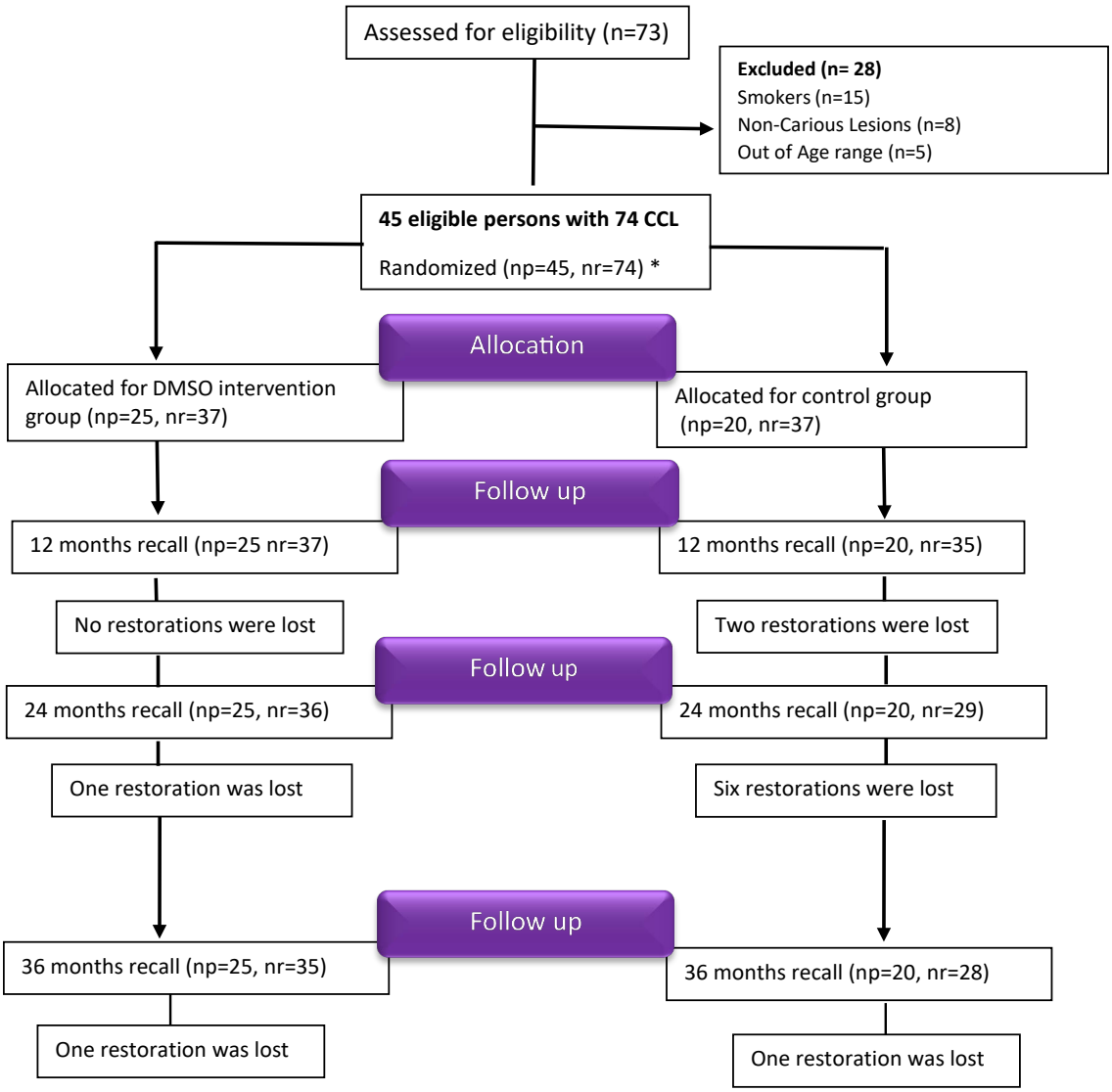


Figure 2. CONSORT flow diagram of Study IV, *modified from manuscript IV*. *np= number of patients, *nr= number of restorations.

Table 2. Inclusion and exclusion criteria, *modified from manuscript IV*.

Inclusion criteria	Exclusion criteria
Age ≥18 years Good systemic health condition (ASA-I or II) Co-operative patients signed informed consent Good oral hygiene (OHI-S) Vital painless teeth Good occlusion (minimum of 20 teeth) Occlusal contact with opposing teeth	Cavities deeper than 3 mm after preparation Smoking Xerostomia Bruxism patients Patients seek bleaching treatments Inaccessible to return for recall visits Fractured or visibly cracked teeth Current desensitizing therapy Long-term use of drugs like: anti-inflammatory analgesic or psychotropic drugs Pregnancy or breast-feeding Allergies to resin-based restorative materials Orthodontic patients Abutment teeth for prosthetic appliances Teeth or supporting structures with painful pathology Existing periodontal disease or periodontal surgery within the previous three months

4.2.6 Clinical Restorative Procedures

All restorative procedures were carried out under rubber dam isolation, and local anesthesia was administered when indicated. The cavities were cleaned with a pumice–water slurry applied by a prophylaxis brush, rinsed thoroughly, and gently air-dried without desiccation. Enamel and dentin were etched with 37% phosphoric acid gel for 30, 15 s respectively, rinsed with water for 15 s, and lightly air-dried. The composition and manufacturer details of the materials used are summarized in **Table 1.** In the intervention groups, dentin surfaces were conditioned with DMSO applied by a microbrush for 60 s, followed by gentle air-drying. For *Study III*, the pretreatment concentration was 1% DMSO/H_2O, while in *Study IV* it was 10% DMSO/H_2O. In both trials, Adper™ Single Bond 2 (3M ESPE, St. Paul, MN, USA) was applied according to the manufacturer's instructions and light-polymerized with an LED curing unit (Bluephase 20i, Ivoclar Vivadent, in *Study III*; Elipar DeepCure-S, 3M ESPE, in *Study IV*). Restorations were built using Filtek Z350XT nanohybrid composite (3M ESPE) placed incrementally in ≤2 mm layers, with each increment light-cured for 20 s. Finishing and polishing were performed with abrasive discs and polishing stones. Representative clinical photographs are presented in **Figure 3** (*Study III*) and **Figure 4** (*Study IV*).

4.2.7 Clinical Evaluation

Clinical evaluations of the restorations were conducted at baseline, and after 12, 24, and 36 months, according to the World Dental Federation (FDI) criteria. The assessment included esthetic outcomes such as marginal staining, functional parameters including fracture/retention and marginal adaptation, and biological aspects such as postoperative sensitivity and caries adjacent to the restoration. Each parameter was scored on a 5-point scale, where scores 1,2,3 represented clinically acceptable outcomes, and scores 4,5 represented clinical failures. Evaluations were carried out independently by two blinded examiners, and disagreements were resolved by consensus.

4.2.8 Statistical Analysis

Statistical analysis was performed using Medcalc software, version 22 for Windows (MedCalc Software Ltd, Ostend, Belgium). Chi-square tests were applied for inter-group comparisons at each evaluation time point. Cochran's Q test was used to analyze changes in restoration performance over time within groups, with Bonferroni adjustment for post hoc comparisons. Kaplan–Meier survival analysis was used to estimate restoration survival rates, and the log-rank test was employed to compare differences between groups. A significance level of $p \leq 0.05$ was set for all statistical analyses. Logistic regression analysis was performed to evaluate the effect of patient-related factors on the success rate at each follow-up. A 95% confidence interval and 80% statistical power were applied, and all tests were two-tailed.

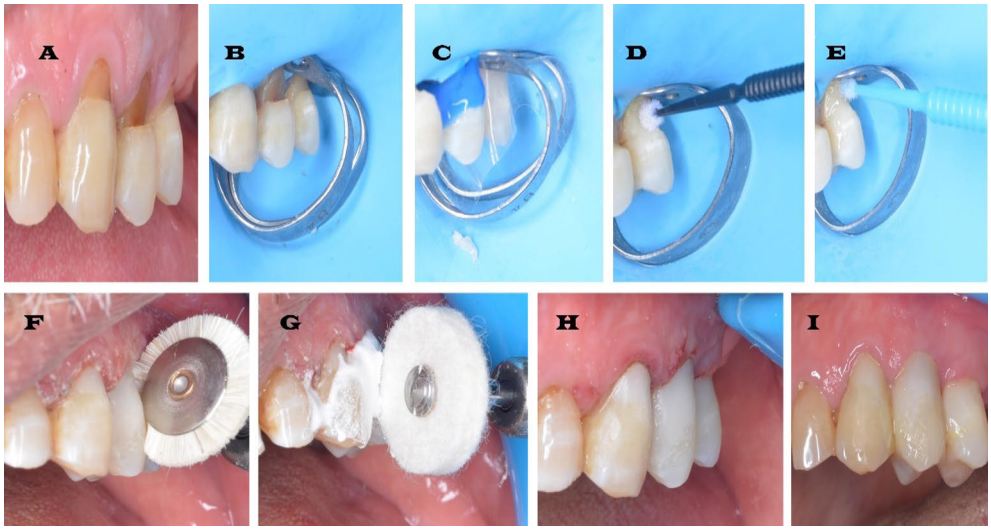


Figure 3. ***Study III*** Restorative procedures performed on teeth #23 and #24. (A) Non-carious cervical lesions (NCCLs); (B) cavity isolation with a rubber dam; (C) selective etching of cavities with 37% phosphoric acid; (D) application of 1%DMSO/H_2O; (E) application of Single Bond 2 adhesive; (F, G) finishing and polishing; (H) immediate postoperative restorations; (I) restorations at baseline follow-up (1 week), *from original publication III*.

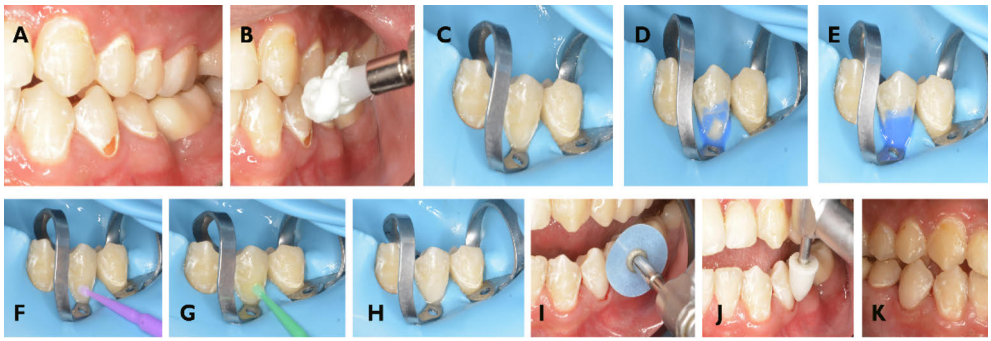


Figure 4. ***Study IV.*** showed restorative procedures of 44, **A**: CCL (Carious Cervical Lesions), **B:** Preoperative Polishing, **C:** Cavity isolated by rubber dam, **D&E**: Cavity etched with 37% phosphoric acid (selective etching), **F:** Application of 10% DMSO/H2O, **G:** Application of Single Bond2, **H:** restored with Filtek Z350 XT**, I&J**: Finishing and polishing, **K:**Immediate restorations.

5 Results

5.1 Microtensile Bond Strength (Study I & II)

The cross-sectional areas of the resin-dentin beams ranged between 0.70 and 0.99 mm² in *Study I* (0.84 ± 0.10 mm²) and between 0.62 and 0.89 mm² in *Study II* (0.79 ± 0.07 mm²), with no significant differences in beam size among the groups ($p > 0.05$). Bond strength means and standard deviations are presented in (**Figure 5,** *Study I*) and (**Figure 6,** *Study II*).

In both studies, untreated dry dentin exhibited the lowest immediate bond strength values, approximately 80–85% lower than the conventional wet-bonding controls ($p < 0.05$). For *Study I*, three-way ANOVA indicated that "bonding protocol" ($p < 0.001$; $\eta^2 = 0.908$), "bonding resin" ($p < 0.001$; $\eta^2 = 0.258$), and "storage time" ($p = 0.005$; $\eta^2 = 0.115$) significantly influenced μTBS values, with significant interactions between "bonding protocol" and "storage time" ($p = 0.046$). In *Study II*, "DMSO pretreatment" ($p < 0.001$; $\eta^2 = 0.88$), "bonding resin composition" ($p < 0.001$; $\eta^2 = 0.67$), and "storage time" ($p < 0.001$; $\eta^2 = 0.40$), as well as their interactions, were statistically significant. In *Study I,* conventional wet bonding (Control Wet) with either SB or SBMP adhesives produced high immediate bond strengths. Dry bonding (Control Dry) showed markedly lower values, whereas DMSO-dry bonding (5% or 50%) restored immediate μTBS to levels comparable to or higher than wet bonding. For the two-step adhesive (SB), both 5% and 50% DMSO-dry protocols produced immediate values similar to wet bonding, while the three-step adhesive (SBMP) showed significantly higher bond strengths with 50% DMSO pretreatment ($p < 0.05$) (**Figure 5,** *Study I*) and (**Figure 6,** *Study II*).

In *Study II*, pretreatment with DMSO markedly enhanced bond strengths compared with untreated dry dentin, independent of resin composition, application time, or storage duration ($p < 0.05$). Application for 60 s yielded significantly higher μTBS values than 20 s within the same bonding protocol ($p < 0.05$). Combinations using Primer+Bond achieved bond strengths approximately 40% greater than those obtained with the neat hydrophobic-rich resin (Bond). For most protocols, immediate bond strengths after DMSO pretreatment under dry conditions were comparable to, or exceeded, the values from conventional wet bonding, with the exception of Bond combined with 20 s pretreatment, which remained significantly lower ($p < 0.05$).

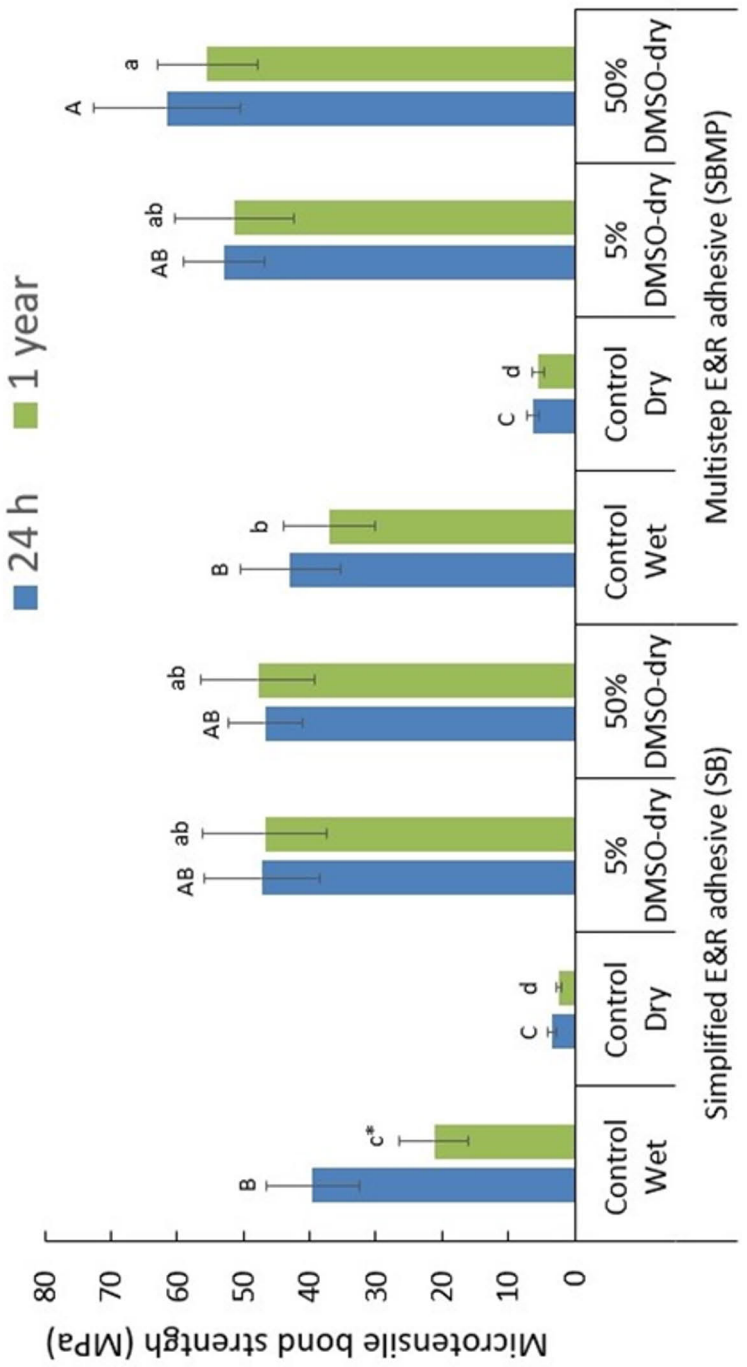


Figure 5. Microtensile bond strength (μTBS, MPa) values were obtained from resin–dentin interfaces. Distinct uppercase letters indicate differences among groups at 24 h, lowercase letters denote differences after 1 year, and asterisks (*) indicate significant differences between ageing periods within the same group (Tukey's test, α = 0.05), *from original publication I*.

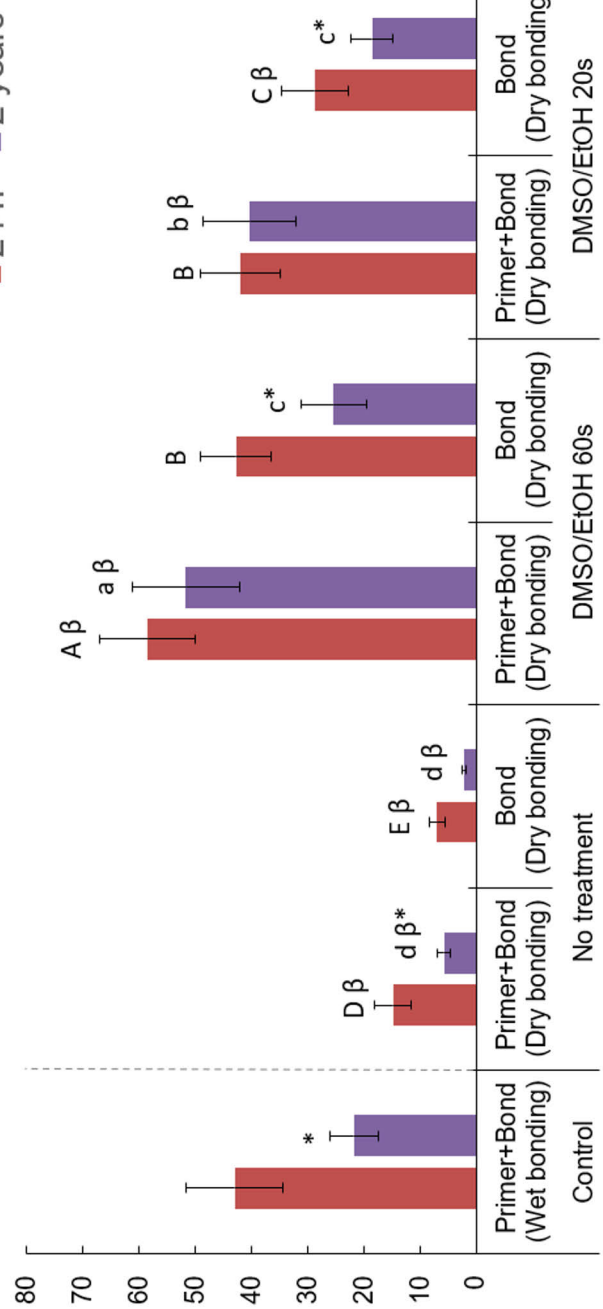
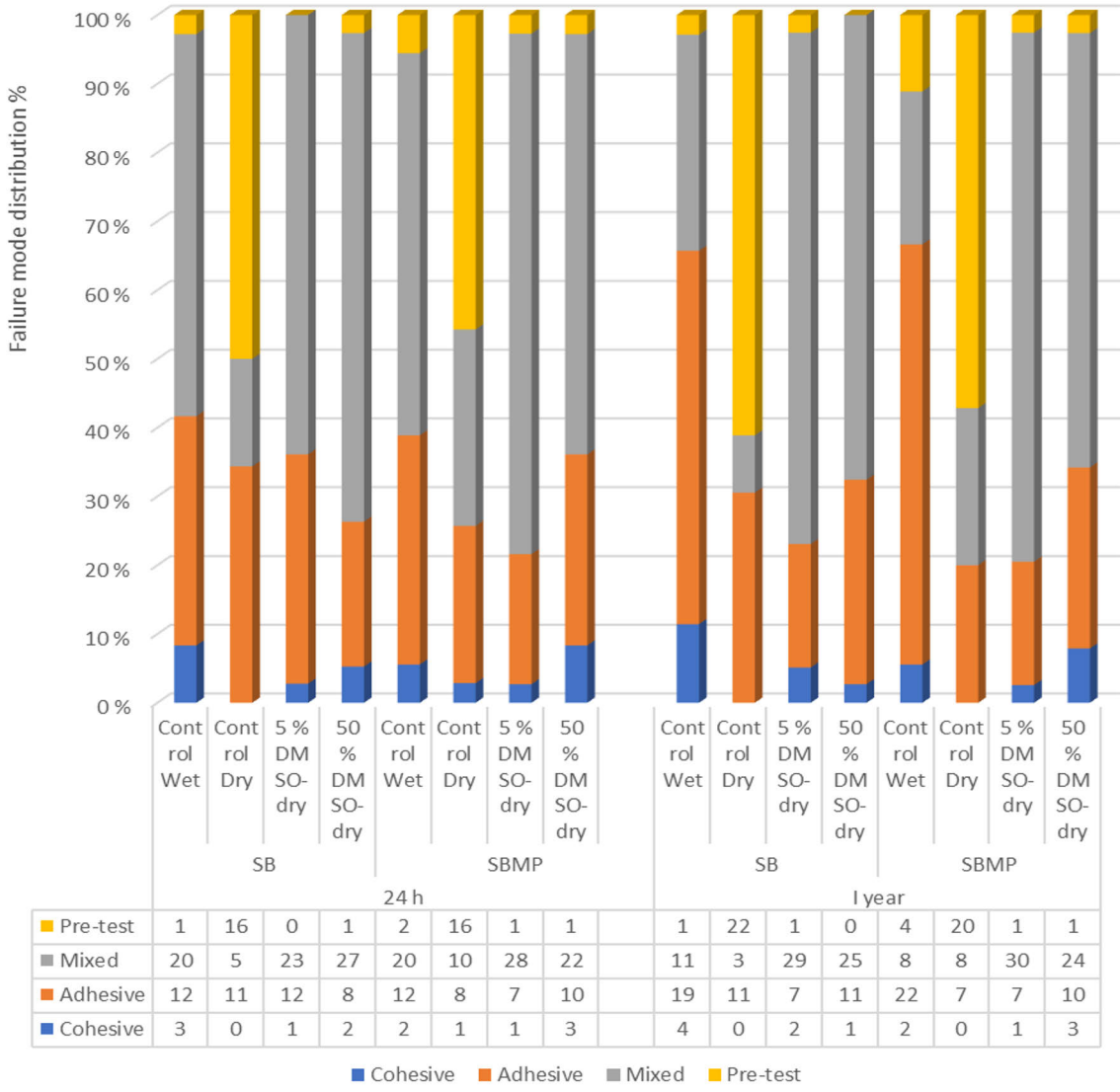
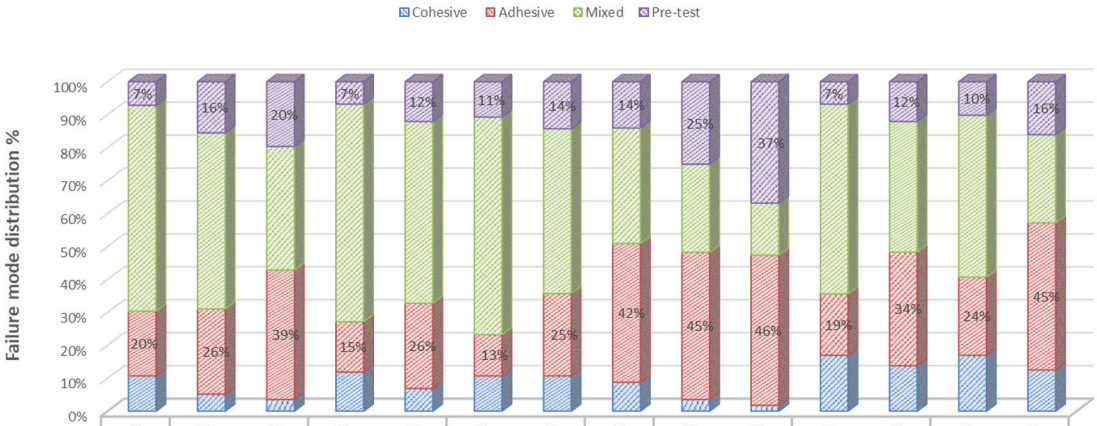


Figure 6. Mean values and standard deviation of microtensile bond strength test. Distinct uppercase letters indicate significant differences among dry-bonded groups at 24 h, lowercase letters represent differences after 2 years, and asterisks (*) denote significant variations between storage times within the same protocol (Tukey's test, $\alpha = 0.05$). The symbol (β) identifies significant differences between the control and dry-bonding groups within the same storage period (Dunnett's test, $p < 0.05$). *modified from original publication II.*



	24 h								1 year							
	SB				SBMP				SB				SBMP			
	Control Wet	Control Dry	5 % DMSO-dry	50 % DMSO-dry	Control Wet	Control Dry	5 % DMSO-dry	50 % DMSO-dry	Control Wet	Control Dry	5 % DMSO-dry	50 % DMSO-dry	Control Wet	Control Dry	5 % DMSO-dry	50 % DMSO-dry
Pre-test	1	16	0	1	2	16	1	1	1	22	1	0	4	20	1	1
Mixed	20	5	23	27	20	10	28	22	11	3	29	25	8	8	30	24
Adhesive	12	11	12	8	12	8	7	10	19	11	7	11	22	7	7	10
Cohesive	3	0	1	2	2	1	1	3	4	0	2	1	2	0	1	3

Figure 7. Fracture patterns, expressed as percentages (%), were recorded for tested specimens after microtensile bond strength evaluation at 24 h and after 1 year of storage in artificial saliva at 37 °C. Failure modes were categorized as follows: **cohesive failure**, occurring exclusively within dentin or resin composite; **adhesive failure**, located at the resin–dentin interface; and **mixed failure**, involving both interfacial failure and cohesive failure within the adjacent substrates, *modified from original publication I*.



	Control (Wet)		No pretreatment (Dry bonding)		DMSO/EtOH 60s (Dry bonding)		DMSO/EtOH 20s (Dry bonding)		Control (Wet)	No treatment (Dry bonding)		DMSO/EtOH 60s (Dry bonding)		DMSO/EtOH 20s (Dry bonding)	
	Primer+Bond		Primer+Bond	Bond	Primer+Bond	Bond	Primer+Bond	Bond	Primer+Bond	Primer+Bond	Bond	Primer+Bond	Bond	Primer+Bond	Bond
	24 h								2 years						
Pre-test	7%		16%	20%	7%	12%	11%	14%	14%	25%	37%	7%	12%	10%	16%
Mixed	63%		53%	38%	66%	55%	66%	50%	35%	27%	16%	58%	40%	49%	27%
Adhesive	20%		26%	39%	15%	26%	13%	25%	42%	45%	46%	19%	34%	24%	45%
Cohesive	11%		5%	4%	12%	7%	11%	11%	9%	4%	2%	17%	14%	17%	13%

Figure 8. Fracture patterns, expressed as percentages (%), of resin–dentin beams evaluated at 24 h and after 2 years. Failure modes were classified as follows: cohesive failure, occurring exclusively within dentin or resin composite; adhesive failure, located at the resin–dentin interface; and mixed failure, involving both interfacial failure and cohesive failure within adjacent substrates, *modified from original publication II*.

Ageing affected the experimental groups differently. In *Study I*, wet bonding with the two-step adhesive (SB) showed ~46% reduction in bond strength after one year, whereas DMSO-dry groups-maintained bond strengths without significant decreases, irrespective of DMSO concentration. For the three-step adhesive (SBMP), ageing had no effect under wet bonding, and the 50% DMSO-dry protocol maintained significantly higher values than wet bonding, by ~50% ($p < 0.05$).

In *Study II*, wet bonding and untreated dry groups showed significant bond strength loss after storage (50–70% lower, $p < 0.05$), whereas Primer+Bond DMSO-dry groups maintained stable μTBS values over time, regardless of application time. DMSO-dry groups bonded with the neat hydrophobic-rich resin (Bond) showed lower bond strengths after ageing but remained statistically comparable to wet bonding.

Fracture analysis revealed predominantly mixed failures at 24 h across most groups, except for untreated dry groups, which showed higher number of adhesive and pre-test failures. After ageing, wet-bonded samples shifted towards predominantly adhesive failures, while DMSO-dry bonded groups largely preserved

mixed fracture patterns with minimal pre-test failures (**Figure 7**, *Study I*) and (**Figure 8**, *Study II*). Across both studies, DMSO pretreatment whether at low or high concentration (*Study I*) or with prolonged application time (S*tudy II*) significantly improved immediate and long-term μTBS under dry bonding conditions, preventing the sharp reductions observed with conventional wet or untreated dry protocols.

5.2 Interfacial Nanoleakage Evaluation (*Study I & II*)

Representative backscattered SEM micrographs showing silver uptake are presented in (**Figure 9, *Study I*** and **Figure 10, *Study II***). Silver deposits were observed across hybrid layers in all bonding protocols.

In *Study I*, the untreated dry-bonding control demonstrated the greatest nanoleakage, with hybrid layers heavily infiltrated by reticular silver deposits that extended through water-filled porous zones. The conventional wet-bonding control exhibited substantially less silver uptake than dry bonding. Among the wet-bonded groups, the two-step adhesive (SB) showed higher nanoleakage than the three-step system (SBMP). In both adhesives, the predominant pattern consisted of reticular deposits located at the base of the hybrid layer, occasionally accompanied by spotted deposits. In contrast, specimens pretreated with 5% or 50% DMSO under dry conditions showed markedly reduced nanoleakage, with hybrid layers characterized by scattered spotted deposits. No significant differences were observed between the two DMSO concentrations.

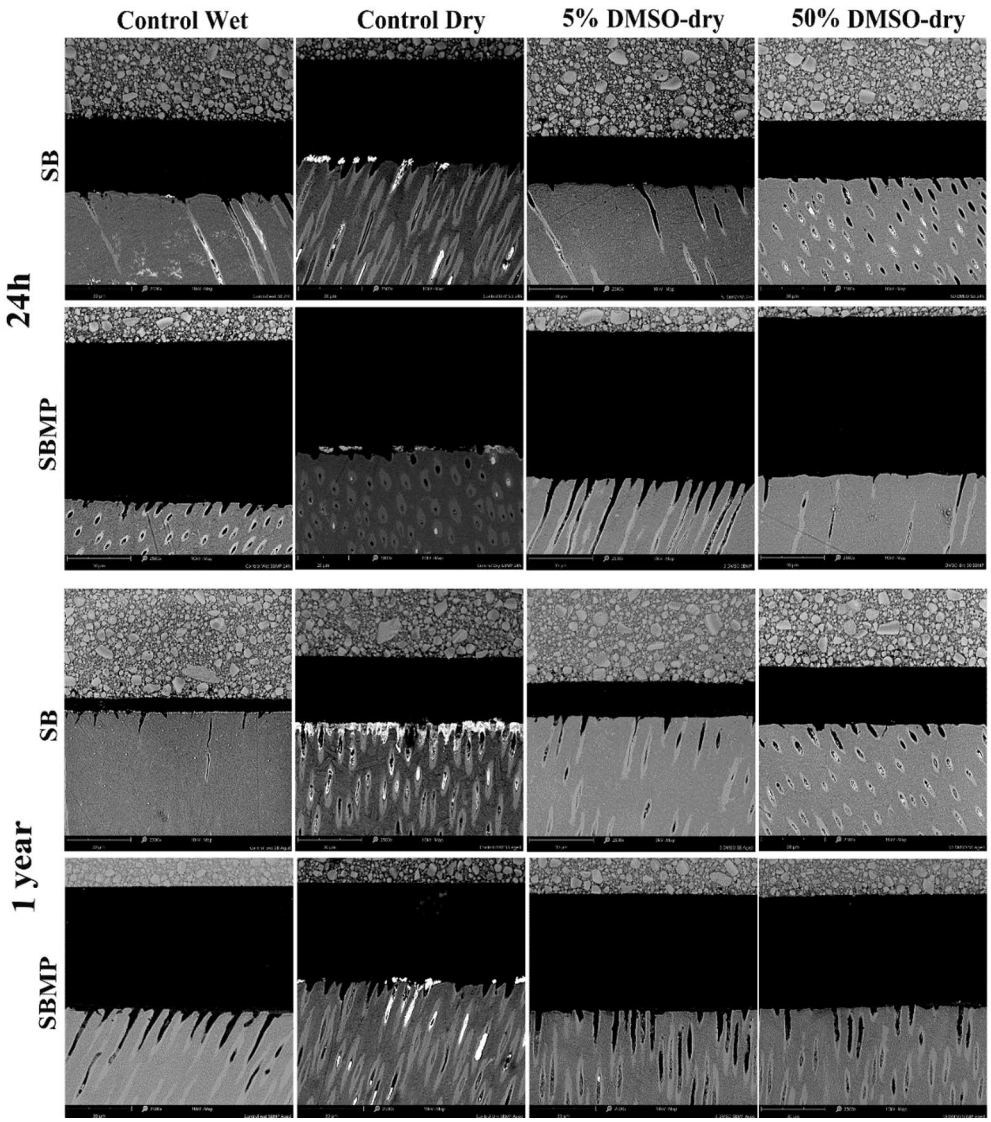


Figure 9. Representative SEM micrographs illustrating nanoleakage patterns in a scale (2500x). Specimens were evaluated after 24 h or following 1 year of ageing in artificial saliva at 37 °C, *from original publication I*.

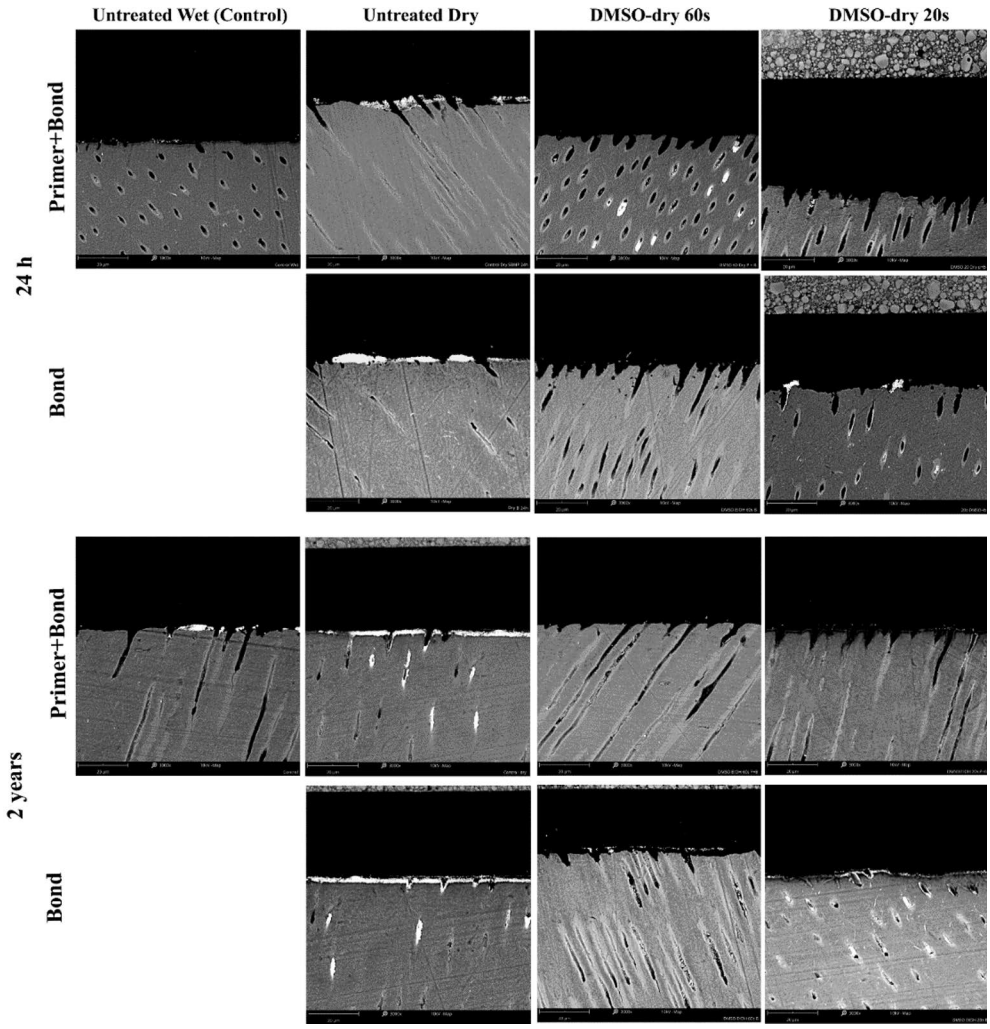


Figure 10. Representative SEM micrographs illustrating nanoleakage patterns in resin-dentin interfaces in a scale (3000x). Nanoleakage evaluation was conducted after 24 h or following 2 years of storage in artificial saliva at 37 °C, *from original publication II*.

In *Study II*, untreated dry bonding also resulted in heavy reticular silver deposits, with the neat hydrophobic-rich resin (Bond) producing the most extensive silver uptake. Bonding dry dentin with the solvated hydrophilic-rich + neat hydrophobic-rich resins (Primer+Bond) showed slightly reduced, but still substantial, reticular deposits. DMSO pretreatments (20 s and 60 s) reduced nanoleakage within hybrid layers for both resins, producing predominantly spotted deposits. For Primer+Bond, nanoleakage levels in both DMSO-dry groups were lower than those of the wet-bonding control. For Bond, the DMSO-dry 60 s group showed spotted deposits with

localized reticular formations, while the DMSO-dry 20 s group presented higher nanoleakage with extensive reticular deposits across hybrid layers.

After aging, both studies demonstrated an increase in nanoleakage for all groups. In *Study I*, the hierarchy was: Control Dry > Control Wet > 5% DMSO-dry = 50% DMSO-dry, irrespective of adhesive type. In *Study II*, the ranking of nanoleakage severity was: Untreated Dry Bond > Untreated Dry Primer+Bond > DMSO-dry 20 s Bond > DMSO-dry 60 s Bond > Wet Primer+Bond (Control) > DMSO-dry 20 s Primer+Bond > DMSO-dry 60 s Primer+Bond. In untreated groups, nanoleakage appeared as continuous reticular patterns spanning the full hybrid layer. DMSO-pretreated samples maintained predominantly spotted silver deposits, especially when bonded with Primer+Bond, irrespective of application time. For Bond, the 60 s pretreatment produced mostly spotted deposits with some localized reticular zones, whereas the 20 s pretreatment resulted in more extensive reticular infiltration.

To sum up, across both studies, untreated dry dentin consistently produced the highest nanoleakage, whereas DMSO pretreatments regardless of concentration (*Study I*) or application time (*Study II*) significantly reduced silver uptake, yielding hybrid layers characterized primarily by spotted rather than reticular nanoleakage patterns.

5.3 Hybrid Layer Characterization (Study I and II)

In *Study I*, hybrid layers with thicknesses ranging from 1.1 to 3.58 μm were identified across all experimental groups (**Figure 11**, **Table 3**, *Study I*). Two-way ANOVA demonstrated that "bonding resin" had no significant effect on thickness, while "bonding protocol" was highly influential ($p < 0.001$; $\eta^2 = 0.922$). The lowest values were obtained with dry bonding (Control Dry), whereas wet bonding produced layers approximately three times thicker ($p < 0.05$). Both 5% and 50% DMSO-dry protocols achieved hybrid layer thicknesses comparable to wet bonding ($p > 0.05$), independent of adhesive type. Morphological analysis showed that dry bonding created thin, irregular interfaces with short resin tags (8–21 μm). In contrast, wet bonding and DMSO-dry pretreatment yielded more continuous hybrid layers with well-distributed resin tags extending 16–38 μm into dentinal tubules. Silica-filler agglomerates were observed across the adhesive layer of the two-step system (SB), and this feature was not altered by DMSO application.

In *Study II*, hybrid layer thickness varied between 0.78 and 3.97 μm (**Figure 12**, **Table 4**, *Study II*). Significant effects were detected for "bonding resin composition" ($p < 0.001$; $\eta^2 = 0.63$), "DMSO pretreatment" ($p < 0.001$; $\eta^2 = 0.91$), and their interaction ($p < 0.001$; $\eta^2 = 0.46$). Untreated dry dentin produced a 50–70% reduction in thickness compared to wet bonding ($p < 0.05$), accompanied by smaller resin tag diameter and extension, particularly when using the neat hydrophobic-rich resin

(Bond). DMSO pretreatment under dry conditions significantly improved hybrid layer formation. For Primer+Bond, both 20 s and 60 s pretreatments increased thickness relative to untreated dry bonding ($p < 0.05$), reaching levels comparable to wet bonding ($p > 0.05$). For Bond, hybrid layer development was time-dependent: 60 s pretreatment restored thickness to wet-bonding values, while 20 s resulted in approximately 50% thinner layers ($p < 0.05$).

Both studies confirmed that untreated dry bonding compromised hybrid layer formation, producing thin and irregular resin tags. Wet bonding and DMSO pretreatments, however, exhibited thicker, more continuous hybrid layers. In *Study I*, the effect of DMSO was concentration-independent, while in *Study II*, prolonged application time (60 s) was essential to optimize hybrid layer formation, particularly when using hydrophobic-rich adhesives.

Table 3. Hybrid layer thickness (μm) and standard deviations, *from original publication I*.

Adhesive	***Control Wet***	***Control Dry***	***5% DMSO-dry***	***50% DMSO-dry***
Simplified E&R (SB)	3.23 ^{Aa} (±0.38)	1.02 ^{Ba} (±0.11)	3.06 ^{Aa} (±0.34)	3.27 ^{Aa} (±0.32)
Multistep E&R (SBMP)	3.58 ^{Aa} (±0.45)	1.10 ^{Ba} (±0.13)	3.14 ^{Aa} (±0.3)	3.39 ^{Aa} (±0.32)

Different uppercase letters denote statistically significant differences among the groups within the same row. Different lowercase letters indicate significant differences between the adhesive systems within each column. All pairwise comparisons were carried out using Tukey's test at a significance level of $\alpha = 0.05$.

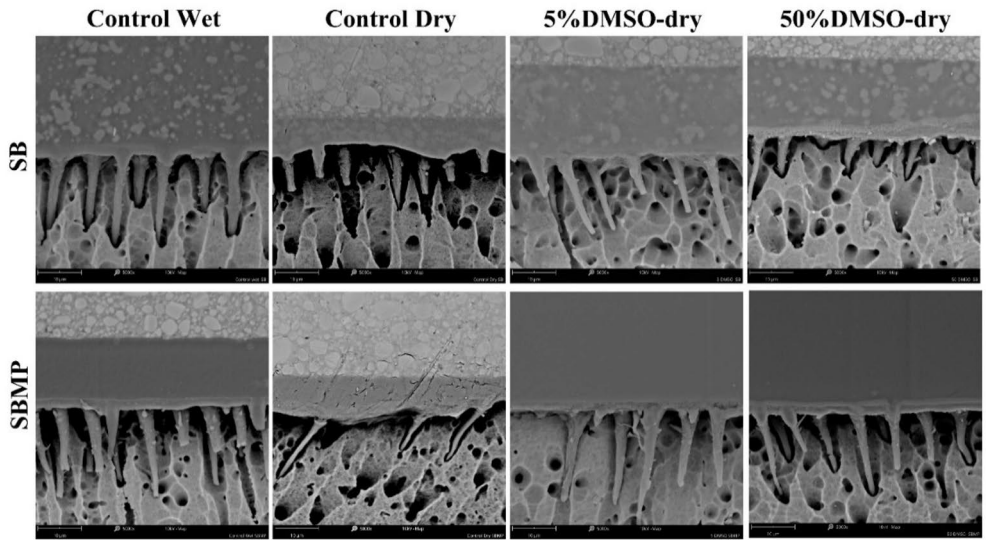


Figure 11. Representative SEM micrographs depicting hybrid layers at resin-dentin interfaces bonded to air-dried dentin pretreated with 5% or 50% DMSO/EtOH solutions in a scale (5000x). Bonding was achieved using either a two-step (SB) or a three-step (SBMP) etch-and-rinse adhesive, *from original publication I*.

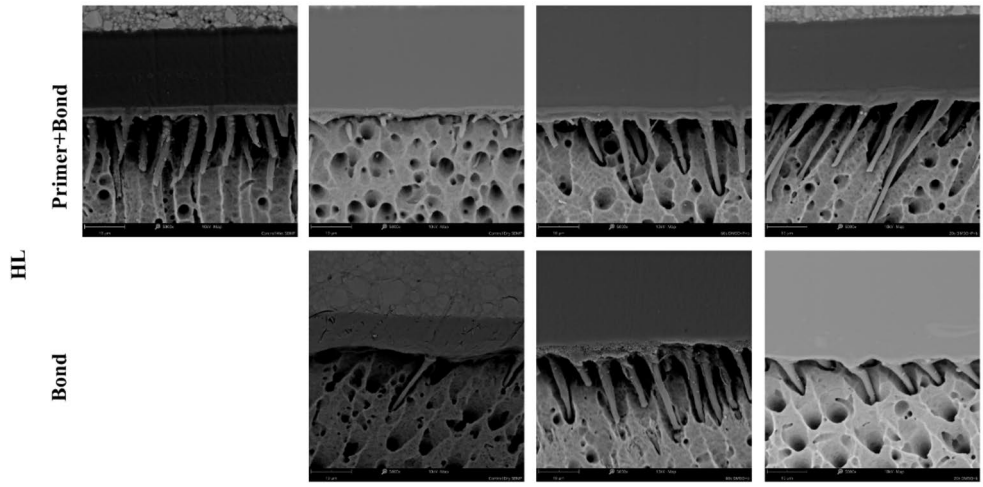


Figure 12. Representative SEM micrographs depicting hybrid layers in a scale (5000x) formed by (Primer + Bond) or (only Bond) from a three-step etch-and-rinse adhesive (Scotchbond Multi-Purpose, 3M ESPE). Dentin was pretreated with 50% DMSO/ethanol solutions (v/v) for 60 or 20 s under dry conditions, *from original publication II*.

Table 4. Hybrid layer thickness (μm), *from original publication II*.

	No pretreatment (Wet bonding)	No pretreatment (Dry bonding)	DMSO/EtOH 60s (Dry bonding)	DMSO/EtOH 20s (Dry bonding)
Primer+Bond	3.25 ±0.37	1.2 $^{Ba\,\beta}$ ±0.14	3.37 ^{Aa} ±0.36	3.00 ^{Aa} ±0.44
Bond	-	0.83 $^{Cb\,\beta}$ ±0.12	3.04 ^{Aa} ±0.41	1.53 $^{Bb\,\beta}$ ±0.24

Uppercase letters identify statistically significant differences among the dentin pretreatment conditions within each row, whereas lowercase letters mark significant differences between the adhesive systems within the corresponding columns. Pairwise comparisons were conducted using Tukey's test ($\alpha = 0.05$). The symbol β denotes groups that differ significantly from the isolated control (No-pretreatment wet-bonding) based on Dunnett's test ($p < 0.05$).

5.4 Clinical effect of DMSO on Non-Carious Cervical Lesions (Study III)

Twenty-nine patients presenting with eighty-two non-carious cervical lesions were enrolled and randomly allocated to either the intervention or control group (41 restorations per group). At the 36-month recall, two participants accounting for four restorations were lost, resulting in an overall follow-up rate of 95%. The mean age of the study population was 30 ± 7.6 years, with no significant age differences between the groups ($p = 0.438$). Gender distribution and the distribution of treated teeth were also comparable between groups ($p > 0.5$) (Ismail et al., 2025). (**Table 5**).

At baseline, both groups showed excellent ratings across all evaluated outcomes. At 12 months, performance remained high, with only one restoration in each group rated as poor for fracture and retention. At 24 and 36 months, deterioration was observed predominantly in the control group, with five and six restorations rated as poor, respectively, compared with three in the DMSO group. For caries adjacent to the restoration and postoperative hypersensitivity, both groups maintained excellent ratings throughout the follow-up, with no significant intergroup differences (**Table 6**).

Marginal adaptation scores remained favorable in both groups, with the DMSO group maintaining a higher proportion of excellent ratings at 36 months (34 excellent, 2 satisfactory) compared with the control group (30 excellent, 1 good, 2 unsatisfactory), although the differences were not statistically significant ($p = 0.57$). In contrast, marginal staining revealed significant differences after 24 and 36 months. At the final recall, the DMSO group presented 31 restorations scored "excellent," two "good," two "satisfactory," and one "unsatisfactory," while the control group showed 22 "excellent," four "satisfactory," six "unsatisfactory," and one "poor" (**Figure 13**), with the difference statistically significant ($p = 0.0126$).

Table 5. Demographic Data gender and teeth distribution, *from original publication III*.

Group	Gender		Row total (RT)
	Male	**Female**	
Control	8 50% CT 61.5% RT	5 38.5% CT 38.5% RT	13 (44.8%)
DSMO	8 50% CT 50% RT	8 61.5% CT 50% RT	16 (55.2%)
Column total (CT)	16 (55.2%)	13 (44.8%)	29
P value	P = 0.5415		
Group	**Teeth**		**Row total (RT)**
	Control	**DSMO**	
Maxillary anterior teeth	18 43.9% CT 47.7% RT	20 48.7% CT 52.6% RT	38 (46.3%)
Maxillary premolars	8 19.5% CT 47.1% RT	9 22% CT 52.9% RT	17 (20.7%)
Mandibular anterior teeth	5 12.2% CT 55.6% RT	4 9.8% CT 44.4% RT	9 (11%)
Mandibular premolars	10 24.4% CT 55.6% RT	8 19.5% CT 44.4% RT	18 (22%)
Column total (CT)	41 (50.0%)	41 (50.0%)	82
P value	P = 0.9195		

Table 6. Overall primary, secondary and tertiary outcome result scores at Baseline and after 12, 24, 36 months, *from original publication III*.

Outcome	Follow-up	Control No	Control Success 1	Control Success 2	Control Success 3	Control Failure 4	Control Failure 5	DSMO No	DSMO Success 1	DSMO Success 2	DSMO Success 3	DSMO Failure 4	DSMO Failure 5	P value
Fracture and Retention	Baseline	41	41 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	41	41 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000
	12 months	41	40 (97.6%)	0 (0%)	0 (0%)	0 (0%)	1 (2.4%)	41	40 (97.6%)	0 (0%)	0 (0%)	0 (0%)	1 (2.4%)	1.0000
	24 months	39	33 (84.6%)	1 (2.6%)	0 (0%)	0 (0%)	5 (12.8%)	39	35 (89.7%)	1 (2.6%)	0 (0%)	0 (0%)	3 (7.7%)	0.7562
	36 months	39	32 (82%)	1 (2.6%)	0 (0%)	0 (0%)	6 (15.4%)	39	35 (89.7%)	1 (2.6%)	0 (0%)	0 (0%)	3 (7.7%)	0.5671
	P value				P < 0.001*						P = 0.004*			
Marginal Adaptation	Baseline	41	41 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	41	41 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000
	12 months	40	38 (95%)	2 (5%)	0 (0%)	0 (0%)	0 (0%)	40	37 (92.5%)	3 (7.5%)	0 (0%)	0 (0%)	0 (0%)	0.6462
	24 months	34	31 (91.2%)	1 (2.9%)	2 (5.9%)	0 (0%)	0 (0%)	36	34 (94.4%)	0 (0%)	2 (5.6%)	0 (0%)	0 (0%)	0.5821
	36 months	33	30 (91%)	1 (3%)	2 (6%)	0 (0%)	0 (0%)	36	34 (94.4%)	0 (0%)	2 (5.6%)	0 (0%)	0 (0%)	0.5707
	P value				P <0.001*						P = 0.004*			
Marginal Staining	Baseline	41	41 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	41	41 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000
	12 months	40	38 (95%)	2 (5%)	0 (0%)	0 (0%)	0 (0%)	40	40 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.1547
	24 months	34	28 (82.4%)	0 (0%)	5 (14.7%)	1 (2.9%)	0 (0%)	36	34 (94.4%)	2 (5.6%)	0 (0%)	0 (0%)	0 (0%)	0.0301*
	36 months	33	22 (66.7%)	0 (0%)	4 (12.1%)	6 (18.2%)	1 (3%)	36	31 (86.2%)	2 (5.5%)	2 (5.5%)	1 (2.8%)	0 (0%)	0.0126*
	P value				P <0.001*						P = 0.003*			
Postoperative Hypersensitivity	Baseline	41	39 (95.1%)	2 (4.9%)	0 (0%)	0 (0%)	0 (0%)	41	38 (92.7%)	3 (7.3%)	0 (0%)	0 (0%)	0 (0%)	0.6465
	12 months	40	39 (97.5%)	0 (0%)	1 (2.5%)	0 (0%)	0 (0%)	40	40 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.3173
	24 months	34	34 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36	36 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.8111
	36 months	33	33 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36	36 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.7180
	P value				P <0.001*						P = 0.004*			
Caries adjacent to the restoration	Baseline	41	41 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	41	41 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000
	12 months	40	40 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	40	40 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000
	24 months	34	34 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36	36 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.8111
	36 months	33	33 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36	36 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.7180
	P value				P <0.001*						P = 0.004*			

*Frequencies and percentages not sharing the same letter throughout follow-up are considered statistically significant, * denotes statistically significant*

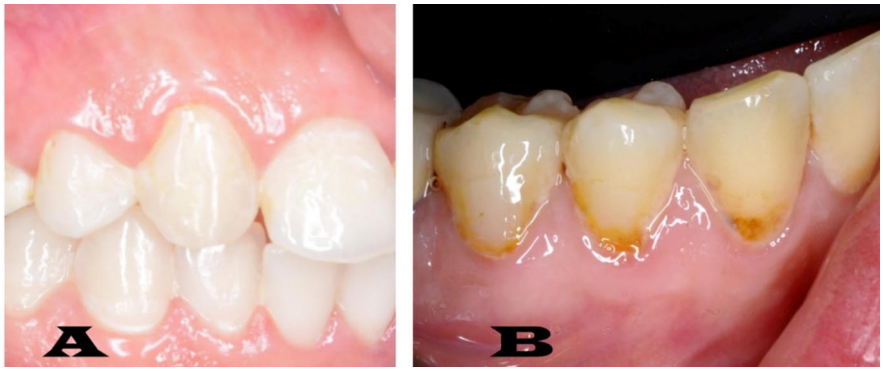


Figure 13. (A) Representative image of tooth #13 showed NCCL restoration after 36-month follow-up with excellent scores across all FDI criteria. (B) Representative image of NCCL restorations rated as unsatisfactory for marginal staining after 36 months*, from original publication III.*

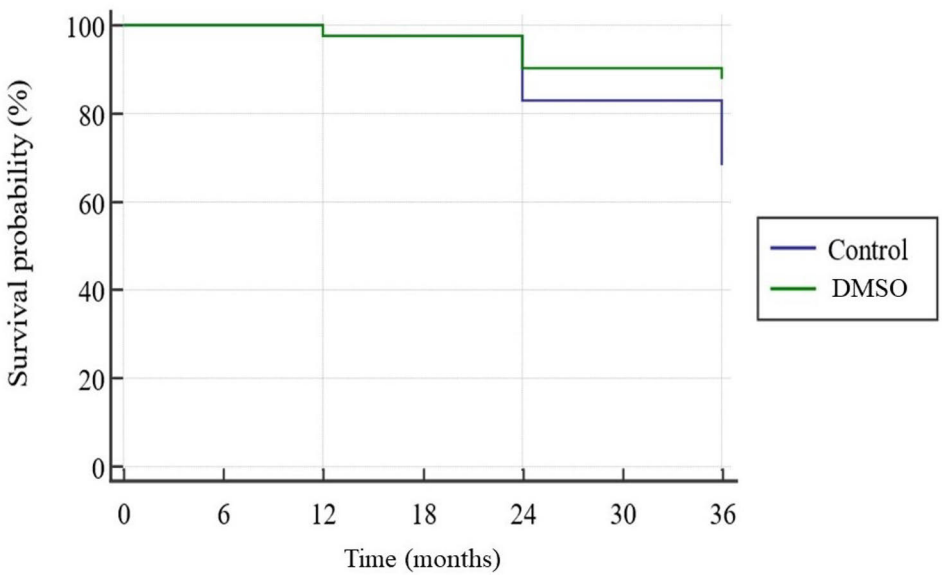


Figure 14. Survival analysis of both adhesives for NCCLs restorations after 36 months*, from original publication III.*

Intergroup comparisons across biological, functional, and esthetic domains showed no significant differences at baseline, 12, and 24 months, except for marginal staining at 36 months, where DMSO demonstrated superior performance ($p = 0.0131$). Intragroup analysis revealed significant deterioration over time for the control group ($p < 0.001$), while the DMSO group also exhibited changes, though to a lesser extent ($p < 0.0083$). Logistic regression showed a patient effect at 12 months ($p = 0.0123$), but not thereafter.

Kaplan–Meier analysis (**Figure 14**) demonstrated survival rates of 87.8% for the DMSO group and 68.3% for the control group after 36 months. Failures were recorded in 13 restorations in the control group and 5 restorations in the DMSO group, defined by FDI scores of 4 or 5. The log-rank test confirmed a statistically significant difference between groups ($p = 0.0408$). The relative risk of failure in the DMSO group was reduced by 61.5% compared with the control (RR = 0.38; 95% CI: 0.1508–0.9809; $p = 0.0454$). After 36 months, DMSO pretreatment in NCCLs significantly improved marginal staining and overall restoration survival, with nearly 20% higher success rates and a markedly reduced risk of failure compared to conventional bonding.

5.5 Clinical effect of DMSO on Carious Cervical Lesions (Study IV)

Forty-five patients with seventy-four carious cervical lesions were recruited and randomly assigned to the control and intervention groups (n=37 restorations per group). All patients attended the follow-up visits, achieving 100% recall rate at 36 months. The demographic distribution of participants is shown in **Table 7**. The mean age of patients was 28.35 ± 6.9 years, with no significant difference between groups ($p = 0.740$). Gender and tooth distribution were also balanced between groups ($p > 0.25$).

At baseline, no statistically significant differences were found between groups in any of the evaluated parameters ($p > 0.05$), except for postoperative sensitivity, where the DMSO group showed significantly fewer cases ($p < 0.05$). After 12 months, intergroup comparisons revealed no significant differences across all criteria. At 24 months, significant improvements were detected in the DMSO group compared to the control group for fracture/retention and marginal discoloration ($p < 0.05$), while other outcomes remained comparable ($p > 0.05$). After 36 months, significant differences were found again in favor of DMSO for fracture/retention ($p < 0.05$), with all other parameters showing no significant intergroup variation ($p > 0.05$).

Intragroup comparisons indicated that restorations in the control group deteriorated progressively across time for all tested outcomes ($p < 0.0083$). In contrast, the DMSO group showed stable performance, with no significant changes across the follow-up period ($p > 0.0083$). Results are summarized in **Table 8**.

Kaplan–Meier survival analysis (**Figure 15**) revealed a success rate of 89% for the DMSO group and 65% for the control group after 36 months. Failures were observed in 13 restorations of the control group and 4 restorations of the DMSO group, due to scores of 4 or 5 in the FDI criteria. The log-rank test indicated a statistically significant difference in survival between groups ($p = 0.0098$). Risk

analysis showed that DMSO pretreatment reduced the risk of failure by approximately 70% compared with the control (RR = 0.30; 95% CI: 0.11–0.86; p = 0.0240).

To conclude, over 36 months, DMSO pretreatment in carious cervical lesions significantly improved restoration survival and reduced failure risk compared with conventional bonding. Performance across other FDI criteria remained stable in the DMSO group, while controls deteriorated progressively over time.

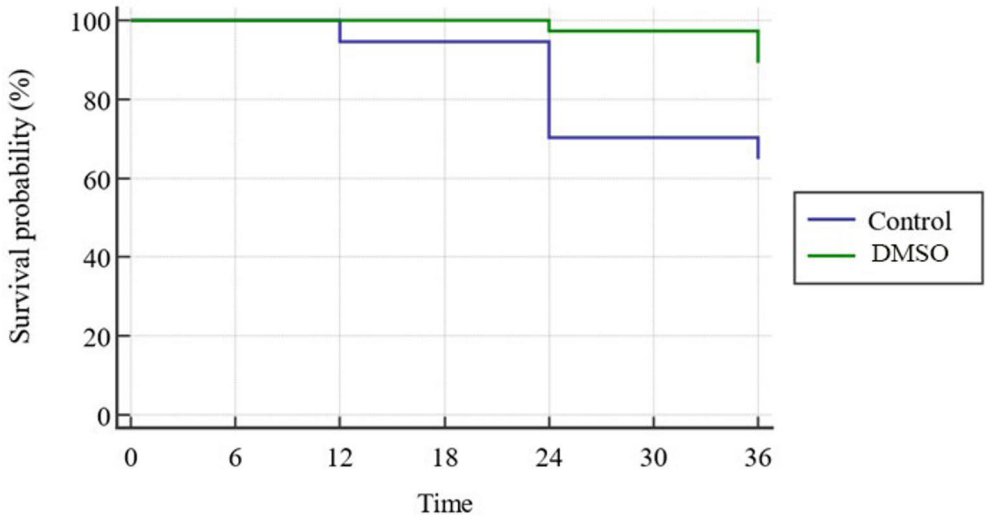


Figure 15. Survival analysis of both adhesives for CCLs restorations after 36 months, *from manuscript IV*.

Table 7. Demographic Data (gender and teeth distribution), *from manuscript IV.*

Group	Gender		Row total (RT)
	Male	**Female**	
Control	7 35.0% RT 35.0% CT	13 65.0% RT 52.0% CT	20 (44.4%)
DSMO	13 52.0% RT 65.0% CT	12 48.0% RT 48.0% CT	25 (55.6%)
Column total (CT)	20 (44.4%)	25 (55.6%)	45
P value	P = 0.2595		
Group	**Teeth**		**Row total (RT)**
	Control	**DSMO**	
Maxillary anterior teeth	23 62.2% RT 53.5% CT	20 54.1% RT 46.5% CT	43 (58.1%)
Maxillary premolars	3 8.1% RT 25.0% CT	9 24.3% RT 75.0% CT	12 (16.2%)
Mandibular anterior teeth	2 5.4% RT 50.0% CT	2 5.4% RT 50.0% CT	4 (5.4%)
Mandibular premolars	9 24.3% RT 60.0% CT	6 16.2% RT 40.0% CT	15 (20.3%)
Column total (CT)	37 (50.0%)	37 (50.0%)	74
P value	P = 0.2828		

Table 8. Frequency and percentage of scores according to FDI criteria showing intergroup comparisons between control and DSMO for all tested parameters within each follow-up and intragroup comparisons within each material between follow-up periods, *from manuscript IV:*

Outcome	Follow-up	Control						DSMO						P value
		No	Success			Failure		No	Success			Failure		
			1	2	3	4	5		1	2	3	4	5	
Fracture and Retention	Baseline	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000
	12 months	37	35 (94.6%)	0 (0%)	0 (0%)	0 (0%)	2 (5.4%)	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.1544
	24 months	37	29 (78.4%)	0 (0%)	0 (0%)	0 (0%)	8 (21.6%)	37	36 (97.3%)	0 (0%)	0 (0%)	0 (0%)	1 (2.7%)	0.0134*
	36 months	37	28 (75.7%)	0 (0%)	0 (0%)	0 (0%)	9 (24.3%)	37	35 (94.6%)	0 (0%)	0 (0%)	0 (0%)	2 (5.4%)	0.0231*
	P value				P < 0.001*						P = 0.194			
Marginal Integrity	Baseline	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000
	12 months	35	33 (94.3%)	2 (5.7%)	0 (0%)	0 (0%)	0 (0%)	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.1431
	24 months	29	26 (89.6%)	1 (3.5%)	2 (6.9%)	0 (0%)	0 (0%)	36	35 (97.2%)	0 (0%)	1 (2.8%)	0 (0%)	0 (0%)	0.2806
	36 months	28	26 (92.8%)	1 (3.6%)	1 (3.6%)	0 (0%)	0 (0%)	35	35 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.1280
	P value				P <0.001*						P = 0.194			
Marginal Discoloration	Baseline	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000
	12 months	35	33 (94.3%)	2 (5.7%)	0 (0%)	0 (0%)	0 (0%)	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.1431
	24 months	29	18 (62.1%)	0 (0%)	8 (27.6%)	3 (10.3%)	0 (0%)	36	33 (91.7%)	0 (0%)	3 (8.3%)	0 (0%)	0 (0%)	0.0027*
	36 months	28	18 (64.3%)	0 (0%)	6 (21.4%)	4 (14.3%)	0 (0%)	35	29 (82.9%)	0 (0%)	4 (11.4%)	2 (5.7%)	0 (0%)	0.0977
	P value				P <0.001*						P = 0.019			
Postoperative Hypersensitivity	Baseline	37	27 (73%)	2 (5.4%)	0 (0%)	8 (21.6%)	0 (0%)	37	34 (91.9%)	3 (8.1%)	0 (0%)	0 (0%)	0 (0%)	0.0071*
	12 months	35	35 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.8137
	24 months	29	29 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36	36 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.3853
	36 months	28	28 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	35	35 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.3778
	P value				P =0.001*						P = 0.194			
Secondary Caries	Baseline	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.0000
	12 months	35	35 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	37	37 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.8137
	24 months	29	29 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36	36 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.3853
	36 months	28	28 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	35	35 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.3778
	P value				P <0.001*						P = 0.194			

*Frequencies and percentages not sharing the same letter throughout follow-up are considered statistically significant, * denotes statistically significant*

6 Discussion

The durability of resin-dentin bond remains one of the greatest challenges to address in adhesive dentistry. While significant improvements have been made in simplifying adhesive protocols and improving initial bond strengths, clinical evidence consistently demonstrates that dentin bonds deteriorate faster than enamel bonds (Frassetto et al., 2016). The degradation of the hybrid layer, a resin-infiltrated collagen matrix, is at the center of this problem. Water-rich demineralized dentin allows infiltration of hydrophilic monomers, but these are prone to hydrolysis and enzymatic breakdown. Furthermore, incomplete resin infiltration leaves exposed collagen fibrils vulnerable to proteolysis by matrix metalloproteinases (MMPs) and cathepsins (Perdigão et al., 2013; Valdez-Montoya et al., 2024). Accordingly, hybrid layer becomes progressively porous and structurally compromised. The clinical consequence is marginal discoloration, loss of retention, secondary caries, and restoration failure (Tjäderhane, 2015).

This clinical problem has driven the search for adjunctive strategies to stabilize the hybrid layer and extend the longevity of bonded restorations. Dimethyl sulfoxide (DMSO) has emerged as one of the most promising approaches because of its unique amphiphilic properties. It is a small molecule capable of diffusing into collagen fibrils and disrupting hydrogen bonds between water and collagen. This action prevents collagen collapse during drying, increases interfibrillar spacing, and facilitates deeper resin infiltration. In addition, DMSO inhibits endogenous dentin enzymes such as MMPs and cysteine cathepsins, which are activated by acid etching (Mehtälä et al., 2017; Zhang et al., 2022). Together, these actions give DMSO a dual mechanistic advantage: improved physical infiltration of resin and reduced biological degradation of collagen. The present thesis provides a comprehensive analysis of DMSO's effects on adhesive interfaces across laboratory models and clinical trials, focusing on the influence of DMSO concentration *(Study I),* DMSO application time, hydrophobicity of the adhesive *(study II***)**, and clinical performance in non-carious *(Study III)* and carious cervical lesions *(Study IV).*

6.1 Reliability of In vitro Testing Methods

The reliability of laboratory testing methods is fundamental for interpreting the outcomes of adhesive dentistry studies. In vitro tests serve as essential predictors of clinical performance and provide standardized conditions to evaluate adhesive strategies. Among the most widely accepted and employed methods are microtensile bond strength testing, nanoleakage analysis, and hybrid layer characterization. Each of these approaches provides complementary insights into the quality, durability, and stability of resin-dentin interfaces.

Microtensile bond strength (μTBS) testing has become the benchmark method for assessing the bonding effectiveness of adhesives. This technique involves sectioning restored teeth into small beams and subjecting them to tensile stress until failure. Its reliability is attributed to the uniform stress distribution across small specimens, which minimizes variability and enhances sensitivity in detecting differences among adhesives (Armstrong et al., 2017). Compared with macroshear or macrotensile tests, μTBS allows higher sample sizes within a single tooth, enabling robust statistical analysis while reducing inter-tooth variability. Despite certain limitations, such as premature failures in weak bonds, μTBS remains the most widely endorsed method by the Academy of Dental Materials for laboratory evaluation of adhesive bonding (Armstrong et al., 2017).

While μTBS measures the mechanical strength of bonds, it cannot fully reveal the long-term chemical and morphological stability of the interface. Nanoleakage analysis complements bond strength tests by visualizing the permeability of the hybrid layer to small tracer molecules, such as silver nitrate. This method reveals nano-porosities and water channels within the adhesive interface that act as pathways for degradation (Hashimoto et al., 2015). The reliability of nanoleakage testing lies in its capacity to simulate hydrolytic challenges and provide early warnings of potential interface breakdown even before bond strength reduction becomes evident. Numerous studies have demonstrated consistent correlations between higher nanoleakage and reduced bond durability, underscoring its role as a reproducible and sensitive diagnostic method (Hashimoto et al., 2002; Hashimoto et al., 2015; Tay et al., 2002).

Hybrid layer characterization provides direct morphological evidence of the resin-dentin interface. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal microscopy are commonly used to evaluate hybrid layer thickness, continuity, and porosity. These imaging modalities are reliable because they offer structural validation of adhesive infiltration and collagen encapsulation, which are central to bond stability (Chou et al., 2025; Tay & Pashley, 2004). The reproducibility of hybrid layer imaging has been enhanced by advances in digital image processing and biomimetic models, which improve interpretation and enable quantitative analysis (Hashimoto et al., 2015). By directly observing the

quality of hybridization, hybrid layer characterization acts as a confirmatory method that complements μTBS and nanoleakage, ensuring that both mechanical performance and morphological integrity are assessed.

In summary, the combined use of microtensile testing, nanoleakage evaluation, and hybrid layer characterization provides a comprehensive and reliable framework for assessing adhesive systems *(Study I & II).* Each method contributes distinct yet complementary information: μTBS quantifies mechanical strength, nanoleakage identifies permeability and vulnerability to degradation, and hybrid layer characterization confirms structural quality. Together, these methods form the backbone of evidence supporting adhesive strategies, ensuring both scientific rigor and clinical relevance.

6.2 Effect of DMSO concentration on bond durability

The influence of various DMSO concentrations on bonding performance to extensively air-dried dentin was unclear. Specifically, the first few studies, which extensively dried the collagen by 30 s heavy blow of air, were only using 50% DMSO due to its collagen-disrupting properties (Stape et al., 2018; Zimmerley et al., 2009) and enhanced wetting ability (Mehtälä et al., 2017; Stape et al., 2021b). However, *(Study I)* confirmed that even small DMSO fractions, such as 5% w/w, produced similar improvements in bond strength and durability without requiring intense re-expansion of dried collagen (Ismail et al., 2023).

Low concentration 5% DMSO provided long-term bond stability and was selected for its reduced toxicity compared to higher concentrations (Gurtovenko & Anwar, 2007). These lower DMSO fractions were diluted mostly in ethanol, not water, facilitating safe and practical application. In contrast to ethanol-wet bonding, which requires complex and sensitive handling steps (Pashley et al., 2007; Sadek et al., 2010), DMSO-dry bonding did not require collagen to be ethanol-saturated beforehand, yet still maintained resin-dentin integrity under dry conditions (Stape et al., 2021a). This flexibility provides a simplified alternative that accommodates clinical challenges in moisture control, broadening the utility of dry bonding strategies (Stape et al., 2021b).

Moreover, findings showed no significant differences in bond strength between low and high DMSO concentrations. Air-drying protocols with either concentration didn't impair resin infiltration. The improvement in bonding with low DMSO content could be attributed to its capability to modify ethanol-collagen interactions, ensuring adequate resin hybridization. These results diverge from conventional ethanol-wet bonding approaches, which necessitate meticulous moisture regulation

to prevent collagen collapse (Pashley et al., 2007). Even so, low DMSO pretreatments enhanced collagen wetting and bonding interface quality.

While water-free adhesives might further reduce moisture in hybrid layers, two water-containing adhesives were used in this study: a simplified system (SB) with <10% water and a multistep system (SBMP) with >40%. Due to water's high hydrogen bonding potential (δh 37.3), it remains crucial for re-expanding collapsed collagen (Pashley et al., 2007). However, aqueous DMSO solutions even at 50% only moderately restore collagen integrity (Mehtälä et al., 2017). Thus, pretreatments with lower DMSO content and reduced δh may have limited re-expansion potential, reinforcing the need to complement with water-based adhesives (Stape et al., 2021b).

Still, DMSO plays a key role in collagen re-expansion by reducing water's self-association (Vishnyakov et al., 2001), improving monomer infiltration (Stape et al., 2015), and enhancing resin diffusion within collagen matrices. SEM results confirmed that hybrid layer formation remained adequate despite the lower DMSO content. No major differences in hybrid layer thickness or bond strength were noted between wet and DMSO-dry bonding groups, demonstrating the efficiency of the tested protocols.

In terms of bond durability, the simplified adhesive (SB) exhibited more bond degradation after aging, while SBMP showed superior long-term performance. This aligns with earlier findings that multistep adhesives containing hydrophobic resin layers offer better resistance to hydrolytic degradation (De Munck et al., 2005; Pashley et al., 2011; Van Meerbeek et al., 2010; 2020). These adhesives form highly crosslinked polymer networks (Chiaraputt et al., 2008) with lower water solubility (Ito et al., 2005; Malacarne et al., 2006), preserving hybrid layer stability.

Interestingly, the DMSO-dry bonding technique equalized bond durability between simplified and multistep adhesives. Under conventional wet-bonding conditions, multistep systems generally demonstrate superior durability compared with simplified adhesives (Ismail et al., 2023; Van Meerbeek et al., 2020), due to the presence of a separate hydrophobic bonding resin layer that reduces interfacial permeability and water sorption. In contrast, simplified adhesives are typically more hydrophilic and solvent-dependent, rendering them more susceptible to residual water entrapment, phase separation, and subsequent hydrolytic degradation (Göstemeyer & Schwendicke, 2016; Van Meerbeek et al., 2020).

The use of DMSO-dry bonding may attenuate these formulation-related differences by modifying the dentin substrate rather than relying solely on adhesive composition. DMSO disrupts hydrogen bonding within the collagen matrix and interferes with water self-association, thereby maintaining interfibrillar spacing even after air-drying (Stape et al., 2016; Zabeu et al., 2023). This facilitates more uniform monomer diffusion into demineralized dentin and reduces the formation of water-rich domains within the hybrid layer (Stape et al., 2018). Consequently, simplified

adhesives may achieve a degree of infiltration and polymer network homogeneity comparable to that of multistep systems under optimized substrate conditions (Ismail et al., 2023).

Moreover, DMSO pretreatment reduces residual water entrapment at the resin-dentin interface, thereby decreasing nanoleakage and limiting hydrolysis of ester bonds within the adhesive polymer (Stape et al., 2025). Given that the long-term vulnerability of simplified adhesives is largely attributed to their higher hydrophilicity and permeability, minimizing interfacial water content may substantially mitigate this disadvantage (Breschi et al., 2025). In addition, the reported inhibitory effect of DMSO on matrix metalloproteinases may contribute to preservation of exposed collagen fibrils at the base of the hybrid layer, further reducing enzymatic degradation over time (Zhang et al., 2022).

Nanoleakage analysis confirmed improved hybrid layer integrity in DMSO-pretreated groups. Fewer silver deposits were seen in aged DMSO-dry bonded samples compared to untreated groups, indicating reduced nanoporosity and water uptake. This was particularly evident in the simplified adhesive, where DMSO substantially preserved resin-dentin quality over time *(Study I).*

The protective mechanisms offered by DMSO such as inhibition of enzymatic activity (Stape et al., 2018), enhancement of monomer conversion (Stape et al., 2021b), and reduction of collagen-associated water (Stape et al., 2016) collectively contributed to stable adhesive interfaces. These effects were observed even at lower concentrations, which reaffirms the relevance of 5% DMSO in minimizing degradation (Ismail et al., 2023).

Collectively, these mechanisms suggest that DMSO-dry bonding standardizes the dentin substrate by optimizing its hydration state and enhancing collagen–monomer interactions. When substrate-related limitations are minimized, the inherent compositional differences between simplified and multistep adhesives exert a reduced influence on long-term bond stability. Thus, the equalization of bond durability observed in (*Study I)* likely reflects a shift from formulation-dependent performance toward substrate-conditioned interfacial stability (Ismail et al., 2023).

While multistep adhesives may benefit slightly more from higher DMSO content, *Study I* supports the practicality and effectiveness of low-concentration DMSO pretreatments for both adhesive types. Although 50% DMSO offered marginally superior bond strength, the difference was not statistically significant compared to 5%, making lower concentrations a safer and more clinically acceptable alternative and that encourage us to test low concentrations clinically 1% DMSO *(Study III),* and 10% DMSO *(Study IV).*

6.3 Effect of DMSO application time on neat hydrophobic resins

(Study II) investigated the impact of varying application times of DMSO pretreatments on bonding performance of both hydrophilic and neat hydrophobic-rich resins to etched dentin under dry conditions. The results indicated that DMSO significantly improved microtensile bond strength, reduced nanoleakage, and promoted hybrid layer stability, with stronger effects observed at longer application times.

Untreated dry bonding consistently produced poor outcomes, with highly porous hybrid layers (<1 µm) and extensive nanoleakage, particularly for the neat hydrophobic-rich resin. These interfaces degraded further after storage, confirming the incompatibility of dry dentin bonding without pretreatment (Pashley et al., 2007).

By contrast, dentin pretreatment with 50% DMSO improved adhesion across both resin systems. Application for 20 s increased bond strength, but 60 s exposure yielded thicker, more uniform hybrid layers and substantially reduced nanoleakage. Microtensile bond testing showed increases up to six-fold compared to untreated dentin. The neat hydrophobic resin was especially dependent on longer DMSO application, highlighting the time-sensitive nature of the protocol (Stape et al., 2025).

Nanoleakage analysis confirmed these results: untreated groups exhibited dense silver deposits throughout the interface, while DMSO-treated specimens especially at 60 s showed only scattered deposits similar to low-permeability bonds. For Primer+Bond resins, nanoleakage after DMSO pretreatment was even lower than conventional wet bonding (Tay et al., 2002), underscoring DMSO's capacity to stabilize hybrid layers. Neat hydrophobic resin, however, required the full 60 s to reach comparable outcomes (Stape et al., 2024).

SEM observations supported these findings. Without DMSO, hybrid layers were severely collapsed. In contrast, 20s DMSO pretreatment improved thickness but remained discontinuous in neat resin groups. 60 s exposure produced continuous hybrid layers, nearly identical to those achieved through wet bonding. This demonstrates that application time directly influences resin infiltration and collagen stability.

Mechanistically, DMSO reduced residual water, enhanced dentin wettability, and facilitated penetration of hydrophobic monomers into interfibrillar spaces (Stape et al., 2016). Longer application allowed greater collagen rearrangement and hybridization, reducing porosity and improving durability (Mehtälä et al., 2017). These results establish proof-of-concept for solvent-free hydrophobic bonding strategies, where DMSO serves as a mediator of stable resin-dentin interfaces.

Study II showed that, viscosity of neat hydrophobic resins still limited complete infiltration compared to solvated systems. While DMSO pretreatment partially compensated for this, uniformity and mechanical performance favored conventional

Primer+Bond systems. Thus, optimization of resin formulations and DMSO protocols is necessary before clinical adoption. Application time of DMSO is critical in defining resin-dentin interface quality. Short exposures improved adhesion but did not fully ensure long-term stability. Sixty-second pretreatment allowed hydrophobic resins to achieve comparable performance to traditional wet bonding, confirming the importance of sufficient application time to maximize bond strength, minimize nanoleakage, and enhance hybrid layer integrity.

6.4 Evaluation of Clinical DMSO application in Non-Caries Cervical Lesions

(*Study III*) is a randomized clinical trial evaluated the role of 1% DMSO/H_2O pretreatment in adhesive performance for non-carious cervical lesions (NCCLs). These lesions are particularly suited for testing adhesion because they lack macroretentive features and involve both enamel and dentin margins, creating higher risk of debonding and interfacial stress (De Munck et al., 2005). The adhesive chosen was Adper Single Bond 2, a two-step etch-and-rinse system. This choice was based on its established reliability and the availability of supportive laboratory data demonstrating performance improvements when combined with DMSO pretreatment (Guo et al., 2017; Salim et al., 2018; Ismail et al., 2023). Additionally, two-step systems offer procedural simplicity compared to three-step protocols, making them more clinically relevant while still sensitive to improvements via pretreatment strategies.

The design followed a parallel-arm randomized controlled trial, the gold standard in clinical research. RCTs eliminate allocation bias, ensure proper concealment, and provide reliable data for long-term outcomes (Pandis, 2011). In (*study III*), randomization was conducted using an independent online generator and concealed with sealed opaque envelopes, strengthening internal validity. Follow-ups occurred at baseline, 1 y, 2 y, and 3 y, in line with clinical evaluation guidelines (Hickel et al., 2010a). At the three-year recall, retention rates exceeded 95% across both groups, fulfilling the ADA requirement of >90% after two years. No significant differences were observed in retention, marginal adaptation, postoperative sensitivity, or secondary caries. However, marginal staining revealed significant improvements in the DMSO group compared to controls. Marginal staining is a sensitive early indicator of interfacial leakage and degradation (Marquillier et al., 2018). The reduced discoloration observed in the DMSO group aligns with its known ability to disrupt collagen-water interactions, increase wettability, and facilitate more uniform adhesive infiltration (Zhang et al., 2022). Plus, DMSO inhibits enzymatic activity of MMPs, further reducing hybrid layer breakdown (Zabeu et al., 2022) and explaining the preservation of esthetic margins in the intervention group.

Although marginal adaptation criteria showed no significant group differences, this is expected since adaptation reflects larger macro-gaps that form later, while staining captures earlier micro-gaps. Thus, the sensitivity of staining explains why significant differences emerged here but not in adaptation (Hickel et al., 2023). Regarding retention, although both groups performed similarly, the DMSO group recorded slightly higher values. Given that retention is influenced by operator skill, patient habits, and occlusal stresses (Van Meerbeek et al., 2010), these results still suggest that DMSO has a stabilizing effect on the interface without negatively impacting performance.

The findings of (*Study III*) reinforce the translational value of DMSO pretreatment. By improving interface stability and reducing marginal staining, it extends the durability and esthetics of NCCL restorations, supporting its incorporation as a simple, cost-effective step in clinical adhesive protocols. Pretreating dentin with 1% DMSO/H_2O significantly reduced marginal staining in NCCL restorations while maintaining excellent retention, adaptation, and caries resistance. Its effect in stabilizing the hybrid layer highlights the potential for DMSO to enhance clinical outcomes in adhesive dentistry.

6.5 Evaluation of Clinical DMSO application in Caries Cervical Lesions

Study IV is a clinical trial investigated the impact of 10% DMSO/H_2O pretreatment on adhesive performance in carious cervical lesions (CCLs). Unlike NCCLs, these lesions are more complex, involving demineralized dentin and higher organic content, conditions that compromise hybrid layer formation and increase the risk of adhesive degradation (Perdigão, 2010). Evaluating DMSO in this context provides further insights into its ability to stabilize bonding in clinically challenging environments.

Participants were randomly allocated into intervention and control groups with high reliability, achieving 100% retention at baseline and strong follow-up rates after 36 months. The absence of significant differences in demographic data confirmed group homogeneity, minimizing confounding factors and supporting the validity of the outcomes (Pandis, 2011). At baseline and 12 months, no significant group differences were observed across retention, marginal adaptation, and postoperative sensitivity, except that the DMSO group displayed less initial sensitivity. This immediate benefit reflects DMSO's hygroscopic and collagen-modifying properties, which improve adhesive diffusion and reduce fluid shifts through dentinal tubules (Stape et al., 2021a). From 24 to 36 months, results revealed clear divergence between groups. Restorations in the DMSO group showed superior survival, with only four failures compared to thirteen in the control group ($P = 0.0098$). Enhanced

long-term retention is consistent with DMSO's ability to inhibit matrix metalloproteinases (MMPs), suppress collagen degradation, and facilitate deeper monomer infiltration into the collagen matrix (Tjäderhane et al., 2013c; Zhang et al., 2022).

Marginal staining results further supported these effects. After 24 months, the control group exhibited significantly more discoloration compared to the intervention group (P = 0.0027). Marginal staining is an established marker of microleakage and early interface degradation (Hickel et al., 2010a; Marquillier et al., 2018). The lower discoloration in the DMSO group indicates reduced permeability and more stable hybrid layers, consistent with in vitro findings of improved nanoleakage resistance in DMSO-pretreated samples (Ismail et al., 2024). Regarding marginal adaptation, both groups performed similarly throughout the trial, showing that DMSO's primary effect was at the microscopic level (staining) rather than in larger structural adaptation gaps. This observation mirrors results in NCCL study (*Study III*), where staining was more sensitive in detecting interfacial weaknesses (Hickel et al., 2010a).

Fracture resistance and retention were significantly better in the DMSO group at 24 and 36 months ($p < 0.05$). These results suggest that DMSO enhances the structural resilience of restorations by preventing collagen collapse and supporting deeper polymerization of resin within dentin (Zabeu et al., 2023). By contrast, control restorations exhibited gradual loss of retention and greater susceptibility to failure, reflecting the natural degradation of adhesive systems over time (Perdigão et al., 2021).

Interestingly, postoperative sensitivity decreased significantly in the DMSO group at baseline, highlighting its immediate desensitizing effect. This aligns with recent findings on DMSO's hygroscopic nature, which helps control dentin wetness and minimize patient discomfort (Maghaireh et al., 2023). Overall, the clinical implications of (*Study IV*) findings are delaying marginal staining, enhancing retention, and reducing postoperative sensitivity, DMSO demonstrated superiority in maintaining restoration integrity in CCLs. These benefits are likely attributable to its multifunctional role as a solvent, collagen stabilizer, and enzymatic inhibitor. Taken together with the NCCL results (*Study III*), this thesis provides the first integrated laboratory to clinical evidence of DMSO's benefits in both sound and caries-affected dentin. Such findings align with the rationale that DMSO strengthens adhesion by simultaneously improving infiltration and reducing enzymatic degradation.

6.6 Strengths and Limitations of this Thesis

The convergence of laboratory and clinical data in this thesis highlights the translational potential of DMSO. While in vitro tests provide a controlled environment for mechanistic insights, clinical studies remain the definitive measure of restorative longevity. The evidence from NCCL and CCL trials (*Study III & IV*) demonstrates that DMSO pretreatment offers clinically measurable benefits. However, it is important to acknowledge that laboratory findings do not always translate seamlessly into clinical practice due to patient variability, operator technique, and complex oral environmental factors.

Strengths of this thesis include systematic evaluation of DMSO concentration and application time, the incorporation of hydrophobic resin systems, and execution of randomized clinical trials with multi-year follow-up. Limitations include the assessment of only selected adhesive systems, which may limit the generalizability, and a follow-up period of three years in NCCLs and CCLs. Longer observation periods and broader comparisons across adhesive systems will be necessary to fully establish DMSO's role in clinical practice.

6.7 Future Directions in DMSO-Enhanced Adhesive Dentistry

Future research should prioritize optimizing protocols for DMSO pretreatment, including ideal concentrations, application times, and compatibility with universal adhesives. Further long-term randomized trials with larger sample sizes are essential to validate clinical effectiveness. Additionally, combination strategies, such as pairing DMSO with collagen cross-linkers, cavity disinfectants or biomimetic remineralization, may offer synergistic benefits.

Despite the promising laboratory and emerging clinical evidence, several barriers may explain why DMSO has not yet been commercially integrated into mainstream adhesive systems. First, regulatory and safety considerations remain significant. Although DMSO is approved for medical use in other fields, its incorporation into dental materials would require comprehensive toxicological, cytotoxicity, and pulpal diffusion studies under dental-specific conditions. Manufacturers may be cautious due to DMSO's well-known penetration-enhancing properties, which raise theoretical concerns regarding increased pulpal transport of unpolymerized monomers.

Second, formulation stability presents a technical challenge. DMSO is highly polar and fully miscible with both hydrophilic and hydrophobic monomers, which may alter viscosity, polymerization kinetics, phase behavior, and long-term storage stability if incorporated directly into adhesive bottles. Controlling residual solvent

content and ensuring consistent evaporation in clinical settings could further complicate product development.

Third, the commercial incentive may be limited. Contemporary universal adhesives already demonstrate clinically acceptable survival rates, and manufacturers may perceive limited market demand for additional pretreatment steps that increase clinical complexity. The trend in adhesive dentistry has historically favored simplification rather than the introduction of adjunctive steps, even when evidence suggests improved durability.

7 Summary/Conclusions

Based on the series of studies described in this PhD thesis, the following conclusions were found:

1. Low-concentration of DMSO pretreatment (5% DMSO) was as effective as high concentration (50% DMSO) in preserving microtensile bond strength, reducing nanoleakage, and improving hybrid layer morphology. This indicates that low concentrations are sufficient to provide the stabilizing effects of DMSO on resin-dentin bonding, balancing efficacy with biocompatibility.

2. Longer application times (60 s) were necessary for optimal outcomes, so (20 s) was not enough for optimal effect of DMSO specially with neat hydrophobic reins.

3. The concept of DMSO-assisted dry bonding was validated. Unlike conventional protocols where collagen collapses upon air-drying, DMSO prevented collapse, allowing infiltration of hydrophobic monomers and generating interfaces with greater hydrolytic stability.

4. The clinical phase of the thesis translated these laboratory findings into practice. 1% DMSO pretreatment in non-carious cervical lesions significantly reduced marginal staining, and enhanced restoration survival compared to controls.

5. Randomized trial in cervical carious lesions confirmed that 10% DMSO application improved bonding to compromised dentin substrates by significantly improve the retention rate of the restorations, further supporting its clinical utility.

The overall conclusion, the results show that low concentrations are sufficient, longer application improves efficacy, and benefits translate into allowing infiltration of hydrophobic monomers and clinical success. The work therefore bridges the gap between mechanistic understanding and clinical implementation, positioning DMSO as one of the most promising adjunctive strategies in adhesive dentistry. By

addressing one of the central challenges of adhesive dentistry (the instability of the hybrid layer) this thesis contributes meaningful evidence toward more durable and predictable restorative outcomes.

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References

Abu-Nawareg, M., Zidan, A. z., Zhou, J., Agee, K., Chiba, A., Tagami, J., & Pashley, D. H. (2015). Adhesive sealing of dentin surfaces in vitro: A review. *American Journal of Dentistry*, *28*(6), 321–332.

Agee, K. A., Prakki, A., Abu-Haimed, T., Naguib, G. H., Nawareg, M. A., Tezvergil-Mutluay, A., Scheffel, D. L. S., Chen, C., Jang, S. S., Hwang, H., Brackett, M., Grégoire, G., Tay, F. R., Breschi, L., & Pashley, D. H. (2015). Water distribution in dentin matrices: Bound vs. unbound water. *Dental Materials*, *31*(3), 205–216. https://doi.org/10.1016/j.dental.2014.12.007

Araújo, J. F. De, Andrade, T., Barros, D. F., Marina, E., Braga, F., Loretto, S. C., Almeida, P. De, & Silva, R. (2013). One-Year Evaluation of a Simplified Ethanol-Wet Bonding Technique : A Randomized Clinical Trial. *Brazilian Dental Journal*, *24*(3), 267–272.

Armstrong, S., Breschi, L., Özcan, M., Pfefferkorn, F., Ferrari, M., & Van Meerbeek, B. (2017). Academy of Dental Materials guidance on in vitro testing of dental composite bonding effectiveness to dentin/enamel using micro-tensile bond strength (μTBS) approach. *Dental Materials*, *33*(2), 133–143. https://doi.org/10.1016/j.dental.2016.11.015

Atay, M. T., Seseogullari-Dirihan, R., Mutluay, M. M., & Tezvergil-Mutluay, A. (2022). Long-term effect of curcuminoid treatment on resin-to-dentin bond strength. *Eur J Oral Sci*, *130*(1), e12837. https://doi.org/10.1111/eos.12837

Balalaie, A., Rezvani, M. B., & Mohammadi Basir, M. (2018). Dual function of proanthocyanidins as both MMP inhibitor and crosslinker in dentin biomodification: A literature review. *Dental Materials Journal*, *37*(2), 173–182. https://doi.org/10.4012/dmj.2017-062

Banting, D., Eggertsson, H., Ekstrand, K., Ferreira, A., Longbottom, C., Reich, E., Ricketts, D., Selwitz, R., Sohn, W., & Zero, D. (2011). *International Caries Detection and Assessment System (ICDAS II) Workshop held in Baltimore, Maryland, March 12th -14th 2005*. *June*, 1–28. https://www.sdpt.net/ICDAS/pdf/ICDAS II criteria document September 10 2211.pdf

Bardsley, P. F. (2008). The evolution of tooth wear indices. *Clin Oral Invest*, *12*(1), 15–19. https://doi.org/10.1007/s00784-007-0184-2

Bayne, S., & Schmalz, G. (2006). Reprinting the classic article on USPHS evaluation methods for measuring the clinical performance of restorative materials. *Clinical Oral Investigations*, *9*, 209–214. https://doi.org/10.1007/s00784-005-0017-0

Bayraktar, N., & Harorli, O. T. (2024). Effects of different surface treatments on surface topography and bond strength in the repair of fiber-reinforced dentin composite. *Journal of Esthetic and Restorative Dentistry*, *36*(6), 930–940. https://doi.org/10.1111/jerd.13215

Breschi, L., Maravic, T., Cunha, S. R., Comba, A., Cadenaro, M., Tjäderhane, L., Pashley, D. H., Tay, F. R., & Mazzoni, A. (2018). Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. *Dental Materials*, *34*(1), 78–96. https://doi.org/10.1016/j.dental.2017.11.005

Breschi, L., Maravic, T., Mazzitelli, C., Josic, U., Mancuso, E., Cadenaro, M., Pfeifer, C. S., & Mazzoni, A. (2025). The evolution of adhesive dentistry: From etch-and-rinse to universal bonding systems. *Dental Materials*, *41*(2), 141–158. https://doi.org/10.1016/j.dental.2024.11.011

Breschi, L., Mazzoni, A., Ruggeri, A., Cadenaro, M., Di Lenarda, R., & De Stefano Dorigo, E. (2008). Dental adhesion review: Aging and stability of the bonded interface. *Dental Materials*, *24*(1), 90–101. https://doi.org/10.1016/j.dental.2007.02.009

Buonocore, M. G. (1955). A Simple Method of Increasing the Adhesion of Acrylic Filling Materials to Enamel Surfaces. *Journal of Dental Research*, *34*(6), 849–853. https://doi.org/10.1177/00220345550340060801

Cardoso, M. V., De Almeida Neves, A., Mine, A., Coutinho, E., Van Landuyt, K., De Munck, J., & Van Meerbeek, B. (2011). Current aspects on bonding effectiveness and stability in adhesive dentistry. *Australian Dental Journal*, *56*(SUPPL. 1), 31–44. https://doi.org/10.1111/j.1834-7819.2011.01294.x

Carrilho, M., Carvalho, R., de Goes, M., di Hipólito, V., Geraldeli, S., Tay, F. R., Pashley, D. H., & Tjäderhane, L. (2007). Chlorhexidine Preserves Dentin Bond in vitro. *Journal of Dental Research*, *86*(1), 90–94. https://doi.org/10.1177/154405910708600115.

Chiaraputt, S., Mai, S., Huffman, B. P., Kapur, R., Agee, K. A., Yiu, C. K. Y., Chan, D. C. N., Harnirattisai, C., Arola, D. D., Rueggeberg, F. A., Pashley, D. H., & Tay, F. R. (2008). Changes in resin-infiltrated dentin stiffness after water storage. *Journal of Dental Research*, *87*(7), 655–660. https://doi.org/10.1177/154405910808700704

Chou, Y. F., Maciel Pires, P., D'Urso, D., Ozan, G., Mazzitelli, C., Maravic, T., Sancaklı, H. Ş., Breschi, L., & Sauro, S. (2025). Effects of a biomimetic dual-analogue primer on the bonding performance of an experimental ion-releasing adhesive system – An in vitro study. *Journal of Dentistry*, *156*(March). https://doi.org/10.1016/j.jdent.2025.105712

Cvar, J. F., & Ryge, G. (1971). *Criteria for the clinical evaluation of dental restorative materials* (p. 39 p.). U.S. Dept. of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bureau of Health Manpower Education, Division of Dental Health, Dental Health Center. https://catalog.hathitrust.org/Record/000171343

De Munck, J., Van Landuyt, K., Peumans, M., Poitevin, A., Lambrechts, P., Braem, M., & Van Meerbeek, B. (2005). A critical review of the durability of adhesion to tooth tissue: Methods and results. *Journal of Dental Research*, *84*(2), 118–132. https://doi.org/10.1177/154405910508400204

De Souza, L. C., Rodrigues, N. S., Cunha, D. A., Feitosa, V. P., Santiago, S. L., Reis, A., Loguercio, A. D., Matos, T. de P., Saboia, V. de P. A., & Perdigão, J. (2019a). Two-year clinical evaluation of proanthocyanidins added to a two-step etch-and-rinse adhesive. *Journal of Dentistry*, *81*, 7–16. https://doi.org/10.1016/j.jdent.2018.12.012

De Souza, L. C., Sousa Rodrigues, N., Araújo Cunha, D., Pinheiro Feitosa, V., Lima Santiago, S., Reis, A., Dourado Loguercio, A., Perdigão, J., & de Paulo Aragão Saboia, V. (2020). Two-year clinical evaluation of a proanthocyanidins-based primer in non-carious cervical lesions: a double-blind randomized clinical trial. *Journal of Dentistry*, 103325. https://doi.org/10.1016/j.jdent.2020.103325

De Souza, M. Y., Jurema, A. L. B., Caneppele, T. M. F., & Bresciani, E. (2019b). Six-month performance of restorations produced with the ethanol-wet-bonding technique: A randomized trial. *Brazilian Oral Research*, *33*, 1–10. https://doi.org/10.1590/1807-3107BOR-2019.VOL33.0052

Donaldson, M., & Goodchild, J. H. (2019). Chlorhexidine allergy: Raising awareness about rare but potentially life-threatening reactions. *General Dentistry*, *67*(2), 7–10.

Fahy, G. M. (1986). The relevance of cryoprotectant "toxicity" to cryobiology. *Cryobiology*, *23*(1), 1–13. https://doi.org/10.1016/0011-2240(86)90013-1

Fang, M., Liu, R., Xiao, Y., Li, F., Wang, D., Hou, R., & Chen, J. (2012). Biomodification to dentin by a natural crosslinker improved the resin-dentin bonds. *Journal of Dentistry*, *40*(6), 458–466. https://doi.org/10.1016/j.jdent.2012.02.008

Favetti, M., Schroeder, T., Montagner, A. F., Correa, M. B., Pereira-Cenci, T., & Cenci, M. S. (2017). Effectiveness of pre-treatment with chlorhexidine in restoration retention: A 36-month follow-up randomized clinical trial. *Journal of Dentistry*, *60*, 44–49. https://doi.org/10.1016/j.jdent.2017.02.014

FDA. U.S. Department of Health and Human Services Food and Drug Administration. (2017). *Q3C — Tables and List Guidance for Industry: Vol. Revision 3* (Issue June).

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm%5Cnhttp://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

Fernandes Pegado, R. E., Botelho do Amaral, F. L., Flório, F. M., Basting, R. T., Stanislawczuk, R., Pereira, F., Muñoz, M. A., Luque, I., Farago, P. V., Reis, A., Loguercio, A. D., Alkattan, R., Ajaj, R., Koller, G., Banerji, S., & Deb, S. (2010). Effect of Different Bonding Strategies on Adhesion to Deep and Superficial Permanent Dentin. *European Journal of Dentistry*, *4*(02), 110–117. https://doi.org/10.1055/s-0039-1697818

Frassetto, A., Breschi, L., Turco, G., Marchesi, G., Di Lenarda, R., Tay, F. R., Pashley, D. H., & Cadenaro, M. (2016). Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability - A literature review. *Dental Materials*, *32*(2), e41–e53. https://doi.org/10.1016/j.dental.2015.11.007

Göstemeyer, G., & Schwendicke, F. (2016). Inhibition of hybrid layer degradation by cavity pretreatment: Meta- and trial sequential analysis. *Journal of Dentistry*, *49*, 14–21. https://doi.org/10.1016/j.jdent.2016.04.007

Grimes, D. A., & Schulz, K. F. (2002). Epidemiology series An overview of clinical research : the lay of the land. *The Lancet*, *359*, 57–61. https://doi.org/10.1016/S0140-6736(02)07283-5

Guo, J., Lei, W., Yang, H., Zhang, Y., Zhao, S., & Huang, C. (2017). Dimethyl Sulfoxide Wet-bonding Technique May Improve the Quality of Dentin Bonding. *The Journal of Adhesive Dentistry*, *19*(3), 229–237. https://doi.org/10.3290/j.jad.a38438

Gurtovenko, A. A., & Anwar, J. (2007). Modulating the structure and properties of cell membranes: The molecular mechanism of action of dimethyl sulfoxide. *Journal of Physical Chemistry B*, *111*(35), 10453–10460. https://doi.org/10.1021/jp073113e

Han, F., Liang, R., & Xie, H. (2021). Effects of phosphoric acid pre-etching on chemisorption between enamel and MDP-containing universal adhesives. *ACS Omega*, *6*(25), 16238–16245. https://doi.org/10.1021/acsomega.1c01016

Hardan, L., Daood, U., Bourgi, R., Enrique, C., Devoto, W., Zarow, M., Jakubowicz, N., Eliezer, J., & Radwanski, M. (2022). Effect of Collagen Crosslinkers on Dentin Bond Strength of Adhesive Systems : A Systematic Review and Meta-Analysis. *Cells*, *15*, 1–34. https://doi.org/10.3390/cells11152417

Hashimoto, M., Ohno, H., Kaga, M., Sano, H., Endo, K., & Oguchi, H. (2002). The extent to which resin can infiltrate dentin by acetone-based adhesives. *Journal of Dental Research*, *81*(1), 74–78. https://doi.org/10.1177/002203450208100116

Hashimoto, M., Yamaguchi, S., & Imazato, S. (2015). Nanoleakage and Durability of Resin/Dentin Bonds. *Current Oral Health Reports*, *2*(4), 195–201. https://doi.org/10.1007/s40496-015-0059-6

Hebling, J., Bianchi, L., Basso, F. G., Scheffel, D. L., Soares, D. G., Carrilho, M. R. O., Pashley, D. H., Tjäderhane, L., & De Souza Costa, C. A. (2015). Cytotoxicity of dimethyl sulfoxide (DMSO) in direct contact with odontoblast-like cells. *Dental Materials*, *31*(4), 399–405. https://doi.org/10.1016/j.dental.2015.01.007

Heintze, S. D., & Rousson, V. (2012). Clinical effectiveness of direct class II restorations - a meta-analysis. *The Journal of Adhesive Dentistry*, *14*(5), 407–431. https://doi.org/10.3290/j.jad.a28390

Hickel, R., Mesinger, S., Opdam, N., Loomans, B., Frankenberger, R., Cadenaro, M., Burgess, J., Peschke, A., Heintze, S. D., & Kühnisch, J. (2023). Revised FDI criteria for evaluating direct and indirect dental restorations—recommendations for its clinical use, interpretation, and reporting. *Clinical Oral Investigations*, *27*(6), 2573–2592. https://doi.org/10.1007/s00784-022-04814-1

Hickel, R., Peschke, A., Tyas, M., Mjör, I., Bayne, S., Peters, M., Hiller, K. A., Randall, R., Vanherle, G., & Heintze, S. D. (2010a). FDI World Dental Federation: Clinical criteria for the evaluation of direct and indirect restorations-update and clinical examples. *Clinical Oral Investigations*, *14*(4), 349–366. https://doi.org/10.1007/s00784-010-0432-8

Hickel, R., Tyas, M., Mjör, I., Peters, M., Hiller, K., Randall, R., & Heintze, S. D. (2010b). FDI World Dental Federation – Clinical Criteria for the Evaluation of Direct and Indirect Restorations. *Journal of Adhesive Dentistry*, *12*, 259–272. https://doi.org/10.3290/j.jad.a19262

Hosaka, K., Nishitani, Y., Tagami, J., Yoshiyama, M., Brackett, W. W., Agee, K. A., Tay, F. R., & Pashley, D. H. (2009). Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. *Journal of Dental Research*, *88*(2), 146–151. https://doi.org/10.1177/0022034508328910

Ioannidis, J. P. A. (2016). Why Most Clinical Research Is Not Useful. *PLOS MEDICINE*, *13*(6), 1–10. https://doi.org/10.1371/journal.pmed.1002049

Ismail, O. A., Hassanein, O. E., Hafez, R., & Mamdouh, M. (2024). Effect of Dimethyl Sulfoxide Primer Application Before a Two-Step Etch-and-Rinse Adhesive on the Microtensile Bond Strength and Nanoleakage. *Journal of International Oral Health*, *16*(3), 246–252. https://doi.org/10.4103/jioh.jioh_11_24

Ismail, O. A., Hassanein, O. E., Hafez, R., Mamdouh, M., Shaalan, O., & Tezvergil-Mutluay, A. (2025). Three years clinical assessment of low concentration of dimethyl sulfoxide primer in non-carious cervical lesions: a randomized control trial. *Journal of Evidence-Based Dental Practice*, 102124. https://doi.org/https://doi.org/10.1016/j.jebdp.2025.102124

Ismail, O. A., Stape, T. H. S., & Tezvergil-Mutluay, A. (2023). Concentration effect of DMSO-dry bonding on the stability of etch-and-rinse bonds. *Dental Materials*, *39*(12), 1113–1121. https://doi.org/10.1016/j.dental.2023.09.013

Ito, S., Hashimoto, M., Wadgaonkar, B., Svizero, N., Carvalho, R. M., Yiu, C., Rueggeberg, F. A., Foulger, S., Saito, T., Nishitani, Y., Yoshiyama, M., Tay, F. R., & Pashley, D. H. (2005). Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. *Biomaterials*, *26*(33), 6449–6459. https://doi.org/10.1016/j.biomaterials.2005.04.052

Jacob, S. W., & Herschler, R. (1986). Pharmacology of DMSO. *Cryobiology*, *23*(1), 14–27. https://doi.org/10.1016/0011-2240(86)90014-3

Jiang, R., Xu, Y., Wang, F., & Lin, H. (2022). Effectiveness and cytotoxicity of two desensitizing agents : a dentin permeability measurement and dentin barrier testing in vitro study. *BMC Oral Health*, *22*(391), 1–11. https://doi.org/10.1186/s12903-022-02424-7

Josic, U., Maravic, T., Mazzitelli, C., Del Bianco, F., Mazzoni, A., & Breschi, L. (2021). The effect of chlorhexidine primer application on the clinical performance of composite restorations: a literature review. *Journal of Esthetic and Restorative Dentistry*, *33*(1), 69–77. https://doi.org/10.1111/jerd.12701

Karadas, M., Atmaca, Y., & Özden, G. (2023). Does dimethyl sulfoxide promote the bonding performance of different universal adhesives to dentin in etch-and-rinse approach? *International Journal of Adhesion and Adhesives*, *127*(August). https://doi.org/10.1016/j.ijadhadh.2023.103520

Koc Vural, U., Kerimova, L., & Kiremitci, A. (2021). Clinical comparison of a micro-hybride resin-based composite and resin modified glass ionomer in the treatment of cervical caries lesions: 36-month, split-mouth, randomized clinical trial. *Odontology*, *109*(2), 376–384. https://doi.org/10.1007/s10266-020-00550-8

Kuhn, E., Farhat, P., Teitelbaum, A. P., Mena-Serrano, A., Loguercio, A. D., Reis, A., & Pashley, D. H. (2015). Ethanol-wet bonding technique: Clinical versus laboratory findings. *Dental Materials*, *31*(9), 1030–1037. https://doi.org/10.1016/j.dental.2015.05.010

Landuyt, K. L. Van, Munck, J. De, Mine, A., Cardoso, M. V, Peumans, M., & Meerbeek, B. Van. (2010). Filler Debonding & Subhybrid-layer Failures in Self-etch Adhesives. *Journal of Dental Research*, *89*(10), 1045–1050. https://doi.org/10.1177/0022034510375285

Liu, Y., Tjäderhane, L., Breschi, L., Mazzoni, A., Li, N., Mao, J., Pashley, D. H., & Tay, F. R. (2011). Limitations in bonding to dentin and experimental strategies to prevent bond degradation. *Journal of Dental Research*, *90*(8), 953–968. https://doi.org/10.1177/0022034510391799

Loguercio, A. D., Bittencourt, D. D., Baratieri, L. M., & Reis, A. (2007). A 36-month evaluation of self-etch and etch-and-rinse adhesives in noncarious cervical lesions. *Journal of the American Dental Association*, *138*(4), 507–514. https://doi.org/10.14219/jada.archive.2007.0204

Loguercio, A. D., Moura, S. K., Pellizzaro, A., Dal-Bianco, K., Patzlaff, R. T., Grande, R. H. M., & Reis, A. (2008). Durability of enamel bonding using two-step self-etch systems on ground and unground enamel. *Operative Dentistry*, *33*(1), 79–88. https://doi.org/10.2341/07-42

Macedo, G. V, Yamauchi, M., & Bedran-Russo, A. K. (2009). Effects of chemical cross-linkers on caries-affected dentin bonding. *Journal of Dental Research*, *88*(12), 1096–1100. https://doi.org/10.1177/0022034509351001

Maghaireh, G. A., Albashaireh, Z. S., & Allouz, H. A. (2023). Postoperative sensitivity in posterior restorations restored with self-adhesive and conventional bulk-fill resin composites: A randomized clinical split-mouth trial. *Journal of Dentistry*, *137*, 104655. https://doi.org/10.1016/j.jdent.2023.104655

Maillet, C., Decup, F., Dantony, E., Iwaz, J., Chevalier, C., Gueyffier, F., Maucort-Boulch, D., Grosgogeat, B., & Le Clerc, J. (2022). Selected and simplified FDI criteria for assessment of restorations. *Journal of Dentistry*, *122*(March). https://doi.org/10.1016/j.jdent.2022.104109

Malacarne, J., Carvalho, R. M., de Goes, M. F., Svizero, N., Pashley, D. H., Tay, F. R., Yiu, C. K., & Carrilho, M. R. de O. (2006). Water sorption/solubility of dental adhesive resins. *Dental Materials*, *22*(10), 973–980. https://doi.org/10.1016/j.dental.2005.11.020

Marquillier, T., Doméjean, S., Le Clerc, J., Chemla, F., Gritsch, K., Maurin, J. C., Millet, P., Pérard, M., Grosgogeat, B., & Dursun, E. (2018). The use of FDI criteria in clinical trials on direct dental restorations: A scoping review. In *Journal of Dentistry* (Vol. 68, Issue October 2017, pp. 1–9). Elsevier. https://doi.org/10.1016/j.jdent.2017.10.007

Mazzoni, A., Scaffa, P., Carrilho, M., Tjäderhane, L., Di Lenarda, R., Polimeni, A., Tezvergil-Mutluay, A., Tay, F. R., Pashley, D. H., & Breschi, L. (2013). Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. *Journal of Dental Research*, *92*(1), 82–86. https://doi.org/10.1177/0022034512467034

Mehtälä, P., Pashley, D. H., & Tjäderhane, L. (2017). Effect of dimethyl sulfoxide on dentin collagen. *Dental Materials*, *33*(8), 915–922. https://doi.org/10.1016/j.dental.2017.04.018

Mello, R. M. M. de, Alcântara, B. A. R., França, F. M. G., Amaral, F. L. B. do, & Basting, R. T. (2022). Dimethyl Sulfoxide Dentin Pretreatments Do Not Improve Bonding of a Universal Adhesive in Etch-and-Rinse or Self-etch Modes. *The Journal of Adhesive Dentistry*, *24*(1), 49–56. https://doi.org/10.3290/j.jad.b2701705

Mortazavi, V., Samimi, P., Rafizadeh, M., & Kazemi, S. (2012). A randomized clinical trial evaluating the success rate of ethanol wet bonding technique and two adhesives. *Dental Research Journal*, *9*(5), 588. https://doi.org/10.4103/1735-3327.104878

Pandis, N. (2011). The evidence pyramid and introduction to randomized controlled trials. *American Journal of Orthodontics and Dentofacial Orthopedics*, *140*(3), 446–447. https://doi.org/10.1016/j.ajodo.2011.04.016

Pashley, D. H. (1986). Dentin permeability, dentin sensitivity, and treatment through tubule occlusion. *Journal of Endodontics*, *12*(10), 465–474. https://doi.org/10.1016/S0099-2399(86)80201-1

Pashley, D. H., Carvalhol, R. M., Nakajima, M., Yoshiyama, M., Shono, Y., Fernandes, C., & Tay, F. (1999). The microtensile bond test: A review. *Journal of Adhesive Dentistry*, *1*(4), 299–309.

Pashley, D. H., Tay, F. R., Breschi, L., Tjäderhane, L., Carvalho, R. M., Carrilho, M., & Tezvergil-Mutluay, A. (2011). State of the art etch-and-rinse adhesives. *Dental Materials*, *27*(1), 1–34. https://doi.org/10.1016/j.dental.2010.10.016.State

Pashley, D. H., Tay, F. R., Carvalho, R. M., Rueggeberg, F. A., Agee, K. A., Carrilho, M., Donnelly, A., & García-Godoy, F. (2007). From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *American Journal of Dentistry*, *20*(1), 7–20.

Pashley, D. H., Tay, F. R., Yiu, C., Hashimoto, M., Breschi, L., Carvalho, R. M., & Ito, S. (2004). Collagen Degradation by Host-derived Enzymes during Aging. *Journal of Dental Research*, *83*(3), 216–221.

Perdigão, J. (2010). Dentin bonding-Variables related to the clinical situation and the substrate treatment. *Dental Materials*, *26*(2), 24–37. https://doi.org/10.1016/j.dental.2009.11.149

Perdigão, J., Araujo, E., Ramos, R. Q., Gomes, G., & Pizzolotto, L. (2021). Adhesive dentistry: Current concepts and clinical considerations. *Journal of Esthetic and Restorative Dentistry : Official Publication of the American Academy of Esthetic Dentistry ... [et Al.]*, *33*(1), 51–68. https://doi.org/10.1111/jerd.12692

Perdigão, J., Reis, A., & Loguercio, A. D. (2013). Dentin Adhesion and MMPs: A Comprehensive Review. *Journal of Esthetic and Restorative Dentistry*, *25*(4), 219–241. https://doi.org/https://doi.org/10.1111/jerd.12016

Reis, A., Pellizzaro, A., Dal-Bianco, K., Gomes, O. M., Patzlaff, R., & Loguercio, A. D. (2007). Impact of Adhesive Application to Wet and Dry Dentin on Long-term Resin-dentin Bond Strengths. *Operative Dentistry*, *32*(4), 380–387. https://doi.org/10.2341/06-107

Sadek, F. T., Mazzoni, A., Breschi, L., Tay, F. R., & Braga, R. R. (2010). Six-month evaluation of adhesives interface created by a hydrophobic adhesive to acid-etched ethanol-wet bonded dentine with simplified dehydration protocols. *Journal of Dentistry*, *38*(4), 276–283. https://doi.org/10.1016/j.jdent.2009.11.007

Sadek, F. T., Pashley, D. H., Nishitani, Y., Carrilho, M. R., Donnelly, A., Ferrari, M., & Tay, F. R. (2008). Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique. *Journal of Biomedical Materials Research. Part A*, *84*(1), 19–29. https://doi.org/10.1002/jbm.a.31290

Salim Al-Ani, A. A., Mutluay, M., Stape, T. H. S., Tjäderhane, L., & Tezvergil-Mutluay, A. (2018). Effect of various dimethyl sulfoxide concentrations on the durability of dentin bonding and hybrid layer quality. *Dental Materials Journal*, *37*(3), 501–505. https://doi.org/10.4012/dmj.2017-213

Salim Al-Ani, A. A. S., Scarabello Stape, T. H., Mutluay, M., Tjäderhane, L., & Tezvergil-Mutluay, A. (2019). Incorporation of dimethyl sulfoxide to model adhesive resins with different hydrophilicities: Physico/mechanical properties. *Journal of the Mechanical Behavior of Biomedical Materials*, *93*(August 2018), 143–150. https://doi.org/10.1016/j.jmbbm.2019.02.015

Salim, I. A., Salim, A. A., Scarabello Stape, T. H., Mutluay, M. M., & Tezvergil-Mutluay, A. (2025). Effect of solvent pretreatments on intraradicular bond strength of bioactive cements. *International Journal of Adhesion and Adhesives*, *138*(January), 103952. https://doi.org/10.1016/j.ijadhadh.2025.103952

Sanmartín-Suárez, C., Soto-Otero, R., Sánchez-Sellero, I., & Méndez-Álvarez, E. (2011). Antioxidant properties of dimethyl sulfoxide and its viability as a solvent in the evaluation of neuroprotective antioxidants. *Journal of Pharmacological and Toxicological Methods*, *63*(2), 209–215. https://doi.org/10.1016/j.vascn.2010.10.004

Sano, H., Takatsu, T., Ciucchi, B., Horner, J. A., Matthews, W. G., & Pashley, D. H. (1995). Nanoleakage: leakage within the hybrid layer. *Operative Dentistry*, *20*(1), 18–25.

Sartori, N., Stolf, S. C., Silva, S. B., Lopes, G. C., & Carrilho, M. (2013). Influence of chlorhexidine digluconate on the clinical performance of adhesive restorations: A 3-year follow-up. *Journal of Dentistry*, *41*(12), 1188–1195. https://doi.org/10.1016/j.jdent.2013.09.004

Schwendicke, F., & Opdam, N. (2018). Clinical studies in restorative dentistry : Design , conduct , analysis. *Dental Materials*, *34*(1), 29–39. https://doi.org/10.1016/j.dental.2017.09.009

Sorsa, T., Tjäderhane, L., Konttinen, Y. T., Lauhio, A., Salo, T., Lee, H.-M., Golub, L. M., Brown, D. L., & Mäntylä, P. (2006). Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Annals of Medicine*, *38*(5), 306–321. https://doi.org/10.1080/07853890600800103

Stape T. H. S. (2021). Dry bonding: New perspectives for collagen hybridization. *Doctoral dissertation*. University of Turku, Turku, Finland

Stape, T. H. S., Ismail, O. A., Capitanio, M., & Tezvergil-Mutluay, A. (2025). Bonding neat hydrophobic-rich resins to etched dentin: A proof of concept. *Dental Materials*, *41*(2), 113–121. https://doi.org/10.1016/j.dental.2024.10.018

Stape, T. H. S., Mutluay, M. M., Tjäderhane, L., Uurasjärvi, E., Koistinen, A., & Tezvergil-Mutluay, A. (2021a). The pursuit of resin-dentin bond durability: Simultaneous enhancement of collagen

structure and polymer network formation in hybrid layers. *Dental Materials*, *37*(7), 1083–1095. https://doi.org/10.1016/j.dental.2021.03.010

Stape, T. H. S., Seseogullari-Dirihan, R., Tjäderhane, L., Abuna, G., Martins, L. R. M., & Tezvergil-Mutluay, A. (2018). A novel dry-bonding approach to reduce collagen degradation and optimize resin-dentin interfaces. *Scientific Reports*, *8*(1), 16890. https://doi.org/10.1038/s41598-018-34726-8

Stape, T. H. S., Tezvergil-Mutluay, A., Mutluay, M. M., Martins, L. R. M., do Prado, R. L., Pizi, E. C. G., & Tjäderhane, L. (2016). Influence of dimethyl sulfoxide used as a solvent on the physical properties and long-term dentin bonding of hydrophilic resins. *Journal of the Mechanical Behavior of Biomedical Materials*, *64*, 220–228. https://doi.org/10.1016/j.jmbbm.2016.07.003

Stape, T. H. S., Tjäderhane, L., Marques, M. R., Aguiar, F. H. B., & Martins, L. R. M. (2015). Effect of dimethyl sulfoxide wet-bonding technique on hybrid layer quality and dentin bond strength. *Dental Materials*, *31*(6), 676–683. https://doi.org/10.1016/j.dental.2015.03.008

Stape, T. H. S., Tjäderhane, L., Tezvergil-Mutluay, A., Yanikian, C. R. F., Szesz, A. L., Loguercio, A. D., & Martins, L. R. M. (2016). Dentin bond optimization using the dimethyl sulfoxide-wet bonding strategy: A 2-year in vitro study. *Dental Materials*, *32*(12), 1472–1481. https://doi.org/10.1016/j.dental.2016.09.015

Stape, T. H. S., Uctasli, M., Cibelik, H. S., Tjäderhane, L., & Tezvergil-Mutluay, A. (2021b). Dry bonding to dentin : Broadening the moisture spectrum and increasing wettability of etch-and-rinse adhesives. *Dental Materials*, *37*(11), 1676–1687. https://doi.org/10.1016/j.dental.2021.08.021

Tay, F. R., & Pashley, D. H. (2003). Water treeing--a potential mechanism for degradation of dentin adhesives. *American Journal of Dentistry*, *16*(1), 6–12.

Tay, F. R., & Pashley, D. H. (2004). Resin bonding to cervical sclerotic dentin: A review. *Journal of Dentistry*, *32*(3), 173–196. https://doi.org/10.1016/j.jdent.2003.10.009

Tay, F. R., Pashley, D. H., & Yoshiyama, M. (2002). Two modes of nanoleakage expression in single-step adhesives. *Journal of Dental Research*, *81*(7), 472–476.

Tezvergil-Mutluay, A., Agee, K. A., Hoshika, T., Uchiyama, T., Tjäderhane, L., Breschi, L., Mazzoni, A., Thompson, J. M., McCracken, C. E., Looney, S. W., Tay, F. R., & Pashley, D. H. (2011). Inhibition of MMPs by alcohols. *Dental Materials*, *27*(9), 926–933. https://doi.org/10.1016/j.dental.2011.05.004

Tezvergil-Mutluay, A., Agee, K. A., Mazzoni, A., Carvalho, R. M., Carrilho, M., Tersariol, I. L., Nascimento, F. D., Imazato, S., Tjäderhane, L., Breschi, L., Tay, F. R., & Pashley, D. H. (2015). Can quaternary ammonium methacrylates inhibit matrix MMPs and cathepsins ? *Dental Materials*, *31*(2), e25–e32. https://doi.org/10.1016/j.dental.2014.10.006

Tjäderhane, L. (2015). Dentin bonding: Can we make it last? *Operative Dentistry*, *40*(1), 4–18. https://doi.org/10.2341/14-095-BL

Tjäderhane, L., Mehtälä, P., Scaffa, P., Vidal, C., Pääkkönen, V., Breschi, L., Hebling, J., Tay, F. R., Nascimento, F. D., Pashley, D. H., & Carrilho, M. R. (2013a). The effect of dimethyl sulfoxide (DMSO) on dentin bonding and nanoleakage of etch-and-rinse adhesives. *Dental Materials*, *29*(10), 1055–1062. https://doi.org/10.1016/j.dental.2013.07.014

Tjäderhane, L., Nascimento, F. D., Breschi, L., Mazzoni, A., Tersariol, I. L. S., Geraldeli, S., Tezvergil-Mutluay, A., Carrilho, M., Carvalho, R. M., Tay, F. R., & Pashley, D. H. (2013b). Strategies to prevent hydrolytic degradation of the hybrid layer - A review. *Dental Materials*, *29*(10), 999–1011. https://doi.org/10.1016/j.dental.2013.07.016

Tjäderhane, L., Nascimento, F. D., Breschi, L., Mazzoni, A., Tersariol, I. L. S., Geraldeli, S., Tezvergil-Mutluay, A., Carrilho, M. R., Carvalho, R. M., Tay, F. R., & Pashley, D. H. (2013c). Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. *Dental Materials*, *29*(1), 116–135. https://doi.org/10.1016/j.dental.2012.08.004

Tomoe, H. (2023). Dimethyl sulfoxide : A review of pharmacology and clinical effect on interstitial cystitis/bladder pain syndrome. *Continence*, *8*, 101058. https://doi.org/https://doi.org/10.1016/j.cont.2023.101058

Turkun, L. S. (2023). New Trends and Criteria in the Minimally Invasive Esthetic Rehabilitation of Anterior Teeth. *Current Oral Health Reports*, *10*(2), 28–35. https://doi.org/10.1007/s40496-023-00333-4

Valdez-Montoya, M., Avendaño-Félix, M. M., Basurto-Flores, J. C., Ramírez-Álvarez, M., Cázarez-Camacho, M. del R., Casillas-Santana, M. Á., Zavala-Alonso, N. V., Sarmiento-Hernández, S. N., Silva-Benítez, E. de L., & Soto-Sainz, J. E. (2024). Role of Metalloproteinases in Adhesion to Radicular Dentin: A Literature Review. *Materials*, *17*(22), 1–17. https://doi.org/10.3390/ma17225674

Van Meerbeek, B., Peumans, M., Poitevin, A., Mine, A., Ende, A. Van, Neves, A., & Munck, J. De. (2010). Relationship between bond-strength tests and clinical outcomes. *Dental Materials*, *26*(2), 100–121. https://doi.org/10.1016/j.dental.2009.11.148

Van Meerbeek, B., Yoshihara, K., van Landuyt, K., Yoshida, Y., & Peumans, M. (2020). From buonocore's pioneering acid-etch technique to self-adhering restoratlves. A status perspective of rapidly advancing dentai adheslve technology. *Journal of Adhesive Dentistry*, *22*(1), 7–34. https://doi.org/10.3290/j.jad.a43994

Vermelho, P. M., Reis, A. F., Maria, G., & Ambrosano, B. (2017). Adhesion of multimode adhesives to enamel and dentin after one year of water storage. *Clinical Oral Investigations*, *21*, 1707–1715. https://doi.org/10.1007/s00784-016-1966-1

Vishnyakov, A., Lyubartsev, A. P., & Laaksonen, A. (2001). Molecular dynamics simulations of dimethyl sulfoxide and dimethyl sulfoxide-water mixture. *Journal of Physical Chemistry A*, *105*(10), 1702–1710. https://doi.org/10.1021/jp0007336

Wendlinger, M., Cardenas, A. F. M., de Siqueira, F. S., Moreira, P. H. de A., Trovão, M. M. A., Stape, T. H. S., Tezvergil-Mutluay, A., & Loguercio, A. D. (2023). Does the application of dimethyl sulfoxide improve resin bonding to eroded dentine? Four-year in vitro evaluation. *Dental Materials*, *39*(11), 1051–1057. https://doi.org/10.1016/j.dental.2023.09.014

Wendlinger, M., Cochinski, G. D., Aguiar Moreira, P. H. de, Cardenas, A. F. M., de Siqueira, F. S. F., Stape, T. H. S., Tezvergil-Mutluay, A., & Loguercio, A. D. (2025). Effect of proanthocyanidin and riboflavin associated with dimethyl sulfoxide in eroded dentine: Six-year in vitro evaluation. *Dental Materials*, *41*(7), 827–838. https://doi.org/10.1016/j.dental.2025.05.002

You, X., Chen, L., Xu, J., Li, S., Zhang, Z., & Guo, L. (2022). Effects of carbodiimide combined with ethanol-wet bonding pretreatment on dentin bonding properties: an in vitro study. *PeerJ*, *10*, e14238. https://doi.org/10.7717/peerj.14238

Zabeu, G. S., Candia Scaffa, P. M., Ciccone Giacomini, M., de Mattos Pimenta Vidal, C., Tjäderhane, L., & Wang, L. (2022). Gelatinolytic activity after dentin pretreatment with dimethyl sulfoxide (DMSO) combined to dental bonding systems: Perspectives for biological responses. *Journal of the Mechanical Behavior of Biomedical Materials*, *130*(January), 105188. https://doi.org/10.1016/j.jmbbm.2022.105188

Zabeu, G. S., Ciccone Giacomini, M., Candia Scaffa, P. M., Tjäderhane, L., Mosquim, V., & Wang, L. (2023). Solvation role of dimethyl sulfoxide on the interaction with dentin bonding systems after 30 months. *Dental Materials*, *39*(5), 478–484. https://doi.org/10.1016/j.dental.2023.03.010

Zhang, M., Sun, Z., Zhang, B., Peng, W., & Guo, L. (2024). Effects of dimethyl sulfoxide pretreatment on the bonding properties of fluorotic dentin of different severity: An in vitro study. *The Journal of Prosthetic Dentistry*, *131*(3), 508–517. https://doi.org/10.1016/j.prosdent.2022.01.033

Zhang, Z., Li, K., Yang, H., Yu, J., & Huang, C. (2022). The influence of dimethyl sulfoxide on resin–dentin bonding: A systematic review: Dimethyl sulfoxide for dentin bonding. *International Journal of Adhesion and Adhesives*, *113* (March 2022), 103037. https://doi.org/10.1016/j.ijadhadh.2021.103037

Zhang, Z., Yu, J., Yao, C., Yang, H., & Huang, C. (2020). New perspective to improve dentin – adhesive interface stability by using dimethyl sulfoxide wet-bonding and epigallocatechin-3-gallate. *Dental Materials*, *39*, 1–12. https://doi.org/10.1016/j.dental.2020.08.009

Zimmerley, M., McClure, R. A., Choi, B., & Potma, E. O. (2009). Following dimethyl sulfoxide skin optical clearing dynamics with quantitative nonlinear multimodal microscopy. *Applied Optics*, *48*(10), D79. https://doi.org/10.1364/AO.48.000D79

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