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DRY BONDING: NEW PERSPECTIVES FOR COLLAGEN HYBRIDIZATION

Thiago Henrique Scarabello Stape

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SARJA – SER. D OSA – TOM. 1600 | MEDICA – ODONTOLOGICA | TURKU 2021

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The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin Originality Check service.

ISBN 978-951-29-8734-4 (PRINT)
ISBN 978-951-29-8735-1 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2021

To my beloved Mother, for your unconditional love, tireless support and everlasting kindness.

UNIVERSITY OF TURKU
Faculty of Medicine
Institute of Dentistry
Department of Cariology and Restorative Dentistry
THIAGO HENRIQUE SCARABELLO STAPE: Dry Bonding: New Perspectives for Collagen Hybridization
Doctoral Dissertation, 178 pp.
Finnish Doctoral Programme in Oral Sciences – FINDOS Turku
December 2021

ABSTRACT

Advancements in dental adhesive technology have revolutionized dental treatments, invariably increasing the number and indications of restorative treatment options. Considering current techniques to bond to tooth tissue, dentin can be characterized as the most challenging mineralized dental substrate for successful long-lasting bonding. Resin-dentin bonding relies, partly or totally, on collagen hybridization to couple methacrylate-based resins to the underlying mineralized dentin. The aim of this study series was to revisit the dry-bonding approach by modifying the application protocol to effectively enable resin bonding to air-dried-etched dentin. Dentin pretreatments containing dimethyl sulfoxide (DMSO) in either ethanolic or aqueous solutions (50 % v/v) were tested to determine whether novel DMSO-dry bonding approaches could improve resin-dentin bonding. Mechanical and physical properties of DMSO-containing resins such as degree of conversion, elastic modulus, flexural strength, water sorption and solubility were evaluated. Resin-dentin interfaces were submitted to long-term microtensile testing, nanoleakage and micropermeability evaluation, in situ zymography and degree of conversion. Indirect assessment of enzymatic activity on DMSO-treated collagen was determined by dry mass loss, hydroxyproline quantification and elastic modulus. Gel zymography was used to determine the effect of DMSO pretreatments on MMP-2 and -9 activity. The wettability of air-dried DMSO-treated collagen by hydrophilic and hydrophobic resins was evaluated by contact angle measurements. This study series produced compelling evidence that DMSO-dry bonding constitutes a feasible alternative to reduce residual water from resin-dentin interfaces by broadening the moisture spectrum of demineralized dentin to substantially drier levels. Bonding methacrylate-based resins to extensively air-dried collagen greatly improved resin-dentin bonding following the DMSO-dry bonding approaches. Higher resin-dentin bond strength was accompanied by better hybrid layer formation with higher monomer conversion and reduced porosities, lower collagenolytic activity, enhanced dentin wettability and lower technique sensitivity. Altogether, such benefits contributed to more efficient collagen hybridization, addressing important issues in resin-dentin bonding with a single bonding protocol.

KEYWORDS: Adhesion; Dentin; Dimethyl sulfoxide; DMSO; Wet bonding; Etch-and-rinse; DMSO-dry bonding.

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Hammaslääketieteen laitos
Kariologia ja Korjaava Hammashoito
THIAGO HENRIQUE SCARABELLO STAPE: Kuivasidostus: uusia näkökulmia kollageenin hybridisaatioon
Väitöskirja, 178 s.
Kansallinen suun terveystieteiden tohtoriohjelma – FINDOS Turku
Joulukuu 2021

TIIVISTELMÄ

Sidostusteknologian kehitys on johtanut suuriin muutoksiin hammashoidossa, ja lisännyt merkittävästi korjaavan hoidon indikaatioita ja hoitotoimenpiteiden määrää. Nykyisillä sidostekniikoilla dentiini asettaa suuria haasteita sidoksen pitkäaikaiskestolle. Sidosaineen ja dentiinin liitos perustuu sidosaineen tunkeutumiseen dentiinin kollageeniverkoston sisään (ns. hybridisaatio), jonka kautta metakrylaattipohjaiset sidosaineet kiinnittyvät dentiiniin. Hybridisaatio on monimutkainen prosessi. Toimenpide täytyy suorittaa nopeasti kosteassa kudoksessa, ja sen tulisi kestää jopa vuosikymmeniä. Tämän tutkimussarjan tavoitteena oli tutkia sidostamista kuivaan kudokseen muokkaamalla protokollaa siten, että se onnistuisi ilmakuivattuun happokäsiteltyyn dentiiniin. Tutkimuksissa testattiin dentiinin esikäsittelyä dimetyylisulfoksidin (DMSO) etanoli- ja vesiseosten (50 til.-%) mahdollisia etuja, ja selvitettiin täysin uudenlaisen DMSO-kuivasidostuksen käyttöä sidoksen parantamisessa. Lisäksi selvitettiin DMSO:ta sisältävien sidosaineiden fyysisiä ja mekaanisia ominaisuuksia, kuten konversioastetta, kimmomoduulia, taivutuslujuutta, veden adsorptiota ja liukenemista. Vanhennettuja sidosrajapintoja tutkittiin mikrosidoslujuustestauksella, nanovuotoanalyysillä, mikropermeabiliteettitestauksella, in situ-zymografialla ja konversioasteanalyysillä. Entsyymien vaikutusta DMSO-käsiteltyyn dentiinikollageeniin tutkittiin epäsuorasti kuivapainomenetystä, hydroksiproliinin vapautumista ja kollageenin kimmomoduulia mittaamalla. Geelizymografialla määritettiin DMSO-esikäsittelyn vaikutusta MMP-2:n ja MMP-9:n aktiivisuuteen. Kuivatun DMSO-käsitellyn dentiinin kostutettavuutta tutkittiin kosketuskulmamittauksilla. Tutkimukset osoittivat DMSO-kuivasidostuksen olevan toteuttamiskelpoinen keino vähentää jäännösvettä sidosrajapinnassa mahdollistamalla sidostaminen kuivaan dentiiniin, ja se paransi sidosta merkittävästi. DMSO-kuivasidostaminen paransi dentiinin kostutettavuutta, hybridikerroksen laatua ja monomeerien konversiota, vähensi kollagenolyyttistä aktiivisuutta ja hybridikerroksen huokoisuutta, paransi välitöntä sidoslujuutta ja sen pysyvyyttä, ja vähensi käsittelyvirheiden riskiä. Kaiken kaikkiaan menetelmällä saavutettiin tehokkaampi dentiinikollageenin hybridisaatio vaikuttamalla useisiin sidostamisen kannalta merkittäviin tekijöihin.

AVAINSANAT: Tarttuvuus; Dentiini; Dimetyylisulfoksidi; DMSO.

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Abbreviations

ANOVA	Analysis of variance
BHT	Butylhydroxytoluene
BisGMA	Bisphenol glycidyl methacrylate
CLSM	Confocal laser scanning microscope
CQ	Camphorquinone
DMSO	Dimethyl sulfoxide
DMSO/H_2O	50 % (v/v) DMSO solution in water
DMSO/EtOH	50 % (v/v) DMSO solution in ethanol
EDMAB	2-ethyl-4-aminobenzoate
EDTA	Ethylenediamine tetraacetic acid
EtOH	Ethanol
h	Unit of time, hour
H_3PO_4	Phosphoric acid
HEMA	2-hydroxyethyl methacrylate
MDPB	12-methacryloyloxydodecylpyridinium bromide
MMP	Matrix metalloproteinase
MPa	Megapascal
mm	Millimeter
NaCl	Sodium chloride
NaN_3	Sodium azide
s	Unit of time, seconds
SBMP	Scotchbond Multipurpose, 3M ESPE
SiC	Silicon carbide
SU	Scotchbond Universal, 3M ESPE
v/v	Volume/volume, dilution
wt %	Weight percentage
g	Unit of mass, gram
Hz	Hertz, cycles per second
min	Unit of time, 60 seconds
µl	Microliter

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. ***Stape THS***, Tezvergil-Mutluay A, Mutluay MM, Martins LR, do Prado RL, Pizi EC, Tjäderhane L. (2016). Influence of dimethyl sulfoxide used as a solvent on the physical properties and long-term dentin bonding of hydrophilic resins. *J Mech Behav Biomed Mater*. 64:220–8. doi: 10.1016/j.jmbbm.2016.07.003.

II. ***Stape THS***, Tjäderhane L, Abuna G, Sinhoreti MAC, Martins LRM, Tezvergil-Mutluay A. (2018). Optimization of the etch-and-rinse technique: New perspectives to improve resin-dentin bonding and hybrid layer integrity by reducing residual water using dimethyl sulfoxide pretreatments. *Dent Mater*. 34:967–77. doi: 10.1016/j.dental.2018.03.010.

III. ***Stape THS***, Seseogullari-Dirihan R, Tjäderhane L, Abuna G, Martins LRM, Tezvergil-Mutluay A. (2018). A novel dry-bonding approach to reduce collagen degradation and optimize resin-dentin interfaces. *Sci Rep*. 8:16890. doi: 10.1038/s41598-018-34726-8.

IV. ***Stape THS***, Mutluay MM, Tjäderhane L, Uurasjärvi E, Koistinen A, Tezvergil-Mutluay A. (2021). The pursuit of resin-dentin bond durability: Simultaneous enhancement of collagen structure and polymer network formation in hybrid layers. *Dent Mater*. 37:1083–95. doi: 10.1016/j.dental.2021.03.010.

V. ***Stape THS***, Uctasli M, Cibelik HS, Tjäderhane L, Tezvergil-Mutluay A. (2021). Dry bonding to dentin: Broadening the moisture spectrum and increasing wettability of etch-and-rinse adhesives. *Dent Mater.* 37:1676–87. https://doi.org/10.1016/j.dental.2021.08.021

1 Introduction

Resin-dentin bonding is a unique form of *in situ* tissue engineering, in which hydrated collagen (Agee et al., 2015) serves as a scaffold for resin infiltration to couple methacrylate-based resins to the underlying mineralized dentin (Van Meerbeek et al., 2020; Liu et al., 2011; Pashley et al., 2011;). Dentin etching with H_3PO_4 for 15 s removes the smear layer and fully demineralizes dentin to an extension of approximately 8 µm (Van Meerbeek et al., 2020; Pashley et al., 2011), causing collagen fibrils to remain literally suspended in water after rinsing (Pashley et al., 2011, 2007). Hence, the etch-and-rinse approach relies on the ability of bonding resins to properly wet dentin surfaces (Van Meerbeek et al., 2020; Pashley et al., 2011), diffuse through the 10 - 30 nm wide interfibrillar collagen spaces (Pashley et al., 2011) and micromechanically interlock within the collagen network upon polymerization. Such intermediate layer, named "hybrid layer", was characterized as a revolutionary resin-collagen "biopolymer" (4 – 6 µm in thickness) in 1982 (Nakabayashi et al., 1982). Dentin hybridization is a rather complex bonding process which must be performed within acceptable clinical times, usually under just a couple of minutes, in hopes of producing a stable link between the bulk adhesive and dentin substrate (Van Meerbeek et al., 2020; Liu et al., 2011; Pashley et al., 2011). Hereof, three-step etch-and-rinse systems (fourth-generation adhesives; released during the early 1990s) were regarded as the first adhesive class to reach favorable clinical outcomes (De Munck et al., 2012, 2005; Van Meerbeek et al., 1994). This was certainly a milestone in the rapidly evolving dental adhesive technology. To date, consistent evidence of favorable long-term bonding performance (Peumans et al., 2014, 2012) still characterizes this class of adhesives (especially those containing ethanol and water as solvents) as a gold-standard (De Van Meerbeek et al., 2020; Munck et al., 2005) amongst the countless attempts made over the last decades to improve dentin bonding. Curiously, three-step etch-and-rinse systems may even outperform self-etch systems when bonded to caries-affected dentin (Isolan et al., 2018), albeit a more laborious technique is required for dentin hybridization.

Nonetheless, the etch-and-rinse approach constitutes several challenges regarding dentin bonding (Van Meerbeek et al., 2020; Liu et al., 2011; Pashley et al.,

2011, 2007). Dentin is a complex histologic structure. It is intrinsically hydrated composed primarily of mineralized collagen forming a dynamic-heterogeneous tissue with different levels of mineralization. Morphologically, it presents a tubular orientation with increasing tubular density and greater diameter in closer proximity to the pulp. In addition, outward dentinal fluid movement upon tubular disocclusion, after smear layer dissolution by etching agents, further complicates matters during clinical application. Considering that water replacement by methacrylate resins is never ideal (Wang & Spencer, 2003), much of the limitations of etch-and-rinse systems can be attributed to poor adhesive diffusion within the demineralized collagen matrix and subsequent low-quality polymer formation (Pashley et al., 2011; Spencer et al., 2004, 2002, 2000; Wang & Spencer, 2003; 2002). Maintenance of collagen interfibrillar spaces is crucial for adequate resin-dentin bonding under the etch-and-rinse approach. Therefrom, the wet-bonding technique gained popularity during the early 1990s (Pashley et al., 2007; Gwinnett, 1992a) due to the effective collagen expansion produced by water (Pashley et al., 2011, 2007). Wet bonding remains as the recommended dentin-bonding technique for the etch-and-rinse approach (Van Meerbeek et al., 2020; Pashley et al., 2011, 2007). Collapse of demineralized collagen microfibrills after air-drying compromises dentin bonding (Pashley et al., 2011, 2007). Stiffening of the collagen matrix through the production of hydrogen bonding between adjacent collagen molecules results in the elimination of the diffusion channels for resin infiltration (Pashley et al., 2011; 2007). However, the required degree of dentin-surface moisture cannot be precisely measured and it is hardly replicable. The lack of or excess moisture contribute to high technique sensitivity of the etch-and-rinse approach (Pashley et al., 2011, 2007). This situation becomes even more complex should the water-removal ability of the solvent content in the bonding resin be taken into consideration. Ideally, dentin moisture levels must be specifically correlated to the adhesive system for optimal bonding. Unfortunately, this is not clinically feasible. Furthermore, residual water entrapped within the hybrid later actively participates in resin-dentin bond hydrolysis (Carrilho et al., 2005) in combination with endogenous enzymes (*e.g.,* MMP, cathepsins and salivary esterases) (Huang et al., 2018a; Tjäderhane et al., 2013a, 2013b; Finer & Santerre, 2004b) and cariogenic bacterium esterases (Huang et al., 2018b). Hydrolysis of resin polymers by esterases (Huang et al., 2018b; Delaviz et al., 2014; Shokati et al., 2010; Kostoryz et al., 2009; Finer et al., 2004a) and collagen by gelatinolytic/collagenolytic enzymes (*i.e.*, matrix metalloproteinases and cysteine cathepsins) (Tjäderhane et al., 2013a, 2013b) have been shown to compromise the micromechanical interlocking within hybrid layer constituents. Reduction of the water content of hybrid layers through a standardized and reproducible procedure could substantially improve long-term bonding performance. The main question

resides on how to perform a simple dry-bonding approach using air-drying without collagen collapse.

In recent years, dimethyl sulfoxide (DMSO) has emerged as one the most versatile solvents to be used in adhesive dentistry (Tjäderhane et al., 2013b) considering its physical-chemical interactions with the constituents of the hybrid layer. DMSO competes with water molecules in collagen interpeptide hydrogen bonding (Vishnyakov et al., 2001), increases collagen-interfibrillar spacing (Zimmerley et al., 2009) resulting in wider interfibrillar spaces which facilitates the diffusion of resin monomers within the collagen network (Stape et al. 2015). Moreover, the oxygen atom in DMSO can hydrogen bond with up to three water molecules (Kiefer et al. 2011), while the methyl groups present on the opposite side of the DMSO molecule form a hydrophobic end. Such DMSO-water molecule rearrangements facilitate inward monomer diffusion into collagen (Stape et al., 2015). DMSO not only improves dentin wetness (Mehtälä et al., 2017), contributing to higher uptake and deeper monomer infiltration within demineralized dentin (Stape et al., 2015), but it disrupts surrounding water-layers thereby improving collagen-monomer interaction with hydrophobic-crosslinking monomers. As a result, more uniform hybrid layers with higher bond strengths (Stape et al., 2016, 2015) can be produced when three-step etch-and-rinse adhesives are associated to dentin pretreatments containing DMSO using the wet-bonding approach (Stape et al., 2016, 2015).

Despite the promising results of DMSO pretreatments following the wet-bonding technique, the possible outcomes on dry-etched dentin are not yet known. DMSO's unique ability to modify the collagen structure (Mehtälä et al., 2017; Zimmerley et al., 2009) and to simultaneously interact with both resin monomers (Geurtsen et al., 1998; Martin et al., 1967) and water (Catalán et al., 2001; Vishnyakov et al., 2001; Luzar & Chandler, 1993; MacGregor, 1967) bring new possibilities to improve resin-dentin bonding even further. Therefore, this thesis investigated the possibility of effectively bonding methacrylate resins via the etch-and-rinse approach in a dry state employing DMSO. The main aim of this study series was to develop a clinically feasible bonding protocol to not only reduce the technique sensitivity of the etch-and-rinse approach, but to effectively optimize resin-dentin interfaces under dry conditions.

2 Review of the Literature

2.1 Tooth structure

2.1.1 Enamel

Enamel **(Figure 1A)** constitutes the coronal outer portion of teeth. It is the most mineralized and stiffest mammal tissue. The elastic modulus of human enamel varies between 20 – 131 GPa, depending on the testing method and tissue area (He & Swain, 2008). Unlike other calcified skeletal structures, fractures of enamel tissue are not repairable. Enamel's inorganic structure, mostly calcium and phosphate in the form of hydroxyapatite, corresponds to 85 % by volume or 96 % by weight. The organic matrix corresponds to 3 % by volume or 1 % by weight. It is a multicomponent system involving free amino acids, glycine, some amino acids bound to the mineral phase and several aggregated complexes of variable sizes. Trace amounts of type I collagen have also been identified in fully mineralized-mature enamel (Açil et al., 2005). After maturation, most of the organic matrix is removed during mineralization and some proteins, mostly ameloblastin and the amino-terminal remnants of amelogenin, are retained. Proteins and collagen lying between minerals have the function of holding hydroxyapatite crystals together to maintain the hierarchical structure of enamel. Water corresponds to 1 – 2 % by weight and it is found in two states loosely and tightly bound (Bachmann et al., 2004). The smallest structural units are needlelike hydroxyapatite crystallites, which are roughly rectangular in cross-section (He & Swain, 2008). In healthy human enamel, hydroxyapatite crystallites are organized and bundled together by organic molecules into larger structures called enamel rods or enamel prisms. Human enamel consists of approximately 5 μm diameter rods encapsulated by protein-rich sheaths that are arranged parallel to each other in a direction perpendicular to the dentin-enamel junction from dentin to the outer enamel surface. The presence of small quantities of protein remnants allows limited movement between adjacent rods reducing stress concentration and crack propagation (He & Swain, 2007).

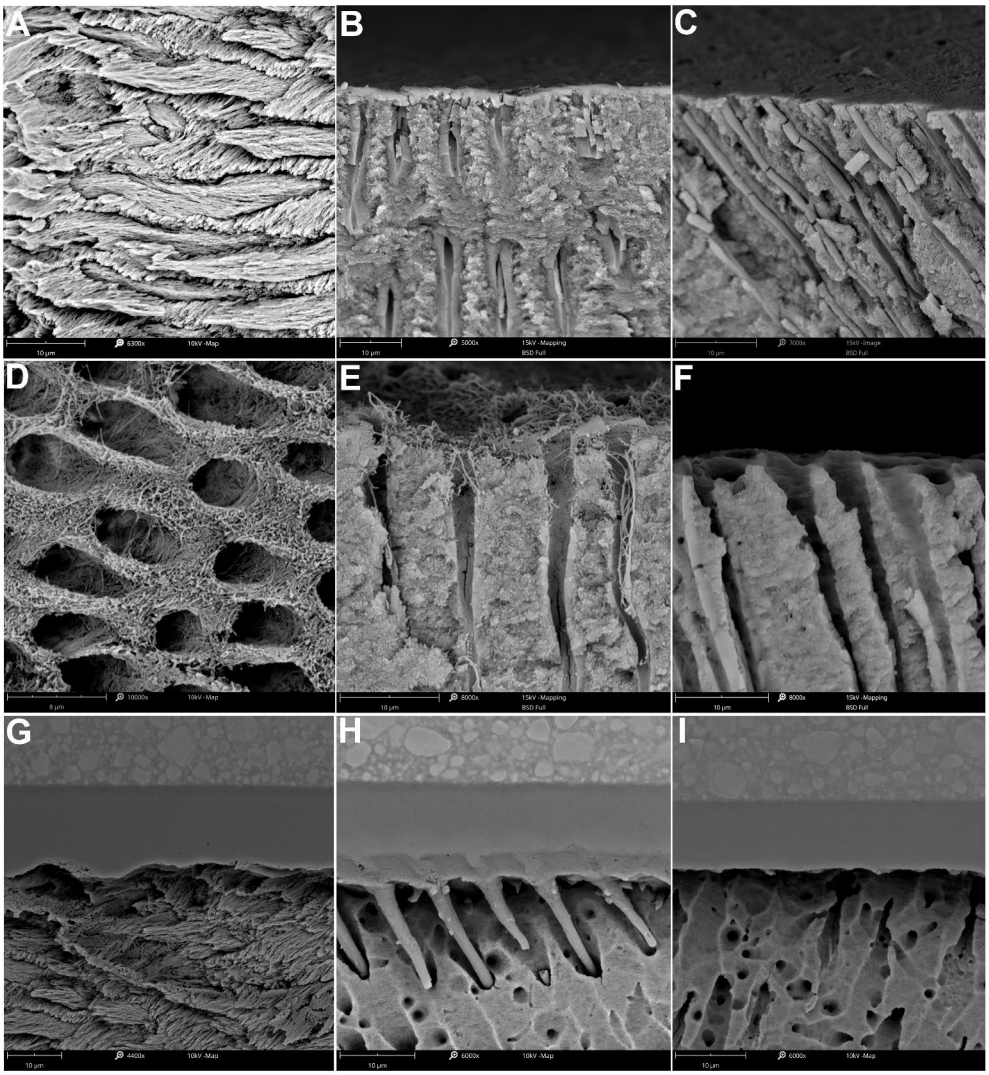


Figure 1. Representative SEM micrographs of sound or 32 % H_3PO_4-etched tooth tissues and bonded interfaces. A: etched enamel; B profile view of cryofractured-mid-coronal dentin with smear layer; C: profile view of cryofractured-mid-coronal dentin and smear plugs; D: occlusal view of mid coronal dentin etched for 15 s; E: profile view of cryofractured-mid-coronal dentin etched for 15 s; F: profile view of cryofractured-mid-coronal dentin etched for 15 s followed by air-drying; G: resin-enamel bonded interface after 30 s etching; H: hybrid layer of an multi-step etch-and-rinse adhesive; I: hybrid layer of a simplified self-etch adhesive.

2.1.2 Dentin

Dentin **(Figure 1 B and C)** is the human tissue with the second-highest degree of mineralization. It is the tissue underlying enamel and it constitutes the bulk of the tooth. Biomechanically, dentin acts as a foundation preventing the propagation of cracks from enamel due to the presence of the dentin-enamel junction (Imbeni et al., 2005). In contrast to enamel, dentin composition is very heterogeneous. Dentin is composed of approximately 70 % minerals, 20 % organic components and 10 % water by weight. Volume percentages account for 50 % minerals, 30 % organic components and 20 % water (Nakabayashi, 1998) of the dentin substrate. Carbonated nanocrystalline apatite minerals, namely hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, compose the majority of dentin´s mineral content. Minerals are distributed in two sites within the collagen matrix. Intrafibrillar minerals occupy the inner portion of the periodically spaced gap zones in the collagen fibril while extrafibrillar minerals are located within the interstices between fibrils. It is estimated that the majority of the mineral content, approximately 70 to 75 %, is located extrafibrillarly (Pidaparti et al., 1996). Approximately 90 % of the organic components of dentin is collagen. Trace amounts of type III and V collagen can be found. Type I collagen is the most abundant (Tjäderhane et al., 2012). This differentiates between dentin collagen and soft tissue collagen along with the fact that dentin collagen is highly crosslinked (Veis & Schlueter, 1964). Dentin collagen is the most cross-linked collagen in the body. The amount of collagen in dentin decreases from superficial to deep dentin. The remaining 10 % of the organic matrix is composed mostly by proteoglycans and numerous non-collagenous proteins including proteoglycans, glycosaminoglycans and phosphorilated proteins (Bertassoni et al., 2012a). At a higher organization level, dentin can be considered as a fiber reinforced tubular structure (Tjäderhane et al., 2012, Kinney et al., 2003). Dentinal tubules radiate continuously from the dentin-pulp border through the entire extension of coronary dentin and from the cementum-dentin junction to the pulp canal. Tubule's diameter range from 0.8 μm, close to the dentin-enamel junction, to 3 μm, close to the pulp. Branching between adjacent tubules and innumerous ramifications form a vast anastomosing system, especially where the density of the main tubules is low (Mjör & Nordahl, 1996). Mantle dentin and the dentin-enamel junction are atubular. Noteworthy, water is primarily located inside dentinal tubules (Tjäderhane et al., 2012). Mineralized collagen contains a trace amount of water (Takahashi et al., 2014). Since the diameter and number of tubules increase with higher proximity to the dentin-pulp border, water distribution is not uniform. The higher the number of tubules present, the wetter the dentin surface. Moisture variations may be up to 20-fold from superficial to deep dentin (Pashley, 1996). Hence, dentin is a highly permeable structure with a pressurized outward flow of dentinal fluids, defined as a pulpar pressure, and also potential inward flow of microbial components depending on pathological conditions.

Peritubular dentin is deposited in the inner portion of each tubule, while a collagen matrix of intertubular dentin is dispersed between dentin tubules. Peritubular dentin lacks collagen fibrils and contains an organic scaffold embedded with mineral (Bertassoni et al., 2012a). It is a hypermineralized structure, mineral content higher than 90 %, with an organic matrix manly composed of glycosaminoglycans (Bertassoni et al., 2012b). Dentinal tubules house the extensions of odontoblast processes conferring the dynamic response of the dentin tissue to external stimulus. Dentin is commonly subdivided into five categories according to their formation stage and location sites including mantle dentin, dentin-enamel junction, primary dentin, secondary dentin and tertiary dentin (Tjäderhane et al., 2012).

2.1.3 Collagen

Collagen **(Figure 1 D – F)** is the main structural protein found in the extracellular matrix present in connective tissues. Approximately 30 – 40 % of the human body is composed of collagen (Verzár, 1964). Collagens are a large family of structurally related extracellular matrix proteins containing a unique triple helical structure. As of 2011, 28 types of collagen have been identified (Ricard-Blum, 2011). Nonetheless, 80 – 90 % of the collagen in the human body consists of types I, II, and III. Type I collagen is the most abundant protein in mammals (Di Lullo et al., 2002). Fibroblasts initially synthetize and secrete procollagen molecules into the extracellular space, where they undergo a series of post-translational modifications to form collagen, also referred to as tropocollagen (Goldberg & Sherr, 1973). A type I collagen molecule consists of three α chains: two α1 and one α2 chains intertwined into a left-handed triple helix (Rainey & Goh, 2009; Okuyama et al., 1977). Type I collagen molecules are further intertwined into a right-handed helix 300 nm in length, 1.4 nm in diameter containing precisely 1050 amino acids wound around one another. They aggregate with their long axes in parallel to form fibrils of various thickness. Collagen molecules (approximately 1.4 nm in diameter and 300 nm in length) are “packed” together to form long-similar fibrillar structures (Bertassoni et al., 2012a). Type I collagen fibrils (100 – 120 nm in diameter) are composed of smaller collagen microfibril bundles (10 – 25 nm in diameter) (Bertassoni et al., 2012a). There is a lateral distance between collagen molecules of approximately 1.26 – 1.33 nm (Bertassoni et al., 2012a), which is filled with water. Such water molecules are tightly bound to collagen connecting and stabilizing neighboring collagen molecules. Three domains compose the collagen molecule: a central triple-helical region, a non-helical aminoterminal (N-telopeptide) region and a carboxyterminal (C-telopeptide) region (Yamauchi & Shiiba, 2008). The triple-helical structure arises from an unusual abundance of three amino acids: glycine (Gly), proline (Pro) and hydroxyproline (Hyp). These amino acids make up the

characteristic repeating pattern Gly-Pro-X or Gly-X-Hyp, where X can be any amino acid (Germann & Heidemann, 1988). Gly is present at every third position. It is the smallest amino acid and it enhances the van Waals forces and hydrogen bonding that holds the triple-helical structure. Each amino acid has a specific function. Although Hyp does not participate directly in hydrogen bonding, it is critical in stabilization and orientation of the molecular arrangement of the triple-helix structure. Since Hyp makes up 10 % of the total collagen amino acids, measurement of Hyp content is commonly used to quantify collagen or its degradation (Comper, 1996). Collagen fibrils are insoluble in water and contain a 67 nm gap (D-period) between neighboring collagen molecules. This characterizes the band pattern through the fibrillar axis observed microscopically by negative staining. Covalent cross-linking occurs within the triple helices and between tropocollagen forming well-organized aggregates. Such inter- and intramolecular covalent cross-links occur between the C-terminal of one collagen molecule and the N-terminal of the neighboring collagen molecule (Yamauchi & Shiiba, 2008). Curiously, dentin collagen does not over turn, meaning that it seldom degrades and when so, it is not replaced (Tjäderhane et al., 2009). Nonetheless, natural cross-linking in dentin collagen accumulates over time (Miura et al., 2014), which may affect the mechanical properties of dentin collagen over time. During dentin maturation, mineral precipitation initially fills the 67 nm gaps between collagen molecules and then the interfibrillar spaces (Landis & Jacquet, 2013).

2.2 Mechanisms involved in resin-dentin bonding

2.2.1 Adhesion

Adhesion is a process of joining two or more solid parts with a flowable adhesive substance also known as bonding agent. The materials of the joined parts, known as adherent substrates, may be composed of different or similar chemical compositions. The adhesive layer is commonly formed by a polymer and its thickness normally does not exceed 0.5 mm. Bonding agents are normally a mixture of monomers, which upon curing are locked into place. Adhesion is a complex of physicochemical processes occurring at the interface of at least two materials brought into an intimate contact, which results in formation of attractive forces between them. The adhesive joint generally consists of two surfaces with the bonding agent filling the gaps between them. From a microscopic perspective, even highly polished materials present a great number of superficial irregularities, which when brought into close contact still present interfacial gaps. Higher material porosity or rougher surfaces generally provide stronger adhesion due to larger interfacial areas and improved interlocking of the bonding agent into the superficial micro-voids. Since there is high

variation in such interfacial irregularities, the adhesive layer is generally not uniform. This is even more noticeable in biological bonded interfaces due to the heterogeneous character of such tissues (*i.e.,* resin-dentin interfaces). Moreover, bonding agents may additionally form chemical bonds with bonding substrates. Hence, adhesion may be greatly improved beyond just mechanical interlocking depending on the chemical composition of the bonding agents and substrates. This is more effective when strong chemical bonds (*i.e.,* ionic or covalent bonds) are formed. In practice, most bonding agents are polymeric materials, so metallic bonds, albeit considered strong, are not normally present in conventional adhesive joints. Adhesion strength, commonly known as bond strength, is the stress required for separation of two adhered parts along the interface. Investigation of bond strengths helps to determine their ability to withstand stresses during application.

2.2.2 Surface wetting

Adequate surface wetting is a primary requirement to achieve good interfacial contact between adhesives and bonding substrates. For uniform spreading along the bonding substrate, the surface tension of bonding resins must be lower than the superficial free energy of bonding surfaces. Good interaction between bonding resins and the bonding substrate is critical for proper adhesion. Several factors affect surface wetting such as adhesive viscosity, surface roughness, substrate and adhesive chemical compositions, surface free energy, bond-promoting effects (*e.g.,* capillary forces in bonding to etched enamel), surface hydrophilicity and hydrophobicity levels and surface hydration. In adhesive dentistry, a positive correlation occurs between dentin wetting and bond strengths (Rosales-Leal et al., 2001).

2.2.3 Smear layer

The term smear layer **(Figure 1B)** was introduced by Boyde and Steward in 1963 (Boyde et al., 1963) and its morphological aspects were later described (Gwinnett, 1984; Mader et al., 1984; Pashley, 1984; Eick et al., 1970). The smear layer is defined as a thin amorphous layer of organic and inorganic matter weakly attached to the tooth surface (Pashley, 1984). It is produced by reduction or instrumentation of dentin, enamel or cementum (Gwinnett, 1984; Pashley, 1984; Eick et al., 1970). The friction of burs and cutting instruments cause plastic and elastic deformation, shattering tooth structures. This results in large amount of debris. Smear layer composition varies according to tooth conditions and cutting instruments. Dentin smear layer is mainly composed by denatured collagen, hydroxyapatite and other cutting debris (Pashley, 1984). Blood cells, saliva, odontoblastic processes and microorganisms can also be part of the organic portion of hybrid layers. In deep

dentin, smear layer tends to contain more organic material than on superficial dentin (Gwinnett, 1984). The inorganic portion of the smear layer is composed mainly of hydroxyapatite crystals identified in the form of granular particles of roughly 0.05 – 0.1 μm in diameter (Gwinnett, 1984; Eick et al., 1970). Particles size varies tremendously according to the cutting method (Pashley, 1984). Smear layer quantity, quality, thickness and density vary widely (Pashley, 1984). Dry-cutting produces thicker smear layers on dentin than preparations performed under water-cooling (Gwinnett, 1984). Grit size also affects smear layer formation with lower grit diamond burs, coarser instruments, producing thicker and denser layers than higher grit finishing burs (Ogata et al., 2001). Hand instruments also produce smear layers. The presence of smear layer in vital teeth restricts dentinal fluid from flowing outwards (Pashley, 1984), which reduces dentin permeability. Morphologically, the smear layer is a bilaminar structure, with an outer layer that lies on the tooth surface, ranging from 1 – 5 μm, and an underlying-deep layer extending inside dentinal tubules. During cavity preparations, debris are forced into dentinal tubules in lengths varying from only a few μm up to 40 μm during root canal preparations. Debris compacted inside dentinal tubules form structures known as smear plugs. Noteworthy, smear layer interferes with surface free energy of dentin, and thus wetting, which directly affects resin-dentin bonding (Suyamaa et al., 2013; Tay et al., 2000; Rosales et al., 1999).

2.2.4 Dentin etching

Suboptimal clinical outcomes were identified in attempts to bond to smear layer covered dentin in the 1970s and 1980s (Van Meerbeek et al., 1994; Vanherle et al., 1991). Resin-dentin bond strengths seldom exceeded 5 – 6 MPa (Tyas et al., 1989). Such low values were insufficient to resist shrinkage stresses of most composites (Kleverlaan & Feilzer, 2005; Versluis et al., 2004), contributing to premature failure of composite restorations. Adhesive formulations available at the time bonded only superficially to the weakly attached smear layer. It became clear that the rather thick-dense smear layer resulting from bur preparations should be dealt with before dentin hybridization. The concept of etching dentin and enamel simultaneously with H_3PO_4 was introduced by Fusayama et al., in 1979 (Fusayama et al., 1979). This was defined as the total-etch protocol. However, dentin etching with high H_3PO_4 concentrations (*i.e.,* 30 – 40 % H_3PO_4) was not initially accepted by the dental communities in Europe and United States. Concerns regarding potential harmful effects to the dentin-pulpar complex halted the general acceptance of this revolutionary total-etch technique. Only in the early 1990s, it was demonstrated that acid etching dentin produces no adverse pulpal reactions (Pashley, 1992). Pulpal inflammatory reactions derived from bacterial leakage and not necessarily as result of acid etching *per se*.

Since dentin collagen is highly cross-linked, it can withstand etching procedures that would otherwise destroy the structure of the dermal collagen (Schlueter et al., 1964). As a result of etching dentin with H_3PO_4 for 15 s, full demineralization takes place **(Figure 1D)**, which exposes collagen fibrils to a depth of approximately 8 µm (Pashley et al., 2011). After rinsing the acid content and solubilized minerals, collagen fibrils lose the mineral support remaining suspended in water **(Figure 1E)**. Dentin etching for the recommended 15 s, does not affect the structural integrity of collagen (Breschi et al., 2003a). Over-etching for longer periods may, however, induce structural changes in the collagen molecules (Breschi et al., 2003b). Incorporation of damaged collagen fibrils into the hybrid layer should be strongly avoided. In fact, less aggressive protocols with shorter etching times and different chelating-etching agents have been proposed to reduce collagen exposure in hybrid layers (Yu et al., 2021; Stape et al., 2018; Feitosa et al., 2013; Salvatore et al., 2011, 2010).

2.2.5 The hybrid layer

Conventional H_3PO_4-etching (*i.e.*, 30 – 40 %) for 15 s solubilizes the entire 50 % (v/v) of dentin's surface and subsurface mineral content **(Figure 1D – E)**, which is then replaced by rinsing water (Pashley et al., 2011, 2007). The resultant dentin composition, considering the intrinsic 20 % (v/v) of water, yields a total water content of 70 % (v/v) which surrounds the 30 % (v/v) collagen fibrils anchored to the underlying mineralized dentin (Pashley et al., 2011). In fact, after etching, collagen fibrils are literally suspended in water. During the subsequent infiltration of methacrylate comonomers, the full extent of the 70 % (v/v) water content should be ideally replaced by resin monomers. Upon polymerization, normally via light-curing, a hybridized "biocomposite" of collagen and resin is formed *in situ*. The bonding resin becomes micro-mechanically interlocked with the demineralized intertubular dentin and through the formation of resin tags **(Figure 1H)**, which are extensions of the resin matrix inside the dentinal tubules. This "biocomposite" was first identified by Nakabayashi et al., in 1982 (Nakabayashi et al., 1982), who coined the term hybrid layer to describe the structure composed of demineralized collagen fibrils reinforced by the bonding resin matrix. Hence, resin-bonded interfaces produced *in vivo* using the etch-and-rinse approach are created at a nanometer scale over a distance of only 5 – 8 µm (Breschi et al., 2018; Pashley et al., 2011) **(Figure 1H)**. Hybrid layers produced by mild or ultra-mild self-etching adhesives are even thinner, below 1 µm (Van Meerbeek et al., 2011) **(Figure 1I)**. Differently from conventional forms of tissue engineering (Baum & Mooney, 2000), where collagen scaffolds are designed to be resorbed in short periods to be later replaced by the host's regenerative tissues, hybrid layers ideally should be stable over time (Breschi et al., 2018). Another critical difference involves the dimensions of porosities found

in most bioengineered scaffolds, roughly 5 – 20 µm, compared to those observed in etched dentin. Dentin collagen interfibrillar spaces between resin-infiltrated collagen fibrils in hybrid layers are only 10 – 30 nm wide (Pashley et al., 2011). Clinically, this rather complex bonding process involving nanometer scales is routinely performed under a couple of minutes. Resin-dentin interfaces are expected to resist cyclic mastication loads, variations in temperature and pH, acid challenges and microbiological attacks in the oral cavity. Unfortunately, complete infiltration of monomers into moist-demineralized dentin is not consistently achieved (Wang & Spencer 2003, 2002; Spencer et al., 2000; Spencer & Swafford, 1999). Resin-dentin interfaces are considered the weakest link in composite restorations (Spencer et al., 2010). The presence of residual solvents in the bonding resin (Cadenaro et al., 2009), the outward dentinal fluid flow towards the hypertonic comonomer blends (Pashley et al., 2011) and the molecular sieving effect on monomers by the proteoglican-collagen matrix (Lu et al., 2014) contribute to incomplete water replacement by resin. Hence, the sealing ability is never ideal since resin-poor-water-filled regions are frequently found within hybrid layers (Tay et al., 2003a, 2002). Noteworthy, adhesive compositions and application modes have a critical effect on hybrid layer integrity (Breschi et al., 2018; Pashley et al., 2011).

2.2.6 Etch-and-rinse vs. self-etch bonding mechanisms

To date, two main approaches are employed for resin-dentin bonding. The etch-and-rinse and self-etch approaches. The terms “etch-and-rinse” and “total-etch” are commonly used interchangeably. Nowadays, all etching procedures are simultaneously performed on enamel and dentin. Hence, the term “total-etch” was replaced by “etch-and-rinse”, since they are both characterized by the same bonding principles and mechanisms. The main difference in the *modus operandi* between the etch-and-rinse and self-etch approaches is the etching step. The etch-and-rinse approach relies on a separate etching step with H_3PO_4 (32 – 40 %) before the application of the bonding agents, while the self-etching approach does not require it. In fact, etching dentin before the use of self-etch adhesives may compromise their bonding effectiveness (Van Meerbeek et al., 2011). The etch-and-rinse approach requires previous collagen exposure to allow subsequent diffusion of the bonding resin into the demineralized dentin matrix. This separate etching step is necessary to allow micromechanical retention via resin-collagen entanglement (Pashley et al., 2011). Differently, the self-etch approach relies on acidic methacrylate monomers to simultaneously demineralize and infiltrate dentin. Moreover, acidic functional monomers, usually composed by carboxyl or phosphate groups, establish chemical interactions (*i.e.,* ionic bonding) with the Ca^{2+} present in tooth hydroxyapatite (Van Meerbeek et al., 2011; Yoshida et al., 2000). Therefore, resin-dentin bonding of self-

etch adhesives are characterized by a two-fold mechanism including micromechanical interlocking and chemical bonding to the tooth structure (Van Meerbeek et al., 2020, 2011). Self-etching bonding also presents lower technique sensitivity than etch-and-rinse bonding due to the absence of the critical moisture-control step of demineralized collagen. If overdried, collagen fibrils collapse hampering monomer diffusion (Pashley et al., 2007) **(Figure 1F)**. However, the smear layer may interfere with the interaction of mild and ultra-mild self-etching adhesives to dentin (Takamizawa et al., 2018; Suyamaa et al., 2013; Kenshima et al., 2006). The dependency on water to hydrolyze and activate carboxyl or phosphate groups also increases their hydrophilicity, which on the long run may invariably contribute to interfacial degradation. Nonetheless, long-term clinical studies performed on non-carious Class V cavities using a three-step etch-and-rinse and a two-step self-etch adhesives indicate little differences between their annual failure rates 3.1 (±2) and 2.5 (±1.5) %, respectively (Peumans et al., 2014). Although three-step etch-and-rinse systems present a more laborious-bonding technique for dentin hybridization, they tend to outperform self-etch systems when bonded to caries-affected dentin (Isolan et al., 2018).

2.3 Limitations in etch-and-rinse bonding

2.3.1 Wet bonding

The concept of wet bonding was introduced in 1992 (Kanca 3rd, 1992). This procedure was then widely adopted due to the high number of scientific publications showing the abrupt post-etching collapse of collagen produced by air-drying (De Munck et al., 2012, 2005; Pashley et al., 2011, 2007). The major disadvantage of wet bonding is its high sensitivity to the correct degree of dentin moisture. Both overwet and overdry conditions greatly affect adhesive performance (Tay et al., 1996a, 1996b). The presence of moisture in demineralized collagen is necessary to secure better infiltration of resin monomers within the collagen matrix. Realistically, complete collagen envelopment by resin is unlikely to happen in a consistent manner under wet conditions (Stape et al., 2015; Spencer et al., 2010, 2004; Wang & Spencer 2003, 2002; Spencer & Swafford, 1999). In the presence of moisture, hydrophobic crosslinking monomers are unable to diffuse through the full extension of demineralized collagen matrix (Wang & Spencer, 2003). Therefore, a layer of unenveloped collagen at the base of the hybrid layer is present in resin-dentin interfaces regardless of the bonding technique used (*i.e.,* etch-and-rinse or self-etch) (Stape et al., 2015; Carvalho et al., 2005). Clearly, the extension of collagen exposure at the base of etch-and-rinse hybrid layers is larger due to greater dentin demineralization (Stape et al., 2015; Carvalho et al., 2005). Furthermore, the bottom

of the hybrid layer is mainly constituted of low molecular hydrophilic monomers (*i.e.,* HEMA) by as little as 10 % of hydrophobic monomers (*i.e.,* BisGMA) (Wang & Spencer, 2003). The main issue produced by such low ratios of hydrophobic monomers with greater hybrid layer depth is inferior polymer quality (Ferracane, 2006; Ito et al., 2005). In the presence of water, physical separation of resin monomers into hydrophobic- and hydrophilic-rich phases invariably occurs depending on the water content. This phenomenon has been named nanophase separation (Ye et al., 2009a; Spencer et al., 2000), which limits the diffusion of dimethacrylate monomers into collagen (Wang et al., 2006; Wang & Spencer, 2003, 2002) and it may also reduce monomer conversion within the hybrid layer (Paulette Spencer & Wang, 2002). Overwet conditions further facilitate the occurrence of nanophase separation. In larger extensions, nanophase separation may be identified as water blisters found in resin-dentin interfaces, which increase the number of flaws at the bonded interface. The necessity of incorporating hydrophilic monomers, such as HEMA, into resin blends reduces the vapor pressure of water. According to the Raoult's law of partial vapor pressure, as the vapor pressure of water drops, it becomes more difficult to remove residual water from collagen. Hence, the formation of an impervious soundly integrated resin-dentin link is less likely to happen under wet conditions (Spencer et al., 2010).

2.3.2 Dry bonding

This technique was originally advocated in the 1980s for etched dentin (*i.e.,* using EDTA, maleic, nitric and citric acid or lower concentrations of phosphoric acid). Etched dentin used to be extensively air-dried until it appeared dull and dry. Air-drying is a far simpler and it is a more consistent moisture control approach compared to blot-drying employed in wet bonding. Although simpler and more reproducible, the dry-bonding approach fell into disuse to newer adhesive systems that employed the wet-bonding technique due to more favorable *in vitro* and clinical outcomes (De Munck et al., 2012, 2005; Van Meerbeek et al., 1994). The inability of resin-solvent blends to re-expand dried-collapsed collagen limits dry-bonding (Manso et al., 2008; Pashley et al., 2007). Conventional dry bonding has a detrimental effect on resin-dentin bonding when dentin is etched with H_3PO_4 (Manso et al., 2008; Reis et al., 2007; Tay et al., 1996a; Gwinnett, 1992a, 1992b; Kanca 3rd, 1992). Several attempts have been proposed to overcome such limitations and reestablish dry bonding (Gu et al., 2019; Sebold et al., 2019; Stape, et al., 2018; Reis et al., 2012; Zander-Grande et al., 2011). The key factor for successful dry bonding is collagen stabilization before air-drying or the development of techniques and chemical compounds capable of re-expanding collagen after the adhesive application. Recent approaches have focused on less aggressive polymeric chelators,

such as high molecular polyacrylic acid (Mai et al., 2017), and chitosan (Gu et al., 2019) to selectively etch mineralized collagen. By preserving intra-collagen minerals, the collapse caused by air-drying is less likely to happen. The use of such selective etching protocols followed by air-drying produce comparable results to the wet-bonding approach. Cross-linkers also showed promising results when applied to H_3PO_4-ecthed collagen (Zhou et al., 2016). Cross-linking completely demineralized collagen with grape seed extract produced comparable results on wet and dry resin-dentin bonding (Zhou et al., 2016). Attempts to apply water-containing adhesives to air-dried dentin may be successful if vigorous adhesive application is performed (Zander-Grande et al., 2011; Dal-Bianco et al., 2006; Reis et al., 2007). Although easily applicable, the effectiveness of vigorous adhesive application may be questionable in specific clinical situations. In cavities with a more challenging geometry, however, such approach may not be as effective as seen in previous *in vitro* studies that employed flat dentin surfaces for bonding (Reis et al., 2007; Dal-Bianco et al., 2006) or even *in vivo* studies with non-carious cervical lesions (Zander-Grande et al., 2011; Perdigão et al., 2005). In the best case scenario, most of the proposed dry-bonding techniques involving fully demineralized collagen (*i.e.,* H_3PO_4 etched) have at best equiparated dry-bonding performance with the traditional wet-bonding technique.

2.4 Degradation of resin-dentin interfaces

2.4.1 Endogenous dentin enzymes

Collagenolytic enzymes can degrade demineralized dentin collagen (Tjäderhane et al., 2015; Tezvergil-Mutluay et al., 2013; Pashley et al., 2004). Unprotected collagen is prone to slow hydrolytic degradation by host collagenolytic enzymes, matrix metalloproteinases (MMP) and cysteine cathepsins (Tezvergil-Mutluay et al., 2010a; Carrilho et al., 2009; Mazzoni et al., 2006). Matrix metalloproteinases, also designated as matrixins, are a group of zinc- and calcium-dependent endopeptidases responsible for pathological and physiological remodeling of extracellular matrix in vertebrates (Visse & Nagase, 2003). Twenty-four different MMPs have been discovered hitherto, of which 23 are found in humans (Visse & Nagase, 2003). They normally consist of a hemopexin domain (~ 200 amino acids), a cysteine residue (~ 80 amino acids) a zinc-containing catalytic domain (~ 170 amino acids) and a hinge region (Visse & Nagase, 2003). MMPs are secreted as proenzymes (proMMP) and their activity is blocked by the cysteine residue in the propeptide domain (Visse & Nagase, 2003). The cysteine switch is the mechanism that renders MMPs inactivity by preventing water from binding to zinc in the catalytic domain (Visse & Nagase, 2003; Van Wart & Birkedal-Hansen, 1990). Removal of the catalytic zinc inactivates

MMPs (Visse & Nagase, 2003). Zinc participates directly in the cleavage of peptide bonds along with other metal ions, such as calcium (Visse & Nagase, 2003). Displacement of the cysteine switch activates MMPs. Different processes can displace the cysteine switch such as heat treatments, low pH, specific chemicals and even self-activation by other proteases (Van Wart & Birkedal-Hansen, 1990). Activation of MMPs in dentin is mostly related to initial demineralization followed by exposure to the low pH of bonding resins (Liu et al., 2011). Mildly acidic resin monomers can activate MMPs by inhibiting TIMP-1, tissue inhibitor of metalloproteinases-1 (Sulkala et al., 2001; Tjäderhane et al., 1998). Alternatively, low pH resin monomers may activate latent forms of MMPs (pro-MMPs) via the cysteine-switch mechanism (Tallant et al., 2010). MMPs are classified in six structural groups: collagenases, gelatinases, stromelsysins, matrilsysins, membrane type and others. To date, mineralized human dentin contains at least MMP-2 (gelatinase), -3 (stromelysin), -8 (collagenase), -9 (gelatinase) and -20 (enamelysin) (Mazzoni et al., 2011a, 2011b, 2007; Sulkala et al., 2007, 2002; Martin-De Las Heras et al., 2000). MMP-2 is the most abundant protease in dentin followed by MMP-9. Host-derived proteases are secreted during tooth development, likely to regulate collagen matrix organization and proteoglycan turn over in predentin during the mineralization phase. After mineralization, they become dormant and fossilized (Tjäderhane & Haapasalo, 2009). Non-collagen bound MMPs can be found in saliva (Sulkala et al., 2001; Tjäderhane et al., 1998), dentinal tubules (Boushell et al., 2008; Sulkala et al., 2002) and likely in dentinal fluid. Cysteine cathepsins are papain-like endopeptidases found in mammals that participate in intracellular proteolysis within lysosomal compartments of living cells or exist as exopepitidases in extracellular matrix degradation (Obermajer et al., 2008; Dickinson, 2002). They also have a major role in collagen degradation. Although cathepsins B, C, L1, L2 and O have been identified in human dentin (Tersariol et al., 2010), cathepsin K accounts for roughly 98 % of their protease activity. While cathepsin K cleaves helical collagen and telopeptides, other cathepsins are capable of only cleaving the non-helical telopeptide portion of collagen (Garnero et al., 1998). This makes Cathepsin K a unique protease that it is both a telopeptidase and a collagenase, cleaving telopeptides as well as the helical region of collagen. Cysteine cathepsins can be located in sound dentin and they are expressed by mature human odontoblasts (Tersariol et al., 2010), albeit they are more abundantly identified in carious dentin (Liu et al., 2011). Cysteine cathepsins are activated in mildly acidic environments (pH 4.5 – 6), which may further stimulate the activation of matrix-bound MMPs (Nagase, 1997). In the presence of glycosaminoglycans, cathepsins can be functional even in neutral pH environments (Obermajer et al., 2008).

2.4.2 Hydrolysis of demineralized collagen

When previously resin-infiltrated collagen matrix becomes exposed, its vulnerability to the attack of proteolytic enzymes increases. As hydrolases, MMPs add water across specific collagen peptide bonds, splitting peptide chains into fragments. Collagenolytic MMPs (MMP-1, -2, -8, and -13) cleave type I collagen within the triple helix at a single site (between amino acids 775 and 776 from the first GXY triplet of the triple helix domain) into a ¾ N-terminal fragment and ¼ C-terminal fragment (Garnero et al., 2003). A larger number of MMPs, including MMP-2 and -9, which are present in human dentin, can attack type I collagen telopeptides to release a long C-telopeptide segment named ICTP (Garnero et al., 2003). Differently, cathepsin K cleaves collagen molecules at multiple sites within the triple helix, which generates fragments of various sizes (Garnero et al., 1998). For cathepsin K, the major telopeptide released during type I collagen degradation is the smaller eight amino acid named CTX (Tjäderhane et al., 2013c; Garnero et al., 2003). In mildly acidic conditions, cathepsins become active and act as endopeptidases, except for cathepsin B, which also has carboxypeptidase activity. Cathepsin B plays a special role in collagen degradation by cleaving collagen's non-helical telopeptides. In doing so, the isoleucine-glycine peptide of the triple helix is exposed granting access to true collagenases (Garnero et al., 1998).

2.4.3 Water sorption and hydrolysis of dimethacrylate-based polymers

Dimethacrylate-based polymers used in dentistry are subject to both hygroscopic and hydrolytic effects, which influence their mechanical properties, dimensional stability and biocompatibility (Ferracane, 2006). Water initially enters the polymer matrix by diffusion into loosely cross-linked or hydrophilic domains of polymer chains. Resin degradation is directly related to water sorption levels (Pucci et al., 2018; Liu et al., 2011). The effect of solvents on polymer networks have been described as the plasticizing effect (Ferracane, 2006). Water acts as a plasticizer molecule, separating polymer chains and producing softening by reducing the effectiveness of polymeric entanglements (Ferracane, 2006). The rate of methacrylate polymer softening matches the rate of solvent uptake (Ferracane, 2006), which begins immediately and may reach a maximum plateau as soon as 24 – 48 h in dental adhesives (Malacarne et al., 2006). Monomer composition and hydrophilicity levels are determinant factors on the diffusion coefficient of water into dental adhesives (Malacarne et al., 2006). Polymers may degrade in aqueous solutions through two primary mechanisms: passive hydrolysis and enzymatic reaction (Lenz, 1993). Passive hydrolysis involves the degradation of polymer networks through oxidation, attack of functional groups or chain scission to produce small molecules (Ferracane, 2006; Lenz, 1993).

Methacrylates can undergo degradation reactions over time, producing formaldehyde via oxidation and methacrylic acid (Ferracane, 2006; Lenz, 1993). Oxidation reactions and potentially transesterification can occur in water (Ferracane, 2006; Lenz, 1993). Hydrolytic attack of water on ester linkages of resin components may be further accelerated by salivary (Delaviz et al., 2014; Shokati et al., 2010; Finer et al., 2004b) and bacterial (Huang et al., 2018) esterases, which may contribute to hybrid layer degradation (Zhang et al., 2016; Delaviz et al., 2014; Spencer et al., 2014; Shokati et al., 2010; Zou et al., 2010b; Breschi et al., 2008; De Munck et al., 2005). Human saliva contains sufficient cholesterol esterase and pseudocholinesterase to degrade dimethacrylates (Finer et al., 2004a; Finer & Santerre 2004b, 2003; Jaffer et al., 2002).

2.5 Classification of dental adhesives

The rapid advance of dental adhesives is depicted by the evolution of their compositions, mode of applications and clinical indications. Although most dental adhesives contain similar components, they may differ significantly considering the ratio of ingredients (Van Landuyt et al., 2007). Most dental adhesives are essentially methacrylate monomers mixed with solvents and photocuring initiators and inhibitors. They are normally classified according to the underlying adhesion strategy, etch-and-rinse or self-etch technique, and whether the primer and adhesive resin are presented in separate “bottles” or combined into a single “bottle” (Van Meerbeek et al., 2003). Currently, commercially available dental adhesives can be classified as three-step or two-step etch-and-rinse systems and two-step or one-step self-etch systems. Three-step etch-and-rinse systems, released in the early 1990s, initially require conditioning of the tooth surface with an acid etchant, normally 30 – 40 % H_3PO_4, followed by the primer and a separate adhesive resin application. In multistep systems, the primer and the solvent-free adhesive resin are presented in separate “bottles”. Aiming for simplification, the two-step etch-and-rinse systems were introduced in late 1990s. The main difference between three-step and two-step etch-and-rinse systems is that the latter is composed by one “bottle”, containing the primer and adhesive resin components. Two-step and one-step self-etch systems follow the same classification principle based on the clinical steps represented by the number of “bottles”. In two-step self-etch systems, released in the early 1990s, there are two separate “bottles”, one containing the acid primer and the other the adhesive resin. One-step self-etch systems, released in the early 2000s, are composed by only one “bottle”. Self-etch adhesives can be further subdivided according to their acidity and self-etch aggressiveness: ultra-mild (pH > 2.5), mild (pH ~ 2) and strong (pH < 1) (Van Meerbeek et al., 2011). In 2011, a new class of multi-mode adhesive system was released and named as universal adhesives. This peculiar class of dental

adhesives was formulated to bring more versatility and simplicity to clinicians who may now select the bonding strategy to be used (etch-and-rinse or self-etch) with the same resin blend. In addition, these systems may also incorporate silane-coupling agents to potentially improve bonding to ceramics and composites. The universal adhesives were initially presented as “one bottle” systems until 2020, when a “two bottle” version was released separating the acidic primer components from the adhesive resin.

2.6 The role of solvents in adhesive dentistry

Solvents are one of the major components of dental adhesives (Van Landuyt et al., 2007) playing an important role in bonding methacrylate-based resins to both dentin and enamel (Carvalho et al., 2003). Dental adhesives are co-monomer blends usually solvated in volatile organic solvents (Van Landuyt et al., 2007). The most used solvents in adhesive dentistry are ethanol, acetone, water and their combinations (Ekambaram et al., 2015; Van Landuyt et al., 2007). Curiously, commercial adhesives systems may contain up to 80 % of solvents by weight (Van Landuyt et al., 2007). Solvents are necessary to dilute high-molecular-weight-viscous monomers and thus facilitate their infiltration into demineralized dentin. In addition, they help in the transportation of co-monomer blends and initiators into the bonding surfaces. Solvent-monomer ratios play a major role in achieving adequate resin-dentin bonding (Carvalho et al., 2003). The physical properties of solvents, such a as vapor pressure, viscosity, surface tension, solubility parameters and hydrogen bonding capacity are critical for proper solvent selection and incorporation into adhesive systems. Solvent’s physical properties influence several aspects in resin-dentin bonding including solvent-monomer diffusion into the collagen matrix, the subsequent solvent-removal ability by evaporation after adhesive application and the capacity to re-expand collapsed collagen when needed. Ideally, neat solvents with Hoy’s solubility parameters for hydrogen bonding higher than 14.8 $(J/cm^3)^{½}$ are able to break interpeptide bonds between collapsed collagen fibrils resulting in matrix re-expansion (Pashley et al., 2007). Molecular size and molar concentrations of solvents are also important to facilitate the re-expansion of dried collagen (Agee et al., 2006). The greater the solubility parameters for hydrogen bonding of the resin blend, the greater the rate and extent of collagen re-expansion (Pashley et al., 2007; Agee et al., 2006). Isolate-monomer incorporation into adhesives are viable only if Hoy’s solubility parameter for hydrogen bonding of the resin blend is higher than 17 $(J/cm^3)^{½}$ (Agee et al., 2006). Hence, the combination of organic solvents with water, to increase the overall hydrogen bonding parameter, is a common practice. Water has high hydrogen bonding parameter without any added cytotoxic effects. Solvents may also present additional benefits to the bonded interface such as inhibition of

endogenous proteases. Some alcohols, including ethanol, propanol and tert-butanol, present matrix metalloproteinase inhibitory potential in a dose-dependent manner *in vitro* (Tezvergil-Mutluay et al., 2011a). The hydroxyl groups of alcohols may form covalent bonds to zinc ions and thereby inhibit metalloproteinases (Tezvergil-Mutluay et al., 2011a). Although the inhibition of soluble unbound metalloproteinases by some alcohols may reach up to 90 % (*i.e.,* tert-butanol), the presence of collagen may change the outcome. Collagen competes with metalloproteinases for alcohol hydrogen bonding, which may affect the dose response during clinical use. In addition, rehydration in the oral cavity may also hamper the inhibitory potential of alcohols. Solvents can also affect polymer formation. For instance, residual ethanol, within certain limits, may improve the degree of conversion of resin blends (Cadenaro et al., 2009). The same was not observed for water-containing resins (Cadenaro et al., 2009). Differently, excess residual solvents have detrimental effects on the formation of polymer networks resulting in lower monomer conversion (Cadenaro et al., 2008) and higher water sorption and solubility (Malacarne-Zanon et al., 2009). In self-etch adhesive systems, solvents (*i.e.,* primarily water and to lower extent ethanol) are required to hydrolyze acid monomers (Ekambaram et al., 2015). To date, there is not a perfect solvent to be used alone in dental adhesive formulations. Each solvent possesses specific chemical/physical properties **(Table 1)**, which confers advantages and disadvantages to the overall bonding performance of adhesive systems. Hence, the combination of solvents at adequate ratios is critical to optimize adhesive the bonding performance of dental adhesives.

2.6.1 Water

Water is present on the dentinal surface and throughout the entire depth of demineralized collagen. It is estimated that dentin etching with H_3PO_4, followed by water rinsing, results in a superficially demineralized layer composed of 30 % (v/v) collagen and 70 % (v/v) water (Pashley et al., 2011). Water is invariably the most abundant compound in the initial stages of the wet-bonding technique. Therefore, water's primary role in dental adhesive as a cosolvent is not necessarily related to its "solvent" effect, but indeed to its ability to maintain collagen interfibrillar spaces during the bonding procedures (Ekambaram et al., 2015). This permits better monomer and solvent diffusion through the extension of demineralized collagen (Pashley et al., 2011, 2002, 2001; Agee et al., 2006; Eddleston et al., 2003). The high hydrogen bonding capacity of water facilitates the rupture of collagen interpetptide bonds after drying (Pashley et al., 2007). Since water has lower vapor pressure than ethanol and acetone, evaporation from the adhesive container is much slower contributing to longer self-life of commercial adhesives (Ekambaram et al., 2015).

Nonetheless, evaporation from bonding surfaces is also challenging. As a result, excess residual water at the hybrid layer has detrimental effects on polymer formation, contributing to subsequent hydrolytic degradation of the bonded interface (Cadenaro et al., 2009, 2008; Malacarne-Zanon et al., 2009). Hence, water-based adhesives may contain cosolvents like ethanol to facilitate the removal of water molecules from bonded interfaces. The incorporation of hydrophilic monomers, such as HEMA, in water-based adhesive formulations further challenges water evaporation (Yiu et al., 2005). In addition, such high hydrophilic character of modern dental adhesives, containing large quantities of hydrophilic monomers to permit bonding to water-saturated dentin surfaces, has been questioned for some time (Tay & Pashley, 2003b). Extensive water sorption, solubility and higher water diffusion coefficients in hydrophilic dental resins is a major cause of concern (Malacarne et al., 2006).

2.6.2 Ethanol

Ethanol, C_2H_5OH, is the solvent most currently used in adhesive dentistry (Ekambaram et al., 2015; Van Landuyt et al., 2007). It is usually combined with water or acetone in adhesive formulations (Ekambaram et al., 2015; Van Landuyt et al., 2007). Ethanol is better of solvent than water for higher molecular weight monomers. Ethanol's higher vapor pressure than water facilitates its removal from commoner mixtures by evaporation (Ekambaram et al., 2015). However, complete evaporation is unlikely to happen (Cadenaro et al., 2009). Co-monomers reduce ethanol's vapor pressure hampering its evaporation (Cadenaro et al., 2009). In general, solvated resins exhibit higher levels of water sorption and solubility compared to their corresponding neat versions. Ethanol-based adhesives are not different (Malacarne-Zanon et al., 2009). Compared to neat resin formulations, ethanol incorporation improves degree of conversion, but it lowers the rate of polymerization (Cadenaro et al., 2009). Increase in degree of conversion of ethanol-solvated methacrylate-based resins occurs at the expense of higher water sorption, diffusion and solubility (Malacarne-Zanon et al., 2009). Ethanol also has high hydrogen bonding capacity [19.4 $(J/cm^3)^{1/2}$], albeit lower than water [42.3 $(J/cm^3)^{1/2}$]. Therefore, ethanol can re-expand air-dried collapsed demineralized collagen, although to a lesser extent than water (Pashley et al., 2007). Water removal from demineralized dentin during air-drying may be more easily accomplished in adhesive systems containing solvents with higher vapor pressure (Ekambaram et al., 2015). Ethanol-containing adhesives also have a stiffening effect on demineralized collagen increasing the width of interfibrillar spaces, which facilitates resin diffusion (Carvalho et al., 2003). It is important to note that resin-dentin bond strengths may be directly correlated with the width of collagen interfibrillar spaces (Carvalho et al.,

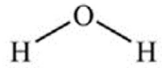
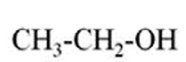
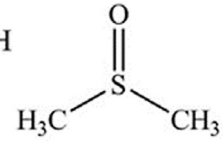
2003). Wider interfibrillar spaces produce higher bond strengths (Carvalho et al., 2003). Ethanol-containing adhesives present the risk of solvent evaporation from containers upon inappropriate storage and from frequent "bottle" opening in clinical practice, which may reduce shelf-life. Another disadvantage is that the negative impact of residual ethanol on water sorption and solubility are greater as the hydrophilicity of the resin blends increase (Malacarne-Zanon et al., 2009).

2.6.3 Dimethyl sulfoxide (DMSO)

DMSO, $(CH_3)_2SO$, is a dipolar aprotic solvent with high solvent power for a great variety of inorganic and organic compounds (Martin et al., 1967). It is a colorless, highly transparent and hygroscopic liquid with a slightly bitter taste (Martin et al., 1967). DMSO possesses a high dielectric constant and low surface energy. It mixes with water in an exothermic reaction and it is also miscible with alcohols, acetone, chloroform, ethers and other organic solvents (Capriotti & Capriotti, 2012). DMSO has the proper polarity to break down water's self-associative tendencies and to form stable complexes with water since DMSO-water interactions is 1.3-fold stronger than water-water complexes (Mehtälä et al., 2017). DMSO reduces the surface tension and cohesive forces of water and thus improves wetting (Mehtälä et al., 2017). At concentrations above 80 % (v/v), DMSO overextends collagen resulting in swelling (Mehtälä et al., 2017) by destabilization of the collagen structure and disruption of inter molecular bonds (Zimmerley et al., 2009). Water contributes to collagen triple helix shape and stability (Bertassoni et al., 2012a). In high concentrations, DMSO may interfere with water-collagen hydrogen bonding (Mehtälä et al., 2017; Zimmerley et al., 2009). Water molecules may be replaced by DMSO due to stronger DMSO-water interaction than water-water (Kirchner & Reiher, 2002) **(Figure 2)**. Modifications in hydrogen bonding within the triple helix certainly contribute to alterations in the collagen structure when submitted to high concentrations of DMSO (Mehtälä et al., 2017). Therefore, DMSO concentrations above 80 % (v/v) should be strictly avoided in resin-dentin bonding. DMSO is also able to dissolve virtually all methacrylate-based dental monomers (Geurtsen et al., 1998). Acute toxicity resulting from oral, dermal or parenteral intake is very slight (Capriotti & Capriotti, 2012). Long-term oral or dermal administrations also produce only slight toxicity (Capriotti & Capriotti, 2012). 70 % DMSO solutions on dermal administrations are usually tolerated without symptoms (Capriotti & Capriotti, 2012). Considering dental applications, DMSO causes no to minor cytotoxic effects on the pulp tissue (Hebling et al., 2015). Besides its great solvent capability, DMSO is considered an outstanding infiltration facilitator in contemporary medical practice. Recently, DMSO has been considered for dental adhesive applications, showing promising results on long-term resin-dentin bonding (Tjäderhane et al., 2013b). The

benefits of DMSO (*i.e.,* 0.004 to 50 %) on resin-dentin bonding have been shown in several *in vitro* studies published by different research groups (Szesz et al., 2021; Salim Al-Ani et al., 2018; Guo et al., 2017; Stape et al., 2016, 2015; Tjäderhane et al., 2013b). DMSO improvements in resin-dentin bonding have been attributed to the increase in collagen interfibrillar interspacing (Zimmerley et al., 2009), better dentin wettability (Mehtälä et al., 2017), higher monomer penetration within demineralized dentin (Stape et al., 2015) and lower activity of endogenous hydrolytic enzymes (Tjäderhane et al., 2013c). Pure DMSO has low hydrogen bonding capacity [13.1 $(J/cm^3)^{1/2}$], suggesting reduced re-expansion potential for air-dried collagen. In addition, DMSO presents low vapor pressure (0.42 mmHg at 20 °C), which hampers its subsequent removal by evaporation. When used as a dentin pretreatment, DMSO removal by blot- or air-drying before adhesive application is a critical step to avoid excessive monomer dilution and thereby reduce possible polymerization issues.

Table 1. Physical/chemical properties of organic solvents.

	Water	**Ethanol**	**DMSO**
Molecular weight (g mol^{-1})	18.01	46.07	78.13
Vapor pressure at 20 °C (mmHg)	17.54	44	0.417
Relative polarity	1.0	0.654	0.444
Boiling point (°C)	100	78.5	189
Melting point (°C)	0	-114	19
Density (g/ml)	0.998	0.789	1.092
Dipole moment (D)	1.85	1.7	3.9
Dielectric constant	80.1	24	46.7
Viscosity at 25 °C (cP)	1.005	1.1	1.996
H-bonding capacity $(J/cm^3)^{1/2}$	42.3	19.4	13.1
Surface tension at 20 °C (dyn/cm)	72.8	22.27	43.53
Chemical structure			

Units: g mol^{-1} = gram per mole; °C = degree Celsius; mmHg = millimeter of mercury; g/ml = grams per milliliter; cP = centipoise; J/cm^3 = joule per cubic centimeter; Dyn/cm = dyne per centimeter.

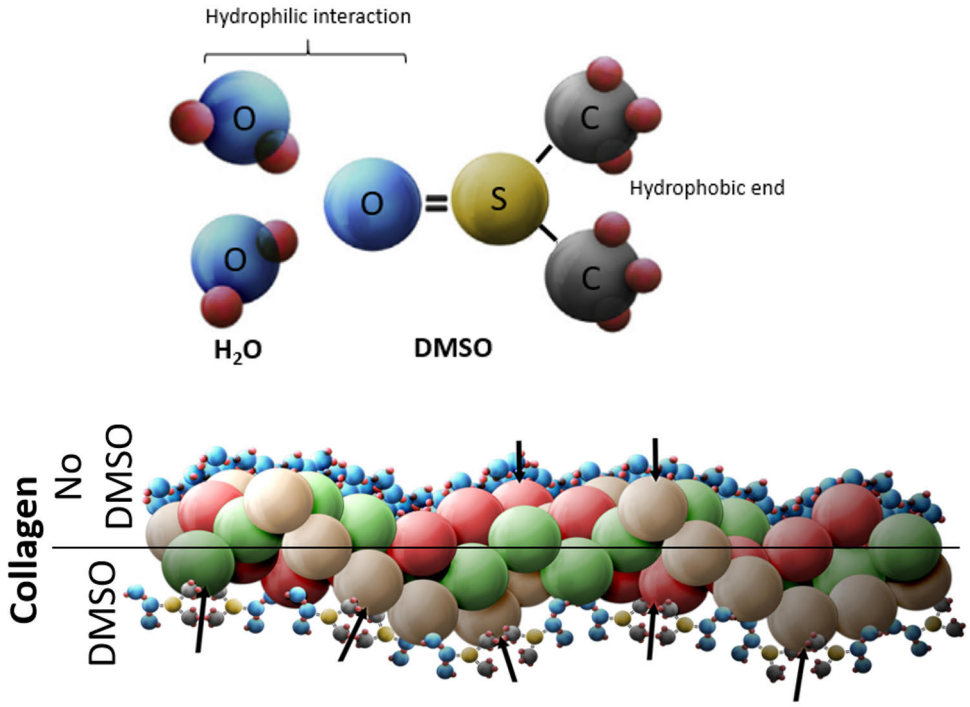


Figure 2. DMSO-water interaction and the proposed water displacement mechanism (Stape et al., 2016). Triple-helical collagen molecules are covered with bound water, which limits chemical interactions of more hydrophobic monomers with collagen. DMSO may disrupt this water layer creating binding sites for more hydrophobic monomers (black arrows).

2.7 Strategies to preserve resin-dentin bonding

Although significant improvements in the past decades have enabled resin-dentin interfaces to last longer, *in vitro* studies still reveal considerable degradation over time. Therefore, several strategies have been proposed to further optimize resin-dentin bonding in attempt to counteract some of the bond-degradation pathways.

2.7.1 Improving resin polymer formation and stability

The importance of proper adhesive polymerization should not be underestimated on hybrid layer formation. A well-polymerized adhesive interface is a prerequisite to achieve long-term stable hybrid layers (Van Meerbeek et al., 2020). Since the degradation of resin-dentin interfaces is directly related to water sorption (Pucci et al., 2018; Liu et al., 2011), hybrid layer composition should be as hydrophobic as possible to limit water uptake. Clearly, the hydrophobicity of adhesives system should comply with the hydrophilic character of dentin to avoid possible

incompatibilities and thus infiltration problems. Hence, adhesive systems should be optimized to contain well-balanced monomer formulations with effective photo-initiator systems. For instance, the use of hydrophobic photoinitiators, such as camphorquinone, for hydrophilic adhesive formulations normally result in suboptimal polymerization, especially in the presence of water (Ye et al., 2009a). The degree of conversion of hydrophilic bonding resins may be improved by the use of hydrophilic photoinitiators such as QTX [2-hydroxy-3-(3,4-dimethyl-9-oxo-9H-thio-xanthen-2-yloxy)-N,N,N-trimethyl-1-propanaminium chloride] or TPO [diphenyl(2,4,6-trimethylbenzoyl)-phosphine oxide (Cadenaro et al., 2010; Ye et al., 2009b). Incorporation of compatible accelerators and bulky/branched esterase-resistant hydrophilic urethane-modified resin monomers also benefits polymer formation. Higher conversion may also reduce the susceptibility of resin-dentin bonded interfaces to esterase hydrolysis by reducing the number of unreacted functional groups (Liu et al., 2011). Proper adhesive application is also critical to produce hybrid layers with better stability. For instance, adequate solvent evaporation and longer curing times than those recommended by manufactures (Van Meerbeek et al., 2020). Careful adhesive application must be performed for resin-dentin bonding regardless of adhesive type. These strategies and tweaks in adhesive formulation certainly benefit polymer formation at the resin-dentin interfaces, but they will not improve the ability of resin monomers to better interact and infiltrate into demineralized dentin.

2.7.2 Ethanol-wet bonding

The ethanol-wet bonding technique was proposed in 2007 (Becker et al., 2007). It aims to eliminate water-rich zones within demineralized collagen by gradually replacing water with ethanol for posterior monomer infiltration (Becker et al., 2007; Tjäderhane, 2015; Liu et al., 2011; Pashley et al., 2007). Ethanol-wet bonding essentially dehydrates collagen to assist the infiltration of more hydrophobic monomers (Tjäderhane, 2015; Liu et al., 2011; Pashley et al., 2007). Hydrophobic resins are considerably difficult to apply to dentin due to its hydrophilic character (Tay et al., 2007). The sequential dehydration steps of ethanol-wet bonding improve miscibility of the solvated adhesive and the collagen matrix, enabling hydrophobic resins to better infiltrate the ethanol-saturated collagen matrix (Tay et al., 2007). Higher uptake of hydrophobic monomers by the hybrid layer reduces water sorption, water solubility and resin plasticization possibly contributing to lower enzymatic hydrolysis of collagen (Sadek et al., 2010a; Hosaka et al., 2009). Ethanol-wet bonding is considered one of the most effective strategies (Van Meerbeek et al., 2020; Pashley et al., 2007) to improve etch-and-rinse hybrid layer formation *in vitro,* especially when combined with hydrophobic-bonding monomers (Li et al., 2012;

Sadek et al., 2010a). There are two versions of the ethanol-wet bonding, a progressive and a simplified technique. The progressive ethanol replacement is based on gradual water removal from collagen via a series of ascending ethanol concentrations (Becker et al., 2007). In the simplified technique, absolute ethanol is applied to water-saturated acid-etched dentin for 1 min, in a single step, prior to the application of ethanol-solvated adhesive resin (Salvatore et al., 2010; Sadek et al., 2010c; Nishitani et al., 2006). Regardless of technique, when ethanol replacement is not meticulously performed to prevent collagen exposure to air, collapse of the collagen matrix prevents optimal infiltration of the adhesive monomers (Tay et al., 2010). Therefore, ethanol-wet bonding is highly technique sensitive and clinically impractical due to the time needed for successive ethanol applications for adequate dentin dehydration (Sadek et al., 2010b, 2010c; Tay et al., 2010). When tested *in vivo*, ethanol-wet bonding was unable to replicate the immediate benefits observed *in vitro* (Kuhn et al., 2015). Ethanol-wet bonding can be greatly compromised by water contamination. As little as 5 % water can reduce bond strengths by 25 % (Sadek et al., 2007). Unfortunately, detrimental effects on composite durability *in vivo* have been reported (Kuhn et al., 2015).

2.7.3 Inhibition of enzymatic activity

Matrix metalloproteinases (MMP) participate in the degradation of exposed collagen fibrils within incompletely resin-infiltrated hybrid layers (Breschi et al., 2018; Liu et al., 2011; Tjäderhane, 2015, 2013a). Although it is not clear whether collagen degradation or resin plasticization occurs first, electron microscopy studies indicate that collagen degradation normally precedes resin loss (Lorenzo Breschi et al., 2018). Lower collagen degradation also results in improved hybrid layer integrity, lower incidence of nanoleakage and improved bond strength durability (Breschi et al., 2018; Hebling et al., 2005). Furthermore, collagen degradation allows higher water flow through the bonded interface, which may accelerate resin plasticization (Breschi et al., 2018). Since the activity of endogenous collagenolytic enzymes is dependent on metal ions (*i.e.,* Zn^{2+} and Ca^{2+}), inhibition of metalloproteinases can occur by a chelating mechanism. Chlorhexidine has been the metalloproteinase inhibitor most commonly studied (Breschi et al., 2018) and it is also considered a cysteine cathepsin inhibitor (Breschi et al., 2010a). Chlorhexidine concentrations as low as 0.2 % solutions have been shown to be non-specific metalloproteinase inhibitors, thereby contributing to extend the longevity of hybrid layers (Kiuru et al., 2021; Breschi et al., 2020, 2010a; Montagner et al., 2014; Carrilho et al., 2007; Hebling et al., 2005). Since chlorhexidine binding to dentin is only electrostatic (Kim et al., 2010a), chlorhexidine leaching may limit the duration the metalloproteinase inhibition over long periods (Van Meerbeek et al., 2020; Breschi et al., 2018).

Nonetheless, demineralized dentin can bind more chlorhexidine than mineralized dentin (Kim et al., 2010a). Sadek et al., reported that chlorhexidine pretreated demineralized dentin bonded with commercial adhesives were preserved after 9 months, but not necessarily after 18 months (Sadek et al., 2010a). Although these findings raise concerns about the effectiveness of metalloproteinase inhibitors on the long run, especially considering chlorhexidine, recent publications may suggest otherwise (Breschi et al., 2020). 0.2 % chlorhexidine may remain in the hybrid layer after 10 years of storage in artificial saliva with preserved MMP inhibitory effect (Breschi et al., 2020). Hence, there is substantial evidence indicating that metalloproteinase inhibition by chlorhexidine is certainly an underlying factor for hybrid layer preservation *in vitro* (Kiuru et al., 2021; Montagner et al., 2014) after long aging periods (Breschi et al., 2020). Quaternary ammonium compounds, positively charged at physiological pH, can inhibit endogenous enzymatic activity of dentin, similarly to chlorhexidine through a cationic mechanism (Breschi et al., 2018; Liu et al., 2011). Benzalkonium chloride is a quaternary ammonium compound which strongly binds to demineralized dentin producing a similar immediate inhibition activity to chlorhexidine (Tezvergil-Mutluay et al., 2011b). Quaternary ammonium methacrylates, such as 12-methacryloyloxydodecylpyridinium bromide (MDPB), also inhibit metalloproteinases (Tezvergil-Mutluay et al., 2011c) presenting antimicrobial properties. MDPB has been incorporated into commercially available adhesive systems. Pharmaceutical agents have also been shown to inhibit metalloproteinases through chelating mechanisms. Polyvinylphosphonic acid, a bisphosphonate, has shown acceptable immediate results, but with questionable longevity (Tezvergil-Mutluay et al., 2011b). Tetracycline and its analogs (doxycycline and minocycline) have shown collagenases and gelatinases inhibitory properties (Sorsa et al., 2006), but their protective effect on hybrid layers has not been documented. Specific metalloproteinase inhibitors, such as galardin, have also shown reduction in hybrid layer degradation (Breschi et al., 2010b). Preventing hybrid layer degradation with the use of enzymatic inhibitors will continue to be an important approach to extend the longevity of hybrid layers until more proactive solutions become clinically available (Tjäderhane, 2015).

2.7.4 Collagen biomodification by crosslinkers

Cross-linking is a naturally occurring phenomenon in dentin collagen. Dentin collagen is reinforced by native inter- and intramolecular cross-links. Enhancement in collagen cross-links causes biomodification of the collagen scaffold, which improves the biomechanical properties of dentin (Bedran-Russo et al., 2014, 2008). The use of cross-linking agents not only improves the short-term mechanical

properties of dentin collagen, but it reduces the susceptibility of dentin collagen to enzymatic degradation by collagenases. Therefore, collagen cross-linking contributes to improved stability of the resin-dentin interface (Van Meerbeek et al., 2020; Breschi et al., 2018; Tjäderhane, 2015; Liu et al., 2011;). Cross-linking agents such as glutaraldehyde, genipin, proanthrocyanidin, riboflavin and carbodiimide have been reported to effectively create additional cross-links in acid-demineralized dentin collagen (Bedran-Russo et al., 2014; Liu et al., 2011). Collagen cross-linking may also offer the possibility to increase hydrophobic monomer uptake by the hybrid layer using a dry-bonding approach with lower risk of collagen collapse during adhesive application (Zhou et al., 2016). Cross-linking agents affect enzymatic degradation by allosteric silencing of collagenolytic enzymes or by altering the enzyme binding site in the collagen molecule (Tjäderhane, 2015; Bedran-Russo et al., 2014; Tezvergil-Mutluay et al., 2012; Liu et al., 2011). Despite the effectiveness of aldehydes [*i.e.,* glutaraldehyde and acrolein (2-propenal)] in hybrid layer preservation, their substantial cytotoxicity makes them inadequate for clinical applications (Bedran-Russo et al., 2014). Carbodiimide hydrochloride has minimal cytotoxicity, but it may have limited cross-linking capacity (Bedran-Russo et al., 2014). Carbodiimide reduces/eliminates collagen degradation at the hybrid layer and preserves bond strength *in vitro*; however, application times may be considered too long for clinical use (Bedran-Russo et al., 2010). Proanthocyanidins are also effective at preserving the hybrid layer producing higher immediate bond strengths with shorter application times (Liu et al., 2013; Fang et al., 2012). Riboflavin has also shown promising results. However, long application times (*i.e.,* 5 min) (Fawzy et al., 2012) requiring blue light or even ultraviolet light activation (*i.e.,* 120 s) (Cova et al., 2011) reduce its clinical acceptability.

2.7.5 Biomimetic demineralization of hybrid layers

In resin-dentin bonding, the mineral phase of dentin is removed by acids, chelating agents or even acidic monomers resulting in collagen exposure. Especially in etch-and-rinse bonding, collagen fibrils are used for anchorage of resins monomers through micromechanical retention. Unfortunately, contemporary etch-and-rinse and self-etch adhesives are incapable of fully replacing water from extrafibrillar and intrafibrillar collagen compartments with resin monomers (Carvalho et al., 2005). As a result, denuded collagen is normally found at resin-dentin interfaces regardless of the bonding mechanism used (Carvalho et al., 2005). Biomimetic remineralization replaces water from resin-sparse regions of the hybrid layer with small apatite crystals that occupy the extrafibrillar and intrafibrillar compartments of the collagen matrix (Niu et al., 2014; Kim et al., 2010b; Tay & Pashley, 2009). In the hybrid layer, water replacement by minerals increases mechanical properties and inhibits

proteolytic hydrolysis. In biomimetic remineralization of the hybrid layer, polyanions bind to collagen and function as analogs of phosphoproteins to regulate physiological mineralization (Niu et al., 2014; Tay & Pashley, 2009). This condition allows calcium binding and promotes apatite nucleation and thus remineralization (Tay & Pashley, 2009). This strategy has great potential, but to date it is still at the proof-of-concept stage (Van Meerbeek et al., 2020; Tjäderhane, 2015).

2.7.6 DMSO-wet bonding

Taking in consideration that the effect of DMSO on water-saturated collagen could potentially benefit resin-dentin interfaces, the first study to investigate and report direct improvements of hybrid layers by DMSO was published by Tjäderhane et al., in 2013 (Tjäderhane et al., 2013b). A quite low-DMSO concentration (0.004 %) was applied on H_3PO_4-etched dentin following the wet-bonding approach. Even at such low concentrations, DMSO inhibited MMP without any negative effects on hybrid layer formation. In addition, higher stability of long-term bond strengths with lower nanoleakage occurred for DMSO-treated dentin, while significant lower values were observed for untreated samples. The term DMSO-wet bonding was first mentioned in 2016 (Stape et al., 2016) to describe the use of higher-DMSO concentrations (*i.e.,* 50 % v/v in water) as pretreatments for H_3PO_4-etched dentin following the wet-bonding approach, albeit the technique was initially described in 2015 (Stape et al., 2015). Higher DMSO concentrations facilitates monomer diffusion into demineralized dentin, resulting in immediate bond strengths that were roughly 50 % higher (Stape et al., 2015). Improvements in collagen wettability by DMSO certainly contributed to better monomer diffusion and thereby higher immediate bond strengths (Mehtälä et al., 2017). DMSO's water displacing mechanism has a major role on the higher hydrolytic stability and reduced water-filled zones of etch-and-rinse hybrid layers (Stape et al., 2016). The proposed disruption of residual-water layers surrounding collagen fibrils by DMSO facilitates the infiltration of hydrophobic monomers to strengthen hybrid layers (Stape et al., 2016, 2015). Improvements in monomer conversion in the hybrid layer certainly contributed to the higher stability of resin-dentin interfaces over time (Stape et al., 2016).

3 Aims

The aim of this study series was to evaluate the central hypothesis that DMSO may improve collagen hybridization of etched dentin under dry conditions. Different uses of DMSO were analyzed, including application form (*i.e.,* as a dentin pretreatment or incorporation into the bonding resins) and DMSO combination with cosolvents (*i.e.,* water or ethanol), to determine limitations and advantages of each bonding approach. The main goal was to identify the most effective application method to potentially optimize the etch-and-rinse technique under dry conditions. Resin-dentin interfaces were characterized to identify possible changes in collagen-hybridization mechanism produced by DMSO.

The specific aims of these studies were to:

I. Evaluate whether the use of DMSO as a potential solvent in adhesive dentistry incorporated in relatively hydrophilic resins could benefit long-term resin-dentin bonding. Assess the impact of DMSO incorporation on the mechanical/physical properties of bonding resins *(Study I)*;
II. Investigate whether immediate resin bonding to air-dried-etched dentin pretreated with binary-DMSO solutions would be comparable to conventional wet-bonding. Evaluate the impact of DMSO-dry bonding on hybrid layer quality *(Study II)*;
III. Evaluate whether DMSO-dry bonding and DMSO incorporation into a hydrophilic primer would permit adequate resin-dentin bonding to air-dried-etched dentin and determine their effect on collagen degradation by host-derived enzymes *(Study III)*;
IV. Examine the effect of DMSO pretreatments on the mechanical properties and integrity of collagen. Investigate polymer formation at the hybrid layer and evaluate the effect of long-term aging on the bonding performance of DMSO-dry bonded resin-dentin interfaces *(Study IV)*.
V. Investigate the possibility of air-drying DMSO-pretreated-etched dentin as the sole form of moisture control in attempt to discard the necessity of maintaining collagen moist before hybridization *(Study V)*.

4 Materials and Methods

4.1 Materials

4.1.1 Sound human dentin

Four hundred and sixty-four extracted sound human third molars were obtained with informed consent from patients (age 18 – 32 years) under a protocol approved by the University of Oulu (#23-2003). Extraction indications were not related to the present study series. Tooth collections were performed in accordance with local guidelines and regulations. Teeth were stored in 0.9 % NaCl containing 0.02 % NaN_3 at 4 °C and used no later than 3 months after extractions.

4.1.2 Commercial and experimental adhesives

Different methacrylate-based bonding resins were used following the etch-and-rinse application mode. **Table 2** displays the composition, classification and application modes for the bonding resins. Neat light curing resin-blends containing 56 % (w/w) 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)]-phenylpropane (BisGMA), 28.65 % 2-hydroxyethyl methacrylate (HEMA), 0.25 % camphorquinone (CQ), 1 % 2-ethyl-4-aminobenzoate (EDMAB), and 0.1 % butylhydroxytoluene (BHT) were produced. This resin was solvated in 20 % ethanol (control) and in gradually increasing DMSO (Dimethyl Sulfoxide, Sigma-Aldrich, St Louis, MO, USA) concentrations to produce six experimental bonding resins containing a gross w/w % of 0, 0.5, 1, 2, 4, and 10 % DMSO. One commercial three-step etch-and-rinse adhesive (Scotchbond Multipurpose, 3M ESPE; SBMP) and a commercial universal adhesive (Scotchbond Universal, 3M ESPE; SU) were used for dentin bonding. In order to produce the DMSO-containing multistep bonding resin, SBMP primer was evaporated at room temperature to remove 10 wt % of the original solvent composition and thus avoid changes in the original monomer-solvent ratio. Subsequently, 10 wt % DMSO was added to replace the evaporated aliquot. The newly formulated DMSO-containing primer was mixed ultrasonically for 60 s. A summary of adhesive use according to the different studies is shown in **Table 3**.

Table 2. Classification, composition and application modes for the bonding resins.

Bonding resin	Classification	Composition	DMSO wt %	pH	Application mode*
Scotchbond Multipurpose (3M ESPE)	3-step etch-and-rinse adhesive	*Primer:* HEMA, polyalkenoic acid methacrylate copolymer and water	0	3.3	a, b, c, d, e
		Adhesive: BisGMA, HEMA, dimethacrylates and photoinitiators	0	-	f, e, i
Scotchbond Multipurpose + DMSO (3M ESPE)	3-step etch-and-rinse adhesive	*Primer:* HEMA, polyalkenoic acid methacrylate copolymer, DMSO and water	10	3.4	a, b, c, d, e
		Adhesive: BisGMA, HEMA, dimethacrylates and photoinitiators	0	-	f, e, i
Scotchbond Universal (3M ESPE)	Universal adhesive	MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate modified polyalkenoic acid copolymer, filler, ethanol, water, initiators and silane	0	2.7	a, b, c, g, e, i
Experimental resins	2-step etch-and-rinse adhesive	BisGMA, HEMA CQ, EDMAB and BHT	0, 0.5, 1, 2, 4 and 10	-	a, b, c, h, e, h, e, i

* Following manufacture's recommendations.

Abbreviations: BisGMA = bisphenol glycidyl methacrylate; TEGDMA = tryethylene glycol dimethacrylate; MDP = methacryloyloxydecyl dihydrogen phosphate; HEMA = 2-hydroxyethyl methacrylate; a: dentin etching for 15 s; b: water rinse 15 s; c: blot drying; d: active Primer application for 10 s; e: gentle blow-drying for 10 s; f: active Adhesive application for 10 s; g: active Universal adhesive application for 20 s; h: active experimental resin application for 10 s; and i: light cure for 10 s.

Table 3. Methods and adhesive use according to the different studies.

Method	Study	Primary Outcome	Samples per group	Adhesive	Study factors and levels	Aging
Microtensile test	I	Dentin bond strength	Tooth (n = 10)	Experimental resins with 0, 2, 4 and 10 % DMSO	*DMSO incorporation:* 0 , 2, 4 and 10 % *Time:* 24 h and 2 years.	24 h 2 years
	II	Dentin bond strength	Tooth (n = 8)	Scotchbond Multipurpose (3M ESPE) Scotchbond Universal (3M ESPE)	*Adhesive:* Multistep and simplified. *Dentin condition*: dry and wet. *DMSO treatment:* DMSO/H_2O, DMSO/EtOH.	24 h
	III	Dentin bond strength	Tooth (n = 8)	Scotchbond Multipurpose Scotchbond Multipurpose + 10 % DMSO	*Dentin condition*: dry and wet. *DMSO treatment*: DMSO/H_2O, DMSO/EtOH, DMSO in resin, no treatment.	24 h
	IV	Dentin bond strength	Tooth (n = 8)	Scotchbond Multipurpose	*Dentin treatment:* no treatment, EtOH-wet bonding, DMSO/H_2O, DMSO/EtOH. *Time*: 24 h and 2.5 years.	24 h 2.5 years
	V	Dentin bond strength	Tooth (n = 8)	Scotchbond Multipurpose	*Dentin treatment:* no treatment, DMSO/H_2O, DMSO/EtOH. *Dentin condition*: dry (different stages) and wet *Time*: 24 h and 2 years.	24 h 2 years

Method	Study	Primary Outcome	Samples per group	Adhesive	Study factors and levels	Aging
SEM Nanoleakage	II	Hybrid layer integrity	Tooth (n = 8) 2 beams/tooth	Scotchbond Multipurpose Scotchbond Universal	*Adhesive:* Multistep and simplified. *Dentin condition*: dry and wet. *DMSO treatment:* DMSO/H_2O, DMSO/EtOH.	24 h
	IV	Hybrid layer integrity	Tooth (n = 8) 3 beams/tooth	Scotchbond Multipurpose	*Dentin treatment:* no treatment, EtOH-wet bonding, DMSO/H_2O, DMSO/EtOH.	2.5 years
	V	Hybrid layer integrity	Tooth (n = 8) 3 beams/tooth	Scotchbond Multipurpose	*Dentin treatment:* no treatment, DMSO/H_2O, DMSO/EtOH. *Dentin condition*: dry (different stages) and wet *Time*: 24 h and 2 years.	2 years
Micropermea -bility	II	Hybrid layer integrity	Tooth (n = 2)	Scotchbond Multipurpose Scotchbond Universal	*Adhesive:* Multistep and simplified. *Dentin condition*: dry and wet. *DMSO treatment:* DMSO/H_2O, DMSO/EtOH.	24 h
In situ zymography	III	Collagenolytic activity within hybrid layer	Tooth (n = 2)	Scotchbond Multipurpose Scotchbond Multipurpose + 10 % DMSO	*Dentin condition*: dry and wet. *DMSO treatment*: DMSO/H_2O, DMSO/EtOH, DMSO in resin, no treatment.	7 days

Method	Study	Primary Outcome	Samples per group	Adhesive	Study factors and levels	Aging
Hydroxyproline quantification	III	Collagen solubilization	Dentin powder (n = 5) 25 mg/sample	DMSO/H_2O, DMSO/EtOH, DMSO in resin	*Dentin condition*: dry and wet. *DMSO treatment*: DMSO/H_2O, DMSO/EtOH, DMSO in resin, no treatment.	-
Elastic modulus	I	Mechanical property of bonding resins	Resin bars (n = 10)	Experimental resins with 0, 0.5, 1, 2, 4 and 10 % DMSO	*DMSO incorporation:* 0, 0.5, 1, 2 , 4 and 10 % DMSO	24 h
	IV	Mechanical property of collagen	Collagen beams (n = 10)	DMSO/H_2O, DMSO/EtOH, EtOH, DMSO and no treatment	*Dentin treatments:* DMSO/H_2O, DMSO/EtOH, EtOH, DMSO and no-treatment. *Time:* 7 days and 30 days	7 days 30 days
Degree of conversion	I	Monomer conversion	Resin discs (n = 8)	Experimental resins with 0, 0.5, 1, 2, 4 and 10 % DMSO	*DMSO incorporation:* 0, 0.5, 1, 2, 4 and 10 % DMSO	-
	IV	Monomer conversion in the hybrid layer	Resin-dentin beams (n = 6)	DMSO/H_2O, DMSO/EtOH, EtOH, DMSO and no treatment	*Dentin treatment:* no treatment, EtOH-wet bonding, DMSO/H_2O, DMSO/EtOH.	-

Method	Study	Primary Outcome	Samples per group	Adhesive	Study factors and levels	Aging
Water sorption and solubility	I	Water interaction with polymer	Resin discs (n = 10)	Experimental resins with 0, 0.5, 1, 2, 4 and 10 % DMSO	*DMSO incorporation:* 0, 0.5, 1, 2, 4 and 10 % DMSO	7 days water immersion
Gel zymography	III	Gelatinolytic activity	Dentin powder (n = 4) 200 mg/sample	DMSO/H_2O, DMSO/EtOH, DMSO in resin	*Dentin condition*: dry and wet. *DMSO treatment*: DMSO/H_2O, DMSO/EtOH, DMSO in resin, no treatment.	-
Flexural Strength	I	Mechanical property of bonding resins	Resin beams (n = 10)	Experimental resins with 0, 0.5, 1, 2, 4 and 10 % DMSO	*DMSO incorporation:* 0, 0.5, 1, 2, 4 and 10 % DMSO	24 h
Loss of dry mass	IV	Indirect indicator of collagen matrix degradation	Dentin beams (n = 10)	DMSO/H_2O, DMSO/EtOH, EtOH, DMSO and no-treatment	*Dentin treatments:* DMSO/H_2O, DMSO/EtOH, EtOH, DMSO and no-treatment.	30 days
Contact angle measurement	V	Wettability	Dentin discs (n = 8)	Scotchbond Multipurpose	*Dentin treatment:* no treatment, DMSO/H_2O, DMSO/EtOH. *Dentin condition*: dry (different stages) and wet *Time*: 24 h and 2 years.	-

4.1.3 Pretreatment solutions

Pretreatment solutions were obtained by diluting DMSO in either distilled water (DMSO/H_2O) or ethanol (99.8 % Ethanol, Sigma-Aldrich, St Louis, MO, USA; DMSO/EtOH) using a graduated cylinder. The 50 % (v/v) binary solutions were prepared 24 h before use. DMSO was poured inside the graduated cylinder followed by the respective solvent (water or ethanol) to reach twice the initial volume of DMSO with minimal splashing of the solutions. The content was transferred to a 100 ml glass reagent bottle, which was then closed and swirled in circular motion for 30 s to mix the binary solutions. Pretreatments were kept from light and at room temperature during use.

4.2 Research methods

Thirteen research methods were included in this study series to evaluate the possibility of employing different dry-bonding approaches containing DMSO to bond methacrylate-based resins to demineralized dentin. Resin-dentin bonding performance was assessed directly or indirectly via mechanical, enzymatic, microscopic and spectroscopic tests. The distribution of methods in accordance with the different studies and the primary outcomes, samples per group, dentin pretreatments, aging periods and study factors are summarized in **Table 3**.

4.2.1 Bonding procedures

To produce resin-dentin beams for the mechanical and microscopic assessments, caries-free third molars were coronally sectioned under water-cooling to expose flat mid-coronal dentin surfaces using a slow speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA). Absence of remaining enamel on dentin surfaces was verified with a stereomicroscope (Leica M60, Leica Microsystems, Wetzlar, Germany) at 40× magnifications. Roots were removed 1 mm below the cervical line and discarded. Exposed dentin surfaces were standardized by wet-polishing with 320-grit SiC paper for 60 s. Crown segments were randomly allocated into their respective groups following each study design. Dentin etching with 32 % H_3PO_4 for 15 s was performed followed by water rinsing for 15 s. For the wet-bonding protocols (*i.e.*, control groups), blot-drying with paper tissues was carefully performed leaving the dentin surface slightly moist, but not overwet. Dry-bonding was performed by a continuous air blast using a 3-way syringe at 10 cm, for 30 s. Dentin pretreatments were performed consisting of active (*Study II, III and V*) or passive (*Study IV*) application of 50 µL DMSO/H_2O or DMSO/EtOH pretreatments on etched dentin followed by blot drying. To standardize moisture control for the wet-bonding procedures, blot-drying was performed until paper filters no longer absorbed

moisture from the bonding surface by capillarity. This produced a partially moist dentin surface following the wet-bonding approach. In *Study V*, pretreatment solutions were further blot-dried or air-dried for 30 s before hybridization. Bonding resins were applied with a slight rubbing motion onto etched dentin surfaces totaling 20 s for Scotchbond Universal (3M ESPE) and 10 s for each of Scotchbond Multipurpose's (3M ESPE) primer and bond resins. The manual pressure was approximately 4.0 g. Composite blocks were built with a nanofilled composite resin (Filtek Z350 XT, 3M ESPE, Shade A2) by layering 2 mm increments. Each increment was light-cured for 20 s using a LED light-curing unit (Elipar Deepcure, 3M ESPE) at 1200 mW/cm^2. Bonding procedures were carried out by a single operator. The restored crown segments were stored in distilled water for 24 h at 37 °C to allow water sorption and postoperative polymerization.

4.2.2 Microtensile bond strength (*Study I, II, III, IV and V*)

Resin-dentin beams were produced with a cross-sectional area of approximately 0.8 mm^2 by sectioning the restored crowns longitudinally in mesio-distal and buccal-lingual directions perpendicular to the bonded interface with a slow-speed diamond saw (Isomet, Buehler Ltd) under water-cooling. Resin-dentin bond strength evaluation followed the Academy of Dental Materials guidelines for non-trimmed microtensile bond strength *(μTBS)* testing (Armstrong et al., 2017). A minimum of 7 beams per tooth were randomly tested on each storage period (*i.e.*, 24 h or after long-term aging) to produce a research design balanced by tooth dependency (Armstrong *et al.*, 2017). For *Study IV and V*, resin-dentin beams were stored in artificial saliva at 37 °C to simulate accelerated aging of bonded interfaces for 2.5 and 2 years, respectively. The artificial saliva (pH 7.4) contained 5 mM HEPES, 2.5 mM $CaCl_2 \cdot H_2O$, 0.05 mM $ZnCl_2$, and 0.3 mM NaN_3 (Tezvergil-Mutluay et al., 2010a). Specimens were tested by a blinded operator. Beams were individually attached to a microtensile testing jig using a cyanoacrylate adhesive (Loctite 416, Henkel Corp., Dublin, Ireland) and tested under tension (Bisco, Schaumburg, IL, USA) at a crosshead speed of 0.5 mm/min until failure. The force (*P*) in N required to fracture the sample and the dimensions of the cross-sectional area (*A*) in mm^2 were recorded with a digital caliper to the nearest 0.01 mm. The tensile bond strength (MPa) was calculated by dividing *P*/*A*. Pretesting failures were recorded and considered as 0 MPa for statistical analyses. Tooth was considered the statistical unit, the bond strength average of resin-dentin beams tested at each period representing the *μTBS* for each tooth. Both surfaces of fractured resin-dentin beams were analyzed in a stereomicroscope (Leica M60, Leica Microsystems) and when in doubt, a scanning electron microscope (Phenom ProX, Phenom-World, Eindhoven, Netherlands) was used to determine the fracture patterns. Fracture modes were

classified as: cohesive (failure exclusive within dentin or resin composite); adhesive failure (failure at resin/dentin interface); and mixed failure (failure at resin/dentin interface with cohesive failure of the neighboring substrates). Statistical analyses were performed by factorial ANOVA followed by the Tukey test ($\alpha = 0.05$).

4.2.3 Nanoleakage evaluation (*Study II, IV and V*)

Nanoleakage evaluation was performed according to a protocol previously described by Tay et al., (Tay et al., 2002). Briefly, resin-dentin beams were initially wet-polished, 2000-grit SiC paper, and coated with nail varnish. Two layers were applied up to 1 mm of the bonded interfaces. Samples were rehydrated in distilled water for 1 h and immersed in 50 % (w/v) ammoniacal silver nitrate (pH 9.5). After 24 h of silver nitrate immersion, samples were thoroughly rinsed in distilled water for 120 s. and immersed in photo-developing solution (Kodak Professional D-76 developer, Kodak Rochester, NY) for 8 h under fluorescent light. This step is necessary to reduce silver ions into metallic silver grains within the water-filled voids along the bonded interface, thereby allowing microscopic visualization. Silver impregnated resin-dentin beams were then embedded in epoxy resin, wet-polished with 600-, 1000-, and 2000-grit SiC paper (Carbimet, Buehler Ltd.,) and 1, 0.25 (MetaDi, Buehler Ltd) and 0.05 µm (MasterPrep, Buehler Ltd) polishing pastes. Embedded samples were ultrasonically cleaned in distilled water after each polishing step for 5 min, air-dried for 2 h, mounted on aluminum stubs, dried in silica overnight and carbon-sputtered. Qualitative or semi-quantitative analyzes of nanoleakage extension was performed using SEM imaging on backscattering mode at 10 – 15 kV (Phenom ProX, Phenom-World). Sequential micrographs (2000 – 3000× magnification) were obtained from all resin-dentin interfaces to detect silver deposition. Total silver nitrate uptake was measured on the 2D acquired micrographs using open-source image software (ImageJ, National Institute of Health, Bethesda, MD, USA). A single blinded experienced examiner evaluated all images. The overall extension of silver uptake in µm along the bonded interface was calculated and converted into percentage values. To determine the predominant nanoleakage patterns, silver impregnated resin-dentin interfaces were evaluated at higher magnifications (4000 – 10000×). For the semi-quantitative analyzes, tooth was considered the statistical unit. In *Study IV*, statistical analyzes was performed by one-way ANOVA followed by Tukey test ($\alpha = 0.05$). As the normality assumption of the nanoleakage data was violated in *Study II*, data was analyzed by Kruskal–Wallis followed by Dunn-Bonferroni multiple comparison test ($\alpha = 0.05$).

4.2.4 Micropermeability evaluation (*Study II*)

The roots of extracted molars were removed 1 mm below the cemento-enamel junction and the pulpal tissue was carefully removed with tweezers. Two teeth (n = 2) were randomly selected for micropermeability analysis under simulated pulpar pressure. Bonding procedures were performed as previously described with the exception that the adhesives were doped with 0.1 wt % rhodamine B (Sigma-Aldricht, Louis, MO, USA) (Sauro et al., 2012). Sodium Fluorescein 5 mM (Sodium Fluorescein, Sigma-Aldricht) was selected as the fluorescent dye used to trace the water-filled spaces along the bonded interface was a solution of under simulated pulpar pressure (20 cm H_2O) for 3 h (Sauro et al., 2012, 2009). Restored crown segments were ultrasonically cleaned in distilled water for 60 s and sectioned into 0.4 mm mesio-distal slabs. Slabs were then slightly polished with 1200-grit SiC paper for 30 s and ultrasonically cleaned in distilled water for 60 s. Resin-dentin bonded interfaces were investigated and representative micropermeability patterns were recorded. Two blinded experienced examiners evaluated the resin-dentin interfaces. Imaging procedures were performed using a confocal laser scanning microscope (Leica SP5 TCS-CLSM, Leica Microsystems) equipped with a 63 × 1.4 NA oil immersion lens using 488 nm Argon and a 633 nm Helium-Neon ion laser illumination. CLSM fluorescence images were obtained from 20 µm optical sections using a 0.5 µm z-step, starting 1 µm below the surface. The z-axis scans were compiled into a single projection using Leica SP5 CLSM image-processing software (Leica, Microsystems).

4.2.5 *In situ* zymography (*Study III*)

Two teeth per group (n = 2) were prepared for qualitative analyses of collagenolytic activity at the hybrid layer via i*n situ* zymography. Freshly reconstituted FITC-conjugated collagen (D-12060, Molecular Probes, Eugene, USA) was actively applied for 60 s on etched dentin after DMSO-pretreatments. For the DMSO-incorporated resin, freshly reconstituted FITC-conjugated collagen was actively applied for 60 s previously to the application of the SBMP primer-containing DMSO. SBMP primer and adhesive were doped with rhodamine B powder 0.1 % (w/w). Bonding procedures were carried as previously described. Samples were stored at 37 °C for 7 days in calcium- and zinc-containing artificial saliva (5 mM HEPES, 2.5 mM $CaCl_2 \cdot H_2O$, 0.05 mM $ZnCl_2$, and 0.3 mM NaN_3, pH 7.4) and sectioned into 0.6 mm slabs. A minimum of 4 slabs were obtained per tooth. Slabs were wet-polished with 600, 1200 and 2000-grit SiC paper and ultrasonically cleaned in distilled water for 5 min. Resin-dentin interfaces were examined using a multiphoton confocal laser microscope (Leica SP5, Heidelberg, Germany) equipped with 63 ×/1.4NA oil immersion lens using a 488 nm argon laser (490 – 540 nm band pass

filter) and a 563 nm laser (580 – 630 bandpass filter). Z-stack scans (0.5 µm) were compiled into single 20 µm projections. FITC fluorescent emissions allowed identification of areas presenting collagenolytic activity due to FITC-conjugated-collagen breakdown by endogenous enzymes. Sequential images of the bonded interface were recorded and qualitatively analyzed to determine the intensity and extension of collagen hydrolysis.

4.2.6 Hydroxyproline quantification (*Study III*)

Collagen solubilization was assessed by hydroxyproline quantification. Ninety extracted human third molars were ground free of enamel, the roots were sectioned off and pulp soft tissues were removed. Dentin fragments were frozen in liquid nitrogen for 5 min followed by trituration at 24 Hz for 2 min in a ball mill (Model MM400, Retsch, Newtown, PA, USA). The produced dentin powder was sieved (Advantech Sonic Sifter, Advantech Mfg., New Berlin, MN, USA) to uniform particle size (< 300 µm). 5 g of dentin powder were demineralized in 10 wt % H_3PO_4 (pH ≈ 0.4) for 10 min, centrifuged at 12000 rpm for 20 min at 4 °C and rinsed twice with 1 ml distilled water. Demineralized dentin powder was dehydrated in a silica desiccator under partial vacuum for 72 h at 4 °C in order to remove loosely bound water and divided (25 mg/sample) into 12 groups (n = 5). Half of the samples were rehydrated with 5 µL/sample distilled water. Wet and dry samples were incubated in a shaking bath for 7 days at 37 °C in 1 mL of tested pretreatment solutions (*i.e.,* DMSO/H_2O and DMSO/EtOH) and in the DMSO-containing primer of SBMP. Dry control samples were incubated in distilled water or SBMP Primer. After the incubation period, 25 µL of the media was collected from each vial, freeze-dried for 72 h (Alpha 1 – 5, Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany) for solvent removal, subsequently re-suspended in 75 µL of distilled water and transferred to individual ampules. Solubilized collagen peptide fragments were assessed following a previously described hydroxyproline quantification protocol (Reddy & Enwemeka, 1996). Specimens were re-suspended with 25 µl water after freeze-drying. Aliquots of standard hydroxyproline (2 – 20 µg) prepared from stock solutions and test samples containing hydroxyproline under 10 µg/ml were mixed with 25 µl of 4 N sodium hydroxide (2 N final concentration) in a total volume of 50 µl in 2 ml Nalgene O-ring tubes. Samples were autoclaved at 120 °C for 20 min. 450 µl of chloramine-T was added to hydrolyzed tubes and mixed gently to allow oxidation for 20 min at room temperature. 500 µl Ehrlich's aldehyde reagent was added to each specimen for chromophore formation by incubating the specimens at 65 °C for 20 min. Absorbance values were obtained in a spectrophotometer (Model UV-A180, Shimadzu, Tokyo, Japan) at 550 nm and plotted against the standard hydroxyproline curves to determine the hydroxyproline release (µg/mg of dry

dentin). Data was analyzed by Kruskal–Wallis one-way ANOVA on ranks and Dunn's multiple comparison tests ($\alpha = 0.05$).

4.2.7 Gel zymography (*Study III*)

Gel zymography was performed in accordance with Mazzoni et al. (Mazzoni et al., 2007) to evaluate the effect of solvent and adhesive components on the gelatinolytic activity of demineralized dentin. Dentin powder (200 mg/sample) was demineralized in 10 wt % H_3PO_4 (pH ≈ 0.4) for 10 min, centrifuged at 12000 rpm for 20 min at 4 °C, rinsed twice with 1 ml distilled water and divided into 12 groups (n = 4) according to dentin condition (wet vs. dry) and treatment solutions (DMSO/H_2O, DMSO/EtOH, DSMO/SBMP). Distilled water, ethanol and SBMP Primer served as controls. For dry groups, demineralized dentin was dehydrated in a desiccator under partial vacuum for 72 h. Dentin powder was treated with 400 µL of the DMSO solutions, vortexed for 60 s and centrifuged to remove the supernatant. Samples were re-suspended in 1.8 mL extraction buffer for 24 h at 4 °C under constant stirring, sonicated for 20 min and centrifuged at 12000 rpm for 20 min at 4 °C. Sample aliquots were concentrated using a centrifugal concentrator device (10000-kDa cut-off, Vivaspin Sartorius Stedim Biotech, Goettingen, Germany) for 30 min at 20 °C (10000 rpm) until the volume was reduced to 20 µL. The Bradford assay was performed to determine the total protein concentrations. 100 µm of protein aliquots were diluted in Laemmli sample buffer and subjected to electrophoresis under non-reducing conditions in 10 % sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel containing 1 mg/mL gelatin, which had been fluorescently labeled with MDPF. A SDS-PAGE molecular weight standard (Dual Color Standards, Bio-Rad), was used along with purified MMP-2 and MMP-9 to allow specific match of corresponding MMP bands. After electrophoresis, gels were washed for 30 min twice in 2.5 % Triton X-100 with agitation and incubated in activation solution for 48 h at 37 °C. Zymography gels were monitored with UV light (Gel Doc XR System, Bio-Rad) to reveal the gelatinolytic bands in triplicate samples. Band intensities were calculated according to the peak area method with digital image analysis software (ImageJ, National Institute of Health, Bethesda, MD, USA).

4.2.8 Collagen apparent modulus of elasticity (*Study IV*)

Mid-coronal dentin discs measuring roughly 0.51 (± 0.06) mm were sectioned perpendicularly to the tooth long axis (Isomet, Buehler Ltd,) under water cooling. Discs were wet-polished with 600-grit SiC paper to remove superficial imperfections and to fine adjust their thickness to approximately 0.5 mm. They were then sectioned mesial-distally to produce rectangular beams (n = 10/group) measuring

approximately 0.5 mm in thickness × 1.7 mm in width × 7 mm in length (Leme-Kraus et al., 2017; Bedran-Russo et al., 2008). A dimple was made on the corner of each beam on the occlusal surface to allow repeated measures on the same beam surface. Beams were then demineralized in 10 % (w/w) phosphoric acid (Leme-Kraus et al., 2017; Tezvergil-Mutluay et al., 2012, 2010a, 2010b; Bedran-Russo et al., 2008) for 7 h at 24 °C under constant stirring. After the demineralization, beams were thoroughly rinsed with distilled water for 10 min to remove dissolved minerals and residual acid. Digital radiography was used to confirm the absence of mineralized dentin. To determine the thickness and width of the demineralized beams, digital images were obtained on a stereo microscope (Leica M60, Leica Microsystems). Dimensional measurements were made using an open source image analysis software (ImageJ, National Institute of Health, Bethesda, MD, USA). Beams were then placed on a 3-point flexure jig, with 5 mm span length between supports, and kept fully immersed in distilled water during testing. Flexural strain was set to 3 % (Bedran-Russo et al., 2008) with a displacement rate of 0.5 mm min^{-1} using a 5 N load cell (SMT1-5N, Interface, Scottsdale, AZ, USA) mounted on a universal testing machine (Autograph AGS-X, Shimadzu, Japan). After maximum displacement, the load was immediately returned to 0 % stress, without further holding, to prevent creep of the demineralized collagen matrix. Specimens were tested by a blinded operator. Load-displacement curves were converted to stress-strain curves and the apparent modulus of elastic (E) in MPa was calculated using the following formula: $E = mL^3/4bh^3\mathrm{E} = \mathrm{mL}^3/4\mathrm{bh}^3$, where m is the steepest slope of the linear portion of the load- displacement curve (N/mm), L is the span length (5 mm), b is the width of the test specimen and h is the beam thickness. The 3-point bending test was selected for evaluation of the apparent elastic modulus due to its nondestructive nature allowing repeated measurements to be performed on the same sample. After baseline measurements, dentin beams (n = 10/group) were distributed into 5 balanced groups, where their mean values were not statistically different. Demineralized beams were then immersed for 2 h in the DMSO solutions used for the bonding protocols (*i.e.,* 50 % DMSO/H_2O and 50 % DMSO/EtOH) to allow proper solvent diffusion throughout the sample (Leme-Kraus et al., 2017; Bedran-Russo et al., 2008). For the EtOH wet-bonding dehydration protocol, the total time in the final EtOH step was 2 h. Undiluted DMSO was also included as a treatment for 2 h. Flexural strengths were determine with beams fully immersed in their respective pretreatment solutions. Beams were then rehydrated for 15 min and retested in distilled water. Subsequently, samples were stored for 7 and 30 days in artificial saliva at 37 ℃ and retested at both periods fully immersed in distilled water. Demineralized dentin beams without any treatment served as a negative control group. Apparent elastic modulus data regarding the effect of "dentin treatments" and "storage time" was analyzed by two-way repeated measures ANOVA and Tukey test

(α = 0.05). As the normality assumption regarding the effect of pretreatments on collagen apparent elastic modulus data was violated, data was analyzed by Kruskal–Wallis followed by Dunn-Bonferroni multiple comparison test (α = 0.05).

4.2.9 Flexural strength and apparent elastic modulus of experimental adhesives (*Study I*)

Except for specimen size, bar-shaped specimens (25 × 2 × 2 mm) were prepared according to ISO 4049 specifications. The experimental adhesives were poured into a stainless-steel mold followed by a Mylar strip and a glass slide. Each specimen was light cured (Bluephase 20i, Ivoclare Vivadent) for 20 s with an output of 1200 mW/cm^2 in four overlapping irradiation zones. Adhesive beams were then removed from the mold. Excess resin from the edges were carefully removed with scalpel blades. Samples (n = 10/group) were stored in distilled water at 37 °C for 24 h prior to testing on a 3-point bending device (Instron, Instron Inc., Canton, MA, USA) in a mechanical testing machine (Instron 4411, Instron Inc.) at a crosshead speed of 1 mm/min until fracture. Flexural strengths (*FS*) were then calculated using the following equation and expressed in MPa: $FS = 3\mathrm{Fl}/2\mathrm{bh}^2$, where F is the maximum load exerted on the specimen in Newton, l is the distance between the supports in mm, b is the width of the specimen in mm, and h is the thickness of the specimen in mm. Elastic modulus, in GPa, was determined (Bluehill Software, Instron Inc.) considering specimen size and the slope of the linear portion of the load-displacement curve for each specimen tested for flexural strength. One-way ANOVA was used for the statistical analyzes followed by the Tukey test (α = 0.05).

4.2.10 Degree of conversion of experimental adhesives (*Study I*)

Absorption spectra of uncured and cured experimental adhesives were obtained by Fourier Transform Infrared Spectroscopy (Spectrum 100 Optica; PerkinElmer, MA, USA) equipped with a HeNe laser. Infrared spectra were recorded in the region between 2000 – 1000 cm^{-1}, with 16 scans, at 4 cm^{-1} spectral resolution using a baseline method (Rueggeberg et al., 1990). Monomer conversion was determined by measuring the decrease of C=C before and after polymerization to an internal aromatic C=C standard. A circumferential (3 mm × 0.8 mm) silicon hollow mold was centralized over the ATR crystal surface and a 5 µL drop of each experimental adhesive was placed inside the mold in direct contact with the ATR crystal. A Mylar strip was placed over the top of the deposited resin to exclude oxygen and prevent solvent evaporation. Light curing was performed at 1 mm distance with a polywave light-emitting diode light-curing unit (Bluephase 20i, Ivoclar Vivadent, Schaan,

Liechtenstein) with an output of 1200 mW/cm^2 for 20 s. Post-curing was allowed to continue up to 180 s and the absorption spectrum was then collected (n = 8)/group. The mean of three readings was used to obtain the ratio of aliphatic/aromatic peaks for uncured adhesives. Degree of conversion was calculated by changes in C=C absorption peak ratios of aliphatic (1638 cm^{-1}) and aromatic (1608 cm^{-1}) peaks in both uncured and cured states obtained from the infrared spectra according to the equation:

$$DC(\%) = \left(1 - \frac{R^{(Cured)}}{R^{(Uncured)}}\right) X\ 100 \qquad \textit{Equation 1}$$

Where *R* is the ratio of aliphatic and aromatic peak intensities at 1638 cm^{-1} and 1608 cm^{-1} in cured and uncured adhesives. One-way ANOVA was used for the statistical analyzes followed by the Tukey test ($\alpha = 0.05$).

4.2.11 Adhesive conversion in the hybrid layer (*Study IV*)

Resin-dentin beams were produced (n = 6) following the previously described DMSO/H_2O and DMSO/EtOH protocols under wet conditions and the ethanol-wet bonding approach. Samples were kept in 100 % humidity for 24 h at 37 ºC before measurements of monomer conversion. The rationale to avoid immersing bonded samples in water was to limit HEMA elution from bonded interfaces to reduce the discrepancy between "apparent" and "real" adhesive conversion values (Zou et al., 2009). Untreated dentin served as control. To precisely locate hybrid layers, samples were embedded in epoxy-resin and wet-polished with 600, 1000 and 2000-grit SiC paper (Buehler Ltd., Lake Bluff, IL, USA), ultrasonically cleaned for 2 min after the last step. Samples were not dehydrated due to the fact that water is a weak Raman scatterer. Raman spectra of hybrid layers were collected using a Raman microscope (Thermo DXR2xi, Thermo Fisher Scientific, Madison, USA) equipped with a 785 nm laser and 400 lines/mm grating resulting in approximately 5 cm^{-1} spectral resolution and 3300 – 50 cm^{-1} spectral range. Spectra were obtained with a 100 × objective at the top half and bottom half of hybrid layers, in arbitrary areas composed by intertubular dentin between dentin tubules. Instrumental calibration was performed according to manufacturer's specifications before each experiment. Specimens were tested by a single blinded operator. The reactive peak at 1639 cm^{-1} is attributed to the methacrylate C=C in both HEMA and BisGMA monomers (Spencer et al., 2000; Colthup, 1950). Upon polymerization, the peak height reduces as C=C are converted into C−C to form polymer chains. An unchanging reference peak at 1609 cm^{-1} is correlated with the aromatic C=C (Spencer et al., 2000; Colthup, 1950). The ratio of double-bond content of monomer to polymer in the hybrid layer

was calculated according to *Equation 1*. Two-way ANOVA was used for the statistical analyzes followed by the Tukey test (α = 0.05).

4.2.12 Water sorption and solubility of experimental adhesives (*Study I*)

DMSO-containing experimental adhesives were poured into cylindrical stainless-steel molds (0.5 mm thick and 9 mm in diameter) followed by a Mylar strip and a glass slide. Experimental adhesives were then light cured (Bluephase 20i, Ivoclare Vivadent) with an output of 1200 mW/cm^2 for 20 s. Polymerized samples (n = 10) were initially stored dried at room temperature for 24 h in the dark and subsequently stored in a desiccator containing dried silica gel at 37 °C for 24 h. Samples were gravimetrically assessed using a calibrated digital balance (resolution of 0.01 mg). Drying/weighting cycles were repeated daily until a constant weight (*M1*) was obtained, when mass variations were inferior to 0.1 mg in a 24 h period. After drying, the specimens were immersed in distilled water at 37 °C for 7 days. Specimens were then blot-dried to remove all visible water on its surface, weighed (*M2*) and placed back in a desiccator containing dried silica gel at 37 °C until a constant weight was achieved (*M3*). The values for water sorption (*Wsp*) and solubility (*Wso*) were calculated as:

$$Wsp = (\mathrm{M2} - \mathrm{M3})/V \qquad Wso = (\mathrm{M1} - \mathrm{M3})/V$$

Where *M1* is the constant initial mass in μg of the specimen, *M2* is the mass in μg of the specimen after immersion in water for 7 days, *M3* is the constant mass in μg of specimen after removal from water and drying and *V* is the volume in mm^3 of the specimen. One-way ANOVA was used for the statistical analyzes followed by the Tukey test (α = 0.05).

4.2.13 Loss of dry mass (*Study IV*)

Loss of dry mass over time provides an indirect measurement of collagen solubilization by endogenous enzymes. Fifty demineralized dentin beams measuring approximately 0.5 mm in thickness × 1.5 mm in width × 6 mm in length were dehydrated in silica under vacuum during 72 h at 24 ℃ and then desiccated to a constant weight (variations lower than 0.1 mg over 6 h; approximately 48 h in total). The initial dry mass (*M1*) was determined gravimetrically to the nearest 0.01 mg using an analytical scale (XS 105, Mettler Toledo, Hightstown, NJ, USA). After initial dry mass measurements, dried dentin beams were rehydrated in distilled water for 2 h before immersion in the treatment solutions for 2 h: EtOH, following the

ethanol-wet bonding protocol, pure DMSO, DMSO/H_2O and DMSO/EtOH (n = 10). The period of 2 h was selected to allow diffusion of treatments throughout most, if not, the entire extension of demineralized dentin beams. Untreated collagen beams served as control. Subsequently, treated beams were rehydrated in artificial saliva for 2 h and placed in separate polypropylene tubes containing 3 ml of artificial saliva. Samples were placed in a shaking-bath to facilitate the diffusion artificial saliva within collagen fibrils. After 30 days of incubation at 37 ℃, collagen beams were rinsed with water for 10 min and sonicated for 5 min in distilled water to remove media salts. Dry mass determination was repeated under the same conditions after dehydration in silica (*M2*). Specimens were tested by a blinded operator. Loss of dry mass (L_{dm}) was calculated according to the equation:

$$Ldm\ (\%) = M2 - M1/M1.$$

Since dry mass data violated the normality and homoscedasticity assumptions for parametric analysis, Kruskal-Wallis followed by Dunn-Bonferroni comparison tests were employed for statistical analysis ($\alpha = 0.05$).

4.2.14 Contact angle measurements (*Study V*)

Dentin discs measuring approximately 2.5 mm in thickness (n = 8/group) from the midcoronal section of sound third molars were transversally sectioned under water-cooling (Isomet, Buehler Ltd). The absence of remaining enamel on the occlusal surfaces was performed with a stereomicroscope (Leica M60, Leica Microsystems) at 40× magnification. The surfaces were then wet-polished with 600-grit SiC paper for 60 s. H_3PO_4-etching (Scotchbond Universal Etchant, 3M ESPE) was performed for 15 s and rinsed for 30 s. Moisture control and DMSO pretreatments were performed as previously described for the bond strength measurements. Control groups consisted of untreated samples air-dried for 30 s (*i.e.*, dry control) or blot-dried (*i.e.*, wet control). To investigate the wettability of etched dentin, contact angle measurements were performed using the sessile drop method. A goniometer (Attension Thetha Lite 101, Biolin Scientific, Espoo, Finland) was used to measure the contact angles of the hydrophilic (Primer, Scotchbond Multi-Purpose: SBMP, 3M ESPE) and hydrophobic (Bond, Scotchbond Multi-Purpose: SBMP, 3M ESPE) bonding resins. 3 μL droplets were deposited on the etched-dentin surfaces with a micropipette after dentin pretreatments and drying conditions of each group. Contact angles were measured up to 240 s. Images were captured at 0.1 s intervals during the initial 20 s, 0.5 s during the subsequent 20 s and after 5 s intervals for the remaining 200 s to evaluate spreading times. Left and right contact angles were averaged by the goniometer software (OneAttension Version 2.9 (r5612), Biolin Scientific, Finland).

A logarithmic fitting model (Grégoire et al., 2011) of the contact angles over time was used to calculate the spread rate constant k for the resins according to DMSO pretreatments and moisture conditions. Factorial ANOVA was used to analyze contact angles at 0.1 and 20 s followed by the Tukey test ($\alpha = 0.05$).

5 Results

5.1 Microtensile bond strength (*Study I, II, III, IV and V*)

The effect of DMSO incorporation into relatively hydrophobic experimental resins was dose dependent ($p < 0.05$). Resins containing 2 and 4 % DMSO had no effects on immediate bond strengths ($p < 0.05$); however, incorporation of 10 % DMSO significantly reduced immediate bond strengths by 22 % ($p < 0.05$). All experimental resins presented significantly lower ($p < 0.05$) bond strengths after the 2-year aging period. No significant differences were observed between control and 4 % DMSO resins after aging. Incorporation of 10 % DMSO significantly reduced ($p < 0.05$) bond strengths (-90 %) after 2 years. Contrary, incorporation of 2 % DMSO produced significantly higher dentin bond strengths (94 %) compared to the control resin after aging **(Table 4)**. Regression analyses of the aged bond strengths estimated 1.58 % as the optimum DMSO concentration to produce the highest dentin bond strengths of DMSO-containing bonding resins after aging **(Figure 3)**.

In general, dry-bonding significantly reduced resin-dentin bond strengths of SBMP by 30 – 50 % ($p < 0.05$) **(Table 5)**. However, DMSO/H_2O and DMSO/EtOH pretreatments, under dry conditions, produced significantly higher bond strengths than the conventional wet-bonding technique without significant differences between DMSO solutions ($p < 0.05$). Similarly, higher bond strengths were not observed for the universal adhesive SU after DMSO pretreatments **(Table 5)**. Partial 10 % replacement of SBMP's *primer* solvent-content by DMSO produced similar bond strength values in both wet and dry bonding conditions ($p < 0.05$). Ethanol-wet bonding had no impact on immediate resin-dentin bonding, producing significantly lower values than DMSO/H_2O and DMSO/EtOH pretreatments ($p < 0.05$). Bond strengths for the ethanol-wet bonding were not affected by long-term aging. Regardless of dentin treatment, DMSO-treated resin-dentin interfaces presented no significant reductions in bond strength ($p < 0.05$) after long-term aging (*i.e.,* 2.5 years). Dry-bonded-untreated samples presented the lowest bond strengths after aging with a significant reduction of up to -85 %. Irrespective of initial dentin hydration or moisture control (blot- or air-drying), DMSO pretreatments produced significantly higher bond strengths (ranging from 30 to 45 %) compared to the

traditional wet-bonding protocol ($p < 0.05$) **(Table 5; Figures 4 and 5)**. No significant differences were detected between DMSO pretreated groups regardless of initial dentin hydration or moisture control ($p < 0.05$). No significant changes were observed for DMSO pretreated groups after aging irrespective of initial dentin hydration or moisture control **(Figure 6)**. Fracture patterns were predominantly mixed for groups tested at 24 h, except for the dry-bonded samples, which were mostly characterized by adhesive failures. In general, untreated samples presented a substantial increase in adhesive failures after long-term aging, while the DMSO-treated groups presented only a minor increase.

Table 4. Microtensile bond strength values (MPa) to dentin of relatively hydrophilic resin blends containing DMSO after long-term aging *(Study I)*.

DMSO Concentration	24 h	2 years
0 %	28.17^{Aa}±5.21 [104-11.5 ^{Aa}/72.1/16.3] (3 %)	8.16 ^{Bb}±1.51 [101 – 59.4^{Bb}/33.7/6.9] (16 %)
2 %	31.75^{Aa}±5.23 [106-10.4 ^{Aa} 74.5/15.1] (2 %)	15.87^{Ab}±2.8 [105 – 32.4^{Ab}/61.0/6.7] (6 %)
4 %	27.73^{Aa}±5.03 [102-11.8 ^{Aa}/74.5/13.7] (3 %)	4.19^{Bb}±0.81 [104 – 60.6^{Bb}/33.7/5.8] (21 %)
10 %	21.91^{Ba}±3.17 [105-19.0 ^{Aa}/68.6/12.4] (4 %)	0.83^{Cb}± 0.1 [102 – 83.3^{Cb}/11.8/4.9] (62 %)

Microtensile bond strength (MPa) to dentin and standard deviation for all groups (n = 10 teeth/group). The number of sticks tested per group and the modes of failure are expressed in % into brackets as [number of tested sticks - adhesive/mix/cohesive]. A minimum of 7 resin-dentin beams were tested per tooth for each storage period. Percentage of premature failures is indicated in parentheses. For the bond strengths and adhesive failures, same superscripts capital letters indicate no significant differences ($p > 0.05$) in columns and same superscript lowercase letters indicate no significant differences ($p > 0.05$) in rows. *(From the supplementary data published in Study I)*

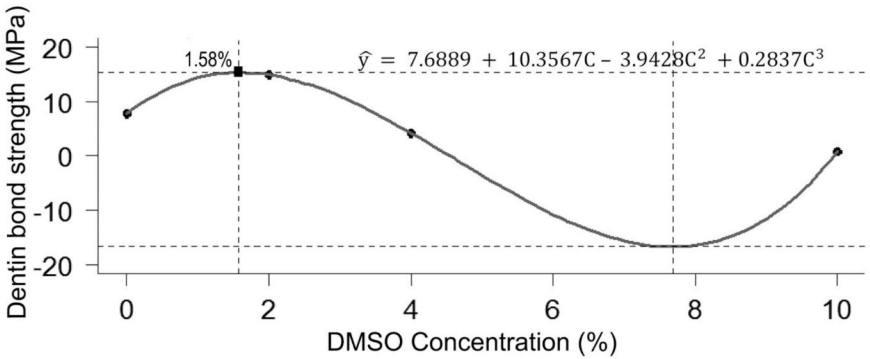


Figure 3. Optimum DMSO concentration determined by orthogonal regression analysis using a third-degree polynomial curve fit. Plotted circular dots represent the aged microtensile bond strength values for each tested DMSO concentrations after aging. The square dot represents the optimum DMSO concentration in the tested resin blends to produce the highest bond strength after long-term aging according to the best fit equation. *(From the supplementary data published in Study I)*

Table 5. Microtensile bond strength of wet- and dry-bonding protocols using DMSO/H_2O and DMSO/EtOH solutions used as dentin pretreatments.

	Scotchbond Multi-Purpose		**Scotchbond Universal**	
	Wet-bonding	Dry-Bonding	Wet-bonding	Dry-bonding
Control	30.13 ±4.99 ^{Ba} [64-25/35/4] (4.5 %)	17.53 ±2.81 ^{Bb} [58-44/12/2] (20.5 %)	27.46 ±4.01 ^{Aa} [69-28/39/2] (5.5 %)	27.58 ±4.55 ^{Aa} [64-22/40/2] (5.9 %)
DMSO/H_2O	43.68 ±7.03 ^{Aa} [64-21/38/5] (3 %)	40.47 ±4.29 ^{Aa} [67-24/35/8] (4.3 %)	30.31 ±3.35 ^{Ab} [64-23/36/5] (7.2 %)	31.13 ±3.66 ^{Ab} [67-22/42/3] (6.9 %)
DMSO/ EtOH	42.86 ±5.87 ^{Aa} [65-22/41/2] (4.4 %)	41.80 ±4.73 ^{Aa} [69-22/41/6] (2.8 %)	32.91 ±3.29 ^{Ab} [66-21/36/9] (4.3 %)	31.78 ±5.61 ^{Ab} [66-19/43/4] (5.7 %)

Dentin bond strength (MPa) means and standard deviation for all groups (n = 8). Similar superscripts capital letters indicate no significant differences within each group (columns) and similar superscript lowercase letters indicate no significant differences between the groups with the same treatment (rows) according to Tukey's studentized range (HSD) test ($p > 0.05$). The total number of tested resin-dentin beams and their failure modes for each group are expressed into brackets as [total number of tested beams - adhesive/mix/cohesive failures]. The percentage of premature failures is indicated in parentheses. *(From the supplementary data published in Study II)*

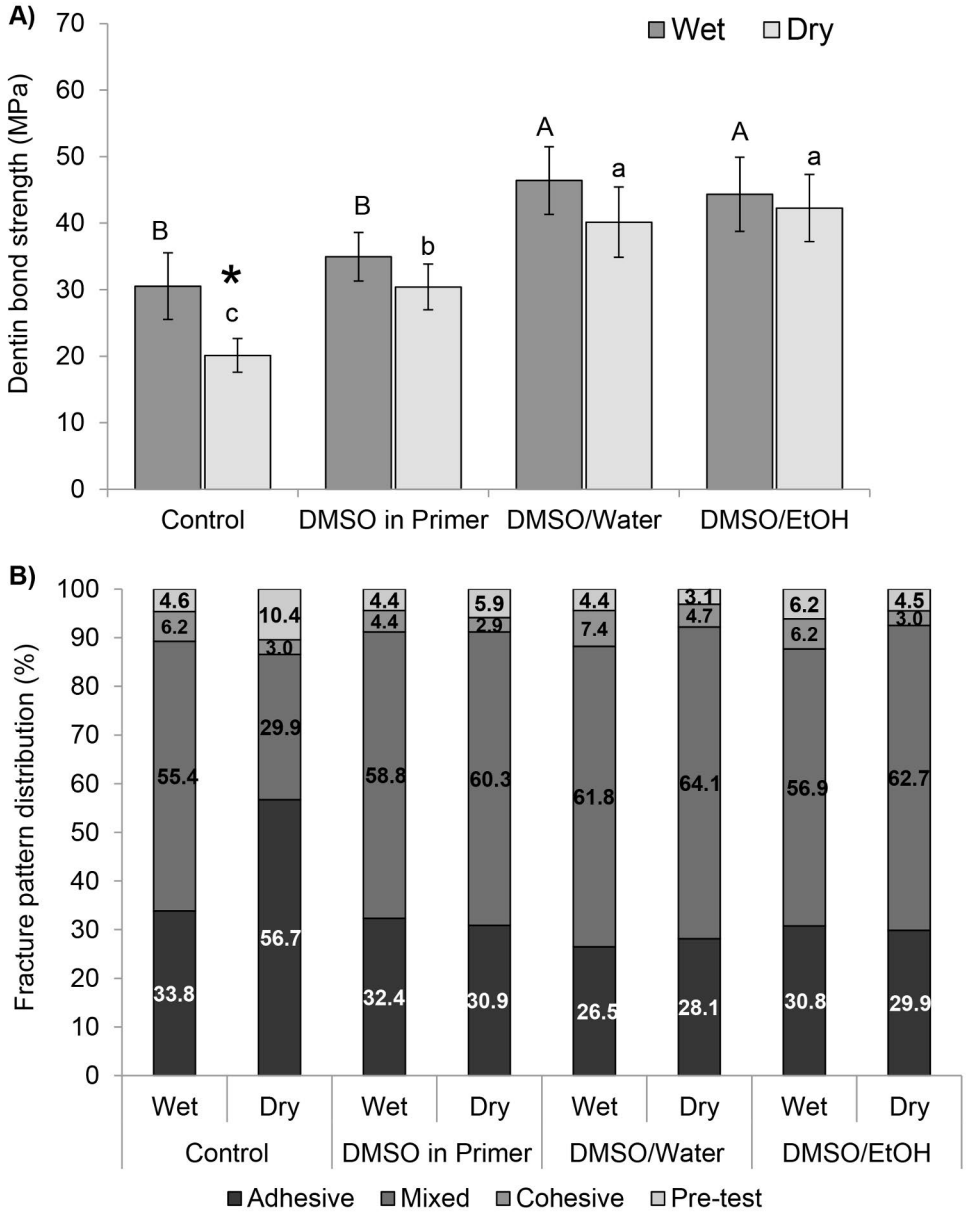


Figure 4. A) Microtensile bond strength of wet and air-dried demineralized dentin bonded with SBMP using DMSO solutions as dentin pretreatments or incorporated into the bonding agent (DMSO/SBMP). Pretreatment solutions consisted of DMSO dissolved in either water (DMSO/H_2O) or ethanol (DMSO/EtOH). Heights of bars indicate mean values (MPa) of 8 teeth per group (n = 8) and standard deviations. Columns identified by different capital letters represent significant differences according to Tukey test ($p < 0.05$) for wet-dentin groups. Columns identified by different lowercase letters represent significant differences for dry-dentin groups. * represent significant differences between wet and dry dentin for each pretreatment. B) Graphical presentation of the percentages of fracture modes for all experimental groups. *(From the supplementary data published in Study III)*

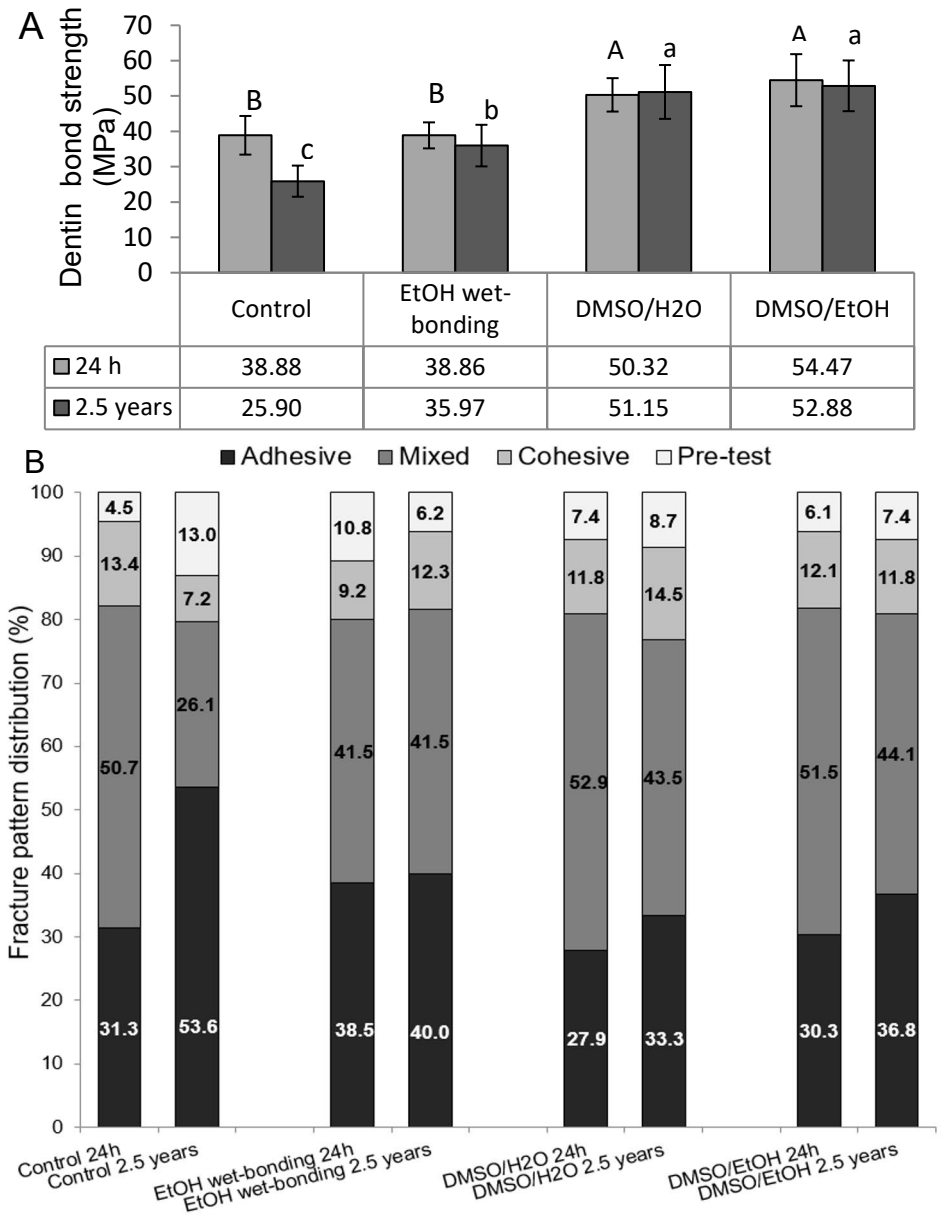
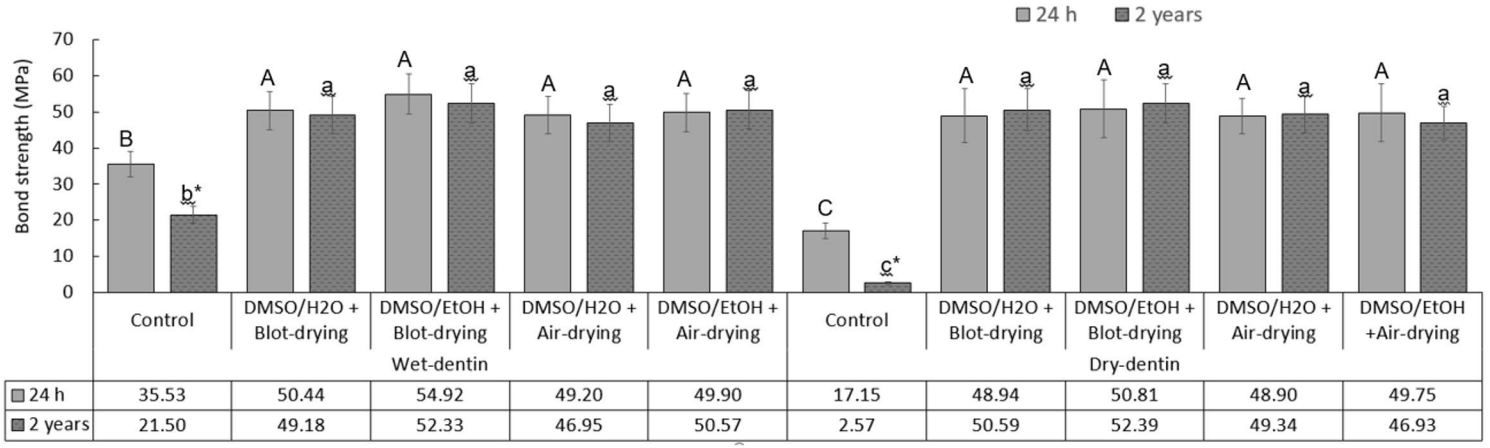


Figure 5. (A) Microtensile bond strength (MPa) means and standard deviations of resin-dentin interfaces (n = 8) bonded (SBMP) with aqueous and ethanolic DMSO solutions or following ethanol-wet bonding as dentin pretreatments at 24 h or 2.5 years of aging in artificial saliva at 37 °C. Different upper case letters indicate significant differences between groups at 24 h. Different lower case letters indicate significant differences between groups at 2.5 years. * indicates significant differences between aging periods within treatments. (B) Fracture patterns (%) for all groups. *(From the supplementary data published in Study IV)*



	Wet-dentin					Dry-dentin				
	Control	DMSO/H2O + Blot-drying	DMSO/EtOH + Blot-drying	DMSO/H2O + Air-drying	DMSO/EtOH + Air-drying	Control	DMSO/H2O + Blot-drying	DMSO/EtOH + Blot-drying	DMSO/H2O + Air-drying	DMSO/EtOH +Air-drying
24 h	35.53	50.44	54.92	49.20	49.90	17.15	48.94	50.81	48.90	49.75
2 years	21.50	49.18	52.33	46.95	50.57	2.57	50.59	52.39	49.34	46.93

Figure 6. Microtensile bond strength (MPa) means and standard deviations of resin-dentin interfaces bonded to wet or dry dentin using aqueous or ethanolic DMSO pretreatments after long-term aging. Tooth was considered the statistical unit (n = 8/group). Different upper case letters indicate significant differences between groups within the 24 h testing period. Different lower case letters indicate significant differences between groups after aging for 2 years. * inidicates significant differences between aging periods within similar treatments. Statistical comparisons were performed by the Tukey test (α = 0.05). *(From the supplementary data published in Study V)*

5.1 Nanoleakage evaluation (*Study II, IV and V*)

All tested resin-dentin beams presented silver deposits at the bonded interface. The extension of silver uptake varied according to the bonding protocols. SU presented significantly higher silver uptake when bonded to acid-etched dentin compared to SBMP ($p < 0.05$). For SU wet bonding to etched dentin, spotted silver grains could be identified along most of the extension of the hybrid layer with few areas presenting reticular deposits **(Figure 7)**. While wet-bonded SBMP presented few discontinuous areas of reticular silver deposits, mostly located at the base of the hybrid layer, dry bonding produced two-fold higher silver uptake than wet bonding ($p < 0.05$) **(Figure 8)**. For the dry-bonding approach, nanoleakage patterns were mostly dense reticular deposits along the entire extension of the bulk of hybrid layer.

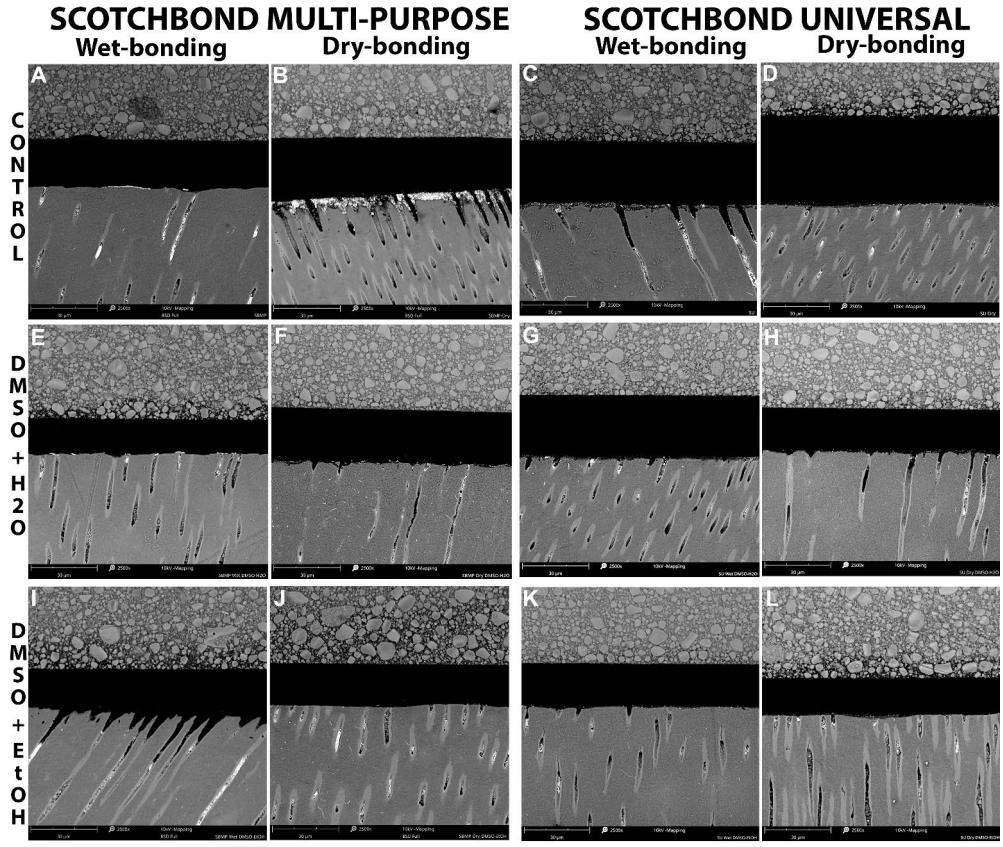


Figure 7. Representative nanoleakage SEM micrographs of DMSO-treated-etched dentin with SBMP and SU following wet- and dry-bonding protocols at 24 h. Silver deposits within the hybrid layer depict the formation of porous water-filled interfaces. *(From the supplementary data published in Study II)*

Regardless of dentin moisture conditions prior to pretreatment application (*i.e.,* dry or wet), DMSO/H_2O produced similar patterns and nanoleakage levels compared to the wet-control group ($p < 0.05$). Significantly lower silver uptake occurred when DMSO/EtOH pretreatments were performed on wet (-54 %) and especially on dry dentin (-71 %) ($p < 0.05$).

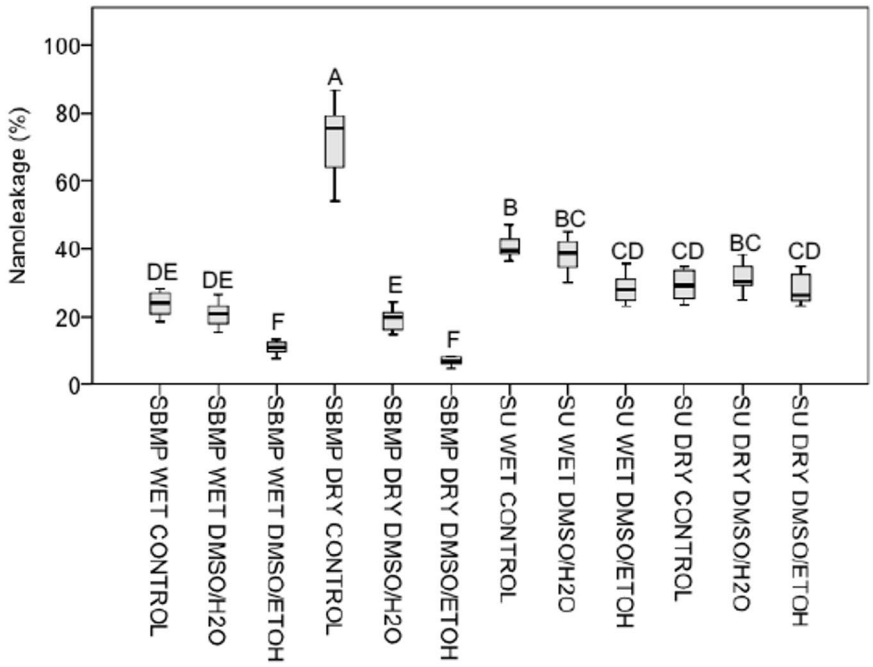


Figure 8. Boxplot of immediate nanoleakage extension (%) within the hybrid layer of wet- and dry-dentin samples (n = 8) bonded with SBMP and SU using DMSO solvated in either water (DMSO/H_2O) or ethanol (DMSO/EtOH) as pretreatments. The box contains 50 % of the data and the middle line of the box represents the median nanoleakage percentage distribution. The whiskers extend between the minimum and maximum value measured. Different capital letters indicate significant differences in nanoleakage percentages according to Dunn-Bonferroni post-hoc test ($p < 0.05$). *(From the supplementary data published in Study II)*

After long-term aging (2 years), untreated samples presented the highest levels of silver uptake, roughly a 2-fold increase when compared to DMSO-treated groups ($p < 0.05$) **(Figure 9)**. No significant differences were observed between aged samples submitted to DMSO/EtOH or ethanol-wet bonding, producing the lowest levels of silver infiltration ($p < 0.05$). DMSO/H_2O and wet bonding produced significantly higher nanoleakage levels than DMSO/EtOH and wet bonding or ethanol-wet bonding; however, values were still significantly lower than in the control group ($p < 0.05$) after aging. Different patterns of silver uptake and nanoleakage levels were invariably identified by SEM after long-term aging ($p < 0.05$), albeit predominant

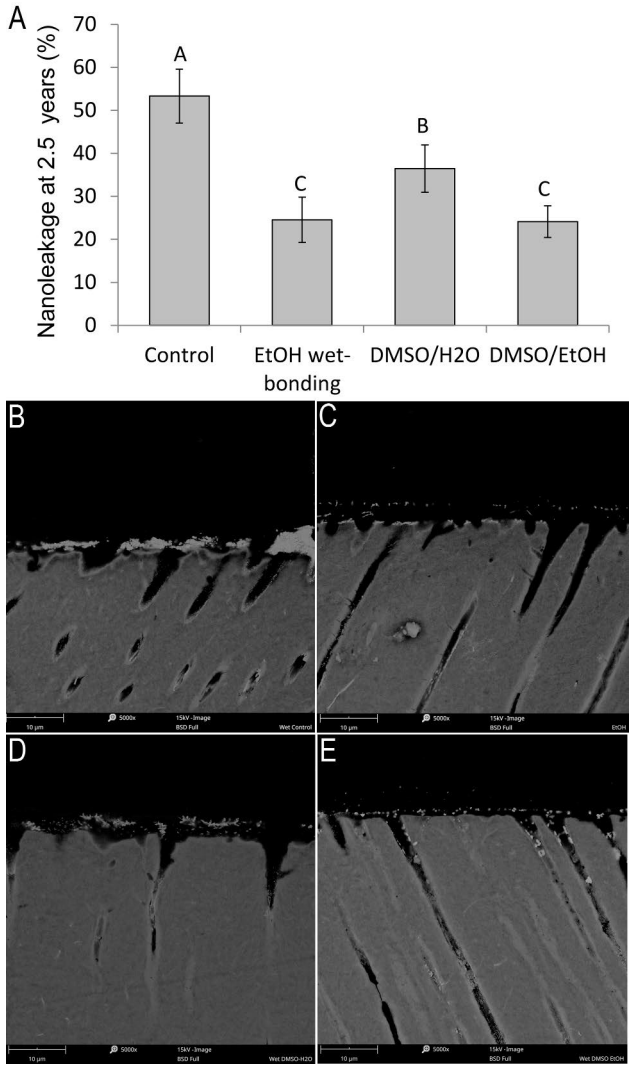


Figure 9. (A) Means and standard deviations of the overall nanoleakage extension (%) along resin-dentin interfaces (n = 8) bonded (SBMP; 3M ESPE) with aqueous and ethanolic DMSO-solutions or following ethanol-wet bonding after 2.5 years of storage in artificial saliva at 37 ℃. Different letters indicate significant differences between groups. Representative SEM micrographs of nanoleakage patterns for (B) untreated dentin; (C) following the ethanol wet-boding approach; (D) DMSO/H_2O; (E) and DMSO/EtOH. *(From the supplementary data published in Study IV)*

patterns were evident according to the dentin treatment. Untreated-control samples were characterized by heavy deposits of silver located within the hybrid layer into the overlying adhesive layers. Distinction between hybrid and adhesive layers was often impossible due to heavy silver deposition after aging **(Figure 10)**. Ethanol-wet bonding produced spotted silver deposits sparsely scattered within the hybrid layer, which was also the predominant pattern in dry and wet DMSO/EtOH groups. Aging also produced light reticular silver deposits characterizing water-trees for DMSO/H_2O pretreated dentin. Irrespective of initial dentin hydration (wet or dry) or moisture control (blot- or air-drying) after DMSO pretreatments, both DMSO/H_2O and DMSO/EtOH produced clearly lower levels of silver uptake at the bonded interfaces compared to wet and dry control groups after aging.

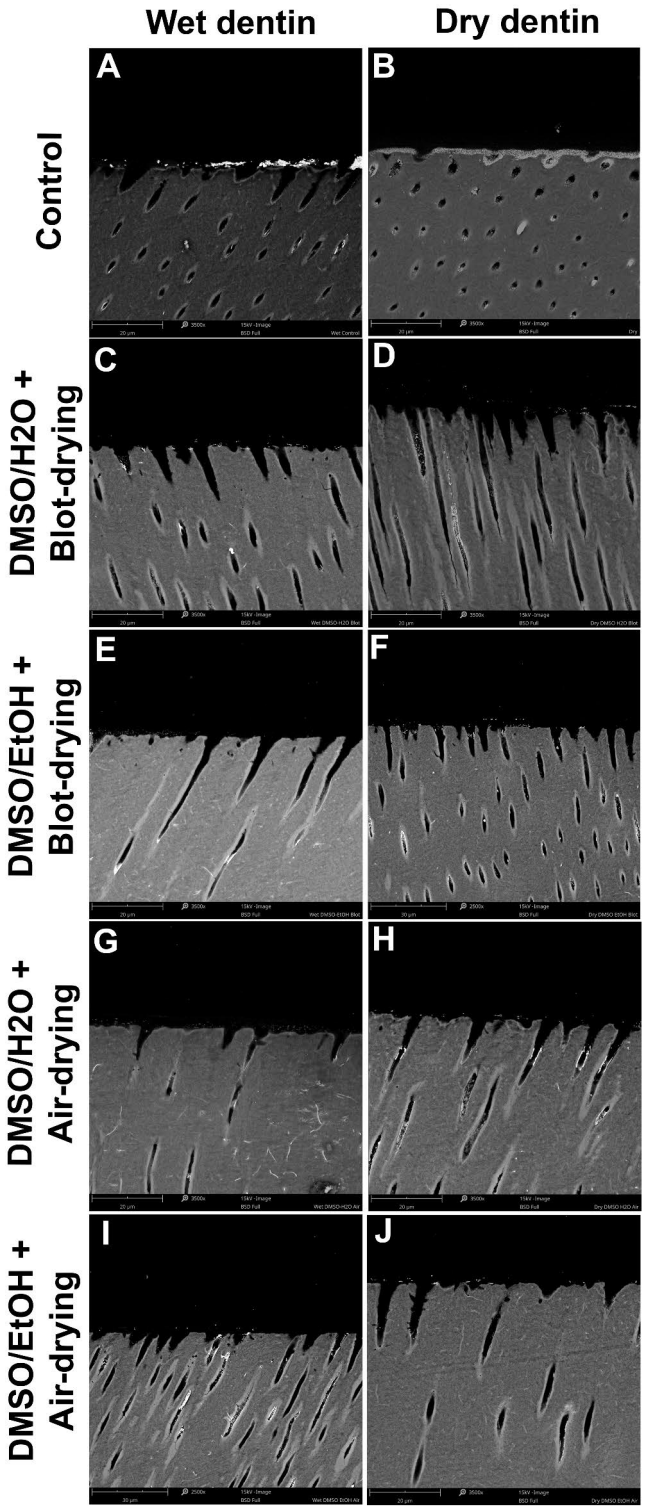


Figure 10. Representative SEM nanoleakage micrographs of aged resin-dentin interfaces bonded to wet or dry dentin (SBMP; 3MESPE) using aqueous or ethanolic DMSO solutions as pretreatments. Moisture control after the application of pretreaments was performed by blot- or air-drying. *(From the supplementary data published in Study V)*

5.2 Micropermeability evaluation (*Study II*)

All samples presented fluorescein at the hybrid layer and/or around resin tags with different extents according to the bonding protocol used. SBMP applied following the wet-bonding technique **(Figure 11A)** produced micropermeability sites throughout the entire bottom and lower half of the hybrid layer: fluorescein easily penetrated around resin tags extending throughout the hybrid layer thickness. Porous zones 3 – 4 μm wide immediately below the hybrid layer were identified. SU **(Figure 11C)** produced a similar micropermeability pattern except that heavy fluorescein deposits around resin tags extending towards the hybrid layer were more evident. In general, wet bonding SU produced wider fluorescent bands below the hybrid layer depicting higher micropermeability compared to SBMP, irrespective of dentin pretreatment. While DMSO/H_2O had no considerable impact on micropermeability levels of wet-bonded SBMP **(Figure 11E)**, it reduced the extension of fluorescein deposits for SU in wet-bonding **(Figure 11G)**. In general, DMSO/EtOH treated dentin presented lower fluorescent sites nearby the hybrid layer compared with wet-bonded SBMP and SU. SBMP-bonded dentin pretreated with DMSO/EtOH **(Figure 11I)** presented fluorescein uptake limited to minimal deposits mostly located around resin tags. DMSO/EtOH pretreated dentin bonded with SU **(Figure 11K)** resulted in diffused-scattered fluorescent sites along the hybrid layer.

SBMP applied following the dry-bonding technique **(Figure 11B)** produced extensive fluorescein deposits throughout most of the hybrid layer and around resin tags forming wide fluorescent bands (roughly 7 – 10 μm). Differently, similar levels of fluorescein uptake in dry-bonded SU **(Figure 11D)** and SU control group (*i.e.,* following the wet-bonding protocol) were evident, but with reduced fluorescein accumulation around dry-bonded resin tags. DMSO/H_2O **(Figure 11F)** and DMSO/EtOH **(Figure 11J)** pretreatments reduced micropermeability levels in dry-bonded SBMP interfaces, with the latter producing only sparse fluorescein deposits mainly around resin tags. Regardless of dentin moisture, both DMSO pretreatments generally reduced the extension of fluorescein bands for dry-bonded SU interfaces. DMSO/H_2O reduced the micropermeability levels for dry-bonded SU **(Figure 11H)**. Comparable results in both wet and dry dentin were observed for DMSO/H_2O. Contrary, DMSO/EtOH reduced micropermeability levels when SU was applied on dry dentin **(Figure 11L)**. Dry bonding SU with DMSO/EtOH produced thin fluorescent bands (1 – 3 μm) dislocated several microns away from the hybrid layer. Nevertheless, dry-bonding SU with DMSO/H_2O or DMSO/EtOH produced higher micropermeability levels compared to dry-bonded SBMP groups bonded with similar DMSO pretreatments.

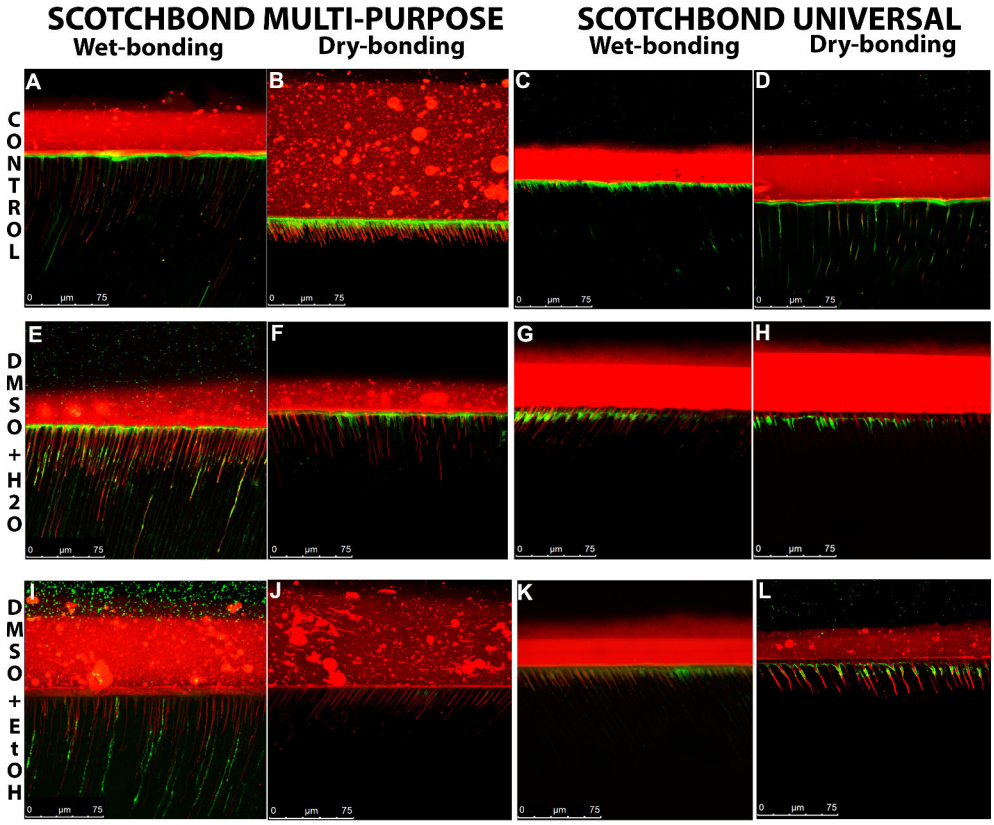


Figure 11. Representative micropermeability confocal laser scanning micrographs of DMSO-treated dentin bonded with SBMP and SU following wet- and dry-bonding protocols. Sodium fluorescein under simulated pulpar pressure was used as a tracer solution to evaluate the dentin sealing ability of the proposed bonding protocols. *(From the supplementary data published in Study II)*

5.3 In situ zymography (Study III)

Signs of collagenolytic activity were detected in all samples at the hybrid layer, underlying intertubular dentin or inside dentinal tubules. Untreated groups presented substantial collagen breakdown in both wet and dry dentin **(Figure 12 A2, E2)**. DMSO/H_2O **(Figure 12 C2, G2)** and DMSO/EtOH **(Figure 12 D2, H2)** produced fewer areas with collagenolytic activity compared to wet- and dry-control groups, respectively. DMSO/EtOH on dry dentin presented the lowest levels of enzymatic activity of all groups **(Figure 12 H2)**. Dentin condition influenced the collagenolytic activity observed in the DMSO-containing resin and DMSO/EtOH producing slightly better inactivation levels in dry conditions than in wet groups. For wet dentin, incorporation of DMSO in the bonding resin **(Figure 12 B2)** produced

fluorescence levels almost similar to control groups; however, a clear reduction was observed on dry dentin **(Figure 12 F2)**.

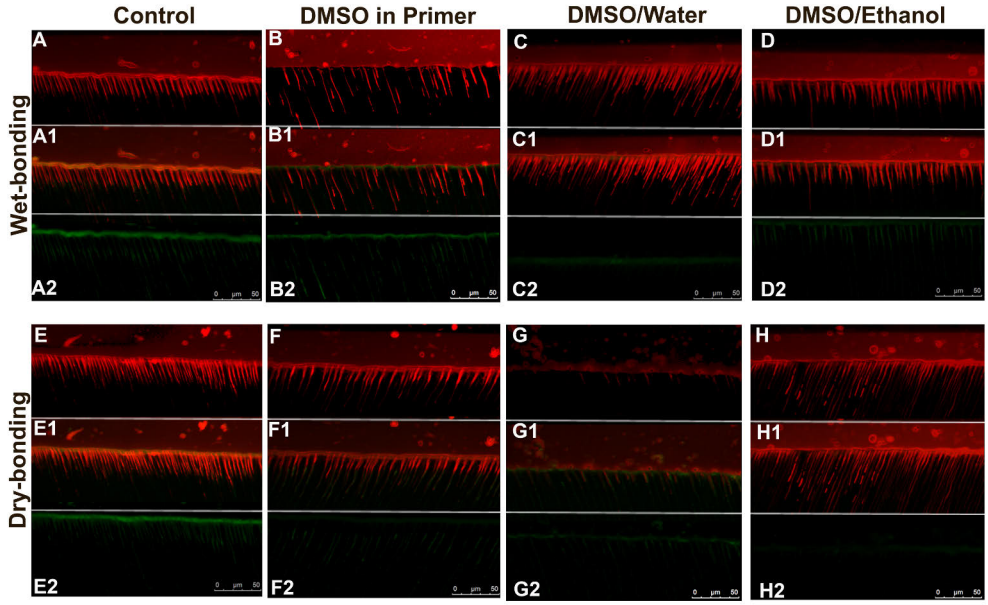


Figure 12. Representative CLSM scans (63 x/1.4NA oil immersion objectives) for *in situ* zymography of wet and air-dried dentin bonded with SBMP. DMSO was solvated in water (DMSO/H_2O) or in ethanol (DMSO/EtOH) and used as dentin pretreatments or incorporated in the bonding agent (DMSO in Primer). Isolated red fluorescence signals, originated from Rhodamine B in adhesive, delineate the morphology of adhesive interface (A – H). Green fluorescence signals designate collagenolytic activity originated from quenched FITC-conjugated collagen breakdown by endogenous enzymes. A1 – H1 depict the localization of collagenolytic activity on the hybrid layer and surrounding areas. A2 – H2 show isolated FITC fluorescence revealing different levels of collagenolytic activity according to the different pretreatments and dentin conditions. While untreated control groups (A2 and E2) exhibited higher FTIC fluorescence signals, pretreatments with DMSO/H2O (C2 and G2) and DMSO/EtOH (D2 and H2) indicate reduced endogenous enzymatic activity especially on dry dentin (F2 and G2). Incorporation of DMSO in the bonding resin produced similar FITC fluorescence signals (D2) to control group in wet condition; however a slight reduction of enzymatic activity in the hybrid layer was observed for the dry-bonding protocol (H2). *(From the supplementary data published in Study III)*

5.4 Hydroxyproline quantification (*Study III*)

Hydroxyproline release (µg/ mg dry dentin) from demineralized dentin is shown in **Figure 13**. Incubation solutions had a significant effect on hydroxyproline release ($p < 0.0001$; Kruskal-Wallis). Hydroxyproline release from wet and dry demineralized dentin incubated in distilled water and in the SBMP primer were not significantly

different ($p < 0.05$), indicating that SBMP *per se* had no impact on collagen degradation regardless of dentin condition (*i.e.*, wet or dry). In contrast, DMSO/H_2O and DMSO/EtOH significantly reduced collagen breakdown when compared to controls ($p < 0.05$) showing reductions in the order of 66 %. No significant differences between DMSO/H_2O and DMSO/EtOH were observed irrespective of dentin condition ($p > 0.05$). The DMSO-containing primer was not significantly different compared to the remaining groups ($p > 0.05$).

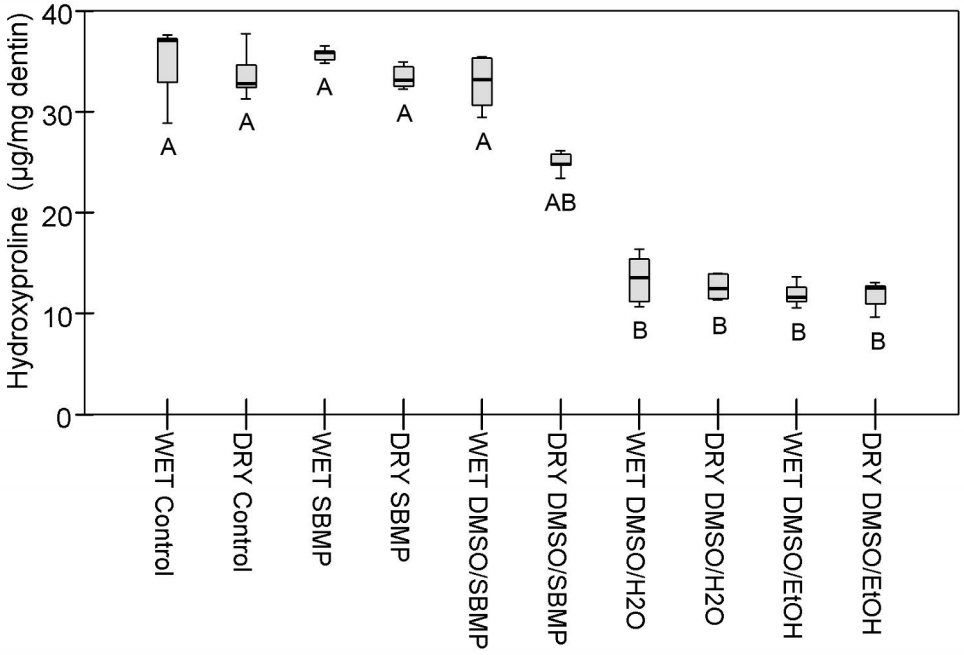


Figure 13. Hydroxyproline content derived from wet and dry demineralized dentin powder (n = 5) treated with DMSO after incubation for 7 days at 37 °C. Treatments consisted of DMSO solvated in water (DMSO/H_2O), ethanol (DMSO/EtOH) or incorporated in the SBMP primer (DMSO/SBMP). SBMP served also as a control incubation solution (Wet SBMP and Dry SBMP). DRY CONTROL Dissolved collagen from the demineralized dentin was expressed as µg hydroxyproline per mg dry mass of the baseline demineralized dentin powder. Groups with different upper case letters were significantly different ($p < 0.05$) according to Dunn's multiple comparison test. *(From the supplementary data published in Study III)*

5.5 Gel zymography (*Study III*)

Zymograms of wet and dry demineralized dentin treatments are shown in **Figure 14 A and B**, respectively. Demineralized dentin exhibited pro- (92 kDa) and active (86 kDa) forms of MMP-9, MMP-2 in active form (66 kDa) and other minor bands

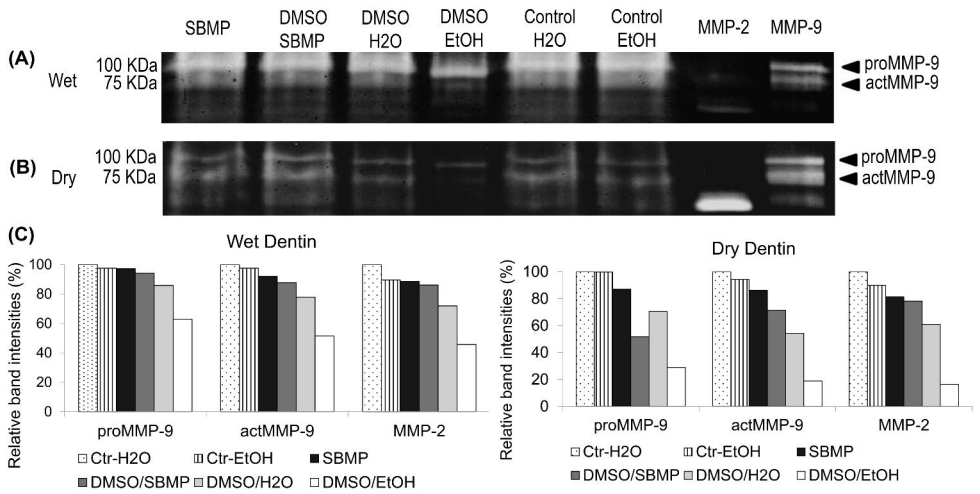


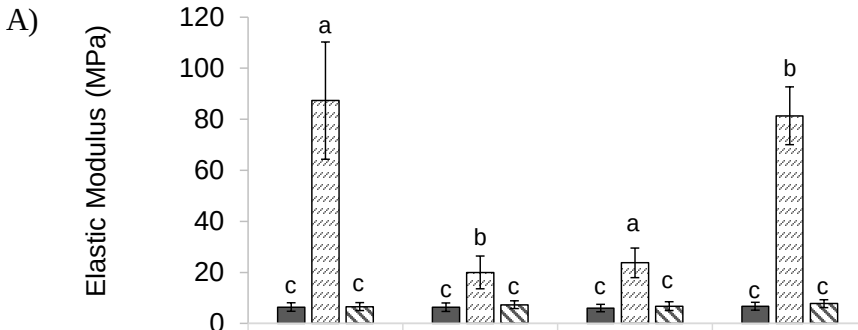
Figure 14. Gelatin zymograms of wet (A) and dry (B) demineralized dentin powder treated with DMSO solvated in water (DMSO/H_2O), ethanol (DMSO/EtOH) or incorporated in SBMP. Control groups consisted of untreated dentin powder (Control H_2O), SBMP and ethanol. Pure MMP-2 and MMP-9 extracts from odontoblasts were used as specific enzyme molecular mass standards. Molecular masses, expressed in kDa, are reported in the standard lane (Std). The graph (C) shows band intensities for proMMP-9, actMMP-9 and actMMP-2 calculated according to the peak area method. Complete inhibition of actMMP-2 and MMP-9 activity was not observed for neither of the DMSO treatments. Nevertheless, fainted bands indicate partial inactivation of MMP-2 and -9. *(From the supplementary data published in Study III)*

with lower molecular weights (not shown). Analysis of band intensities using the peak area method **(Figure 14C)** revealed partial inactivation of MMP-2 and -9. Intensities of pro- and active MMP-9 and MMP-2 bands were similar under wet conditions except for DMSO/EtOH, which exhibited fainter active MMP-2 and MMP-9 bands compared to untreated dentin. A similar trend was observed for DMSO treatments performed on dry dentin, albeit DMSO/H_2O also presented slightly fainter active MMP-9 and MMP-2 bands compared to control.

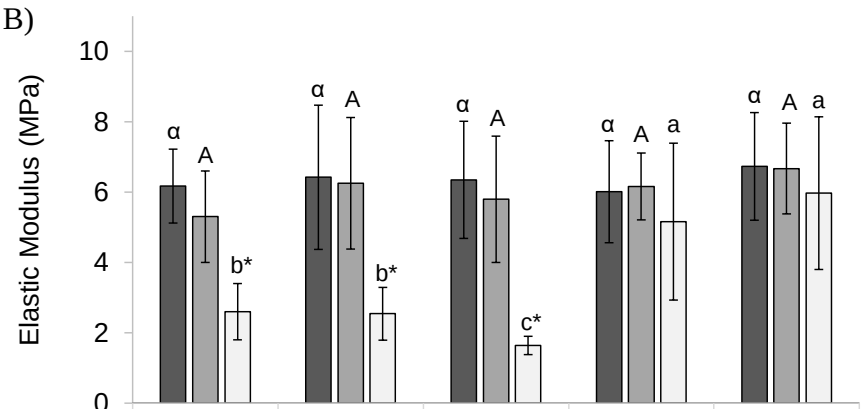
5.6 Collagen apparent modulus of elasticity (*Study IV*)

The average elastic modulus of dentin collagen at baseline (n = 50) was 6.41 MPa ±1.45. Variations in elastic modulus due to the different dentin treatments followed by rehydration are shown in **Figure 15A**. Kruskal-Wallis revealed that "dentin treatments" had a significant effect on the elastic modulus of collagen ($p < 0.001$). EtOH produced a significant 13.8-fold increase in elastic modulus followed by a 12.3-fold increase by DMSO/EtOH. DMSO/H_2O and DMSO produced significantly

lower 3.8 and 3.1-fold increases, respectively. Rehydration significantly reduced elastic modulus to baseline values, regardless of dentin treatment.



	EtOH	DMSO	DMSO/H2O	DMSO/EtOH
Baseline	6.42	6.35	6.01	6.73
Treatment (2 h)	87.31	19.98	23.73	81.37
Rehydration (15 min)	6.59	7.33	6.77	7.73



	Control	EtOH	DMSO	DMSO/H2O	DMSO/EtOH
Baseline	6.17	6.42	6.35	6.01	6.73
7 days	5.30	6.25	5.79	6.16	6.67
30 days	2.60	2.54	1.64	5.16	5.97

Figure 15. Elastic modulus (MPa) means and standard deviations of demineralized dentin beams (n = 10) submitted to (A) different dentin treatments followed by rehydration for 15 min. Different letters indicate significant differences between groups. (B) Elastic modulus means and standard deviations of previously treated and rehydrated beams after incubation in a calcium- and zinc-containing ageing medium for 7 and 30 days at 37 °C. Different Greek letters indicate significant differences between groups at baseline. Different upper case letters indicate significant differences between groups at 7 days. Different lower case letters indicate significant differences between groups at 30 days. No significant differences were detected between baseline and 7 days. * indicates significant differences between baseline and 30 days within treatments. *(From the supplementary data published in Study IV)*

Changes in elastic modulus over time are shown in **Figure 15B**. Repeated measures two-way ANOVA revealed that "dentin treatment" ($p = 0.001$; $\eta_P^2 = 0.1563$) and "storage time" ($p < 0.001$; $\eta_P^2 = 0.2149$) significantly affected the elastic modulus of dentin collagen. Significant interactions between "dentin treatment" and "storage time" were identified ($p < 0.001$; $\eta_P^2 = 0.1635$). No significant differences in elastic modulus occurred between baseline and 7 days of storage regardless of dentin treatment. At 30 days, untreated collagen and EtOH-treated collagen, following the ethanol-wet bonding technique, presented significant reductions in elastic modulus of roughly -55 %. Pure DMSO produced significant reductions in the order of -74 % at 30 days. Differently, collagen treated with DMSO/H_2O and DMSO/EtOH presented no significant reductions in elastic modulus at 30 days.

5.7 Flexural strength and apparent elastic modulus of experimental adhesives (*Study I*)

Incorporation of DMSO into relatively hydrophilic resins significantly affected flexural strengths ($p < 0.0001$) and elastic modulus ($p < 0.0001$). A general trend towards reduction in flexural strength was observed with higher DMSO concentrations **(Figure 16)**. However, resins containing 2 % or less DMSO presented no significant differences compared to the control group. Incorporation of 4 % and 10 % DMSO produced significant reductions in the order of 24 and 37 % ($p < 0.05$) compared to the control resin, respectively. Similarly, resin blends containing 1 – 10 % DMSO presented significantly lower ($p < 0.05$) elastic modulus than resins containing 0 (control) or 0.5 % DMSO **(Figure 17)**.

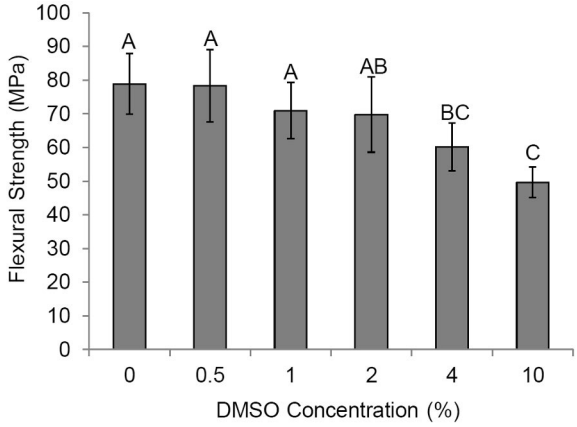


Figure 16. Flexural strength of experimental dental adhesive resins with increasing DMSO concentrations. Mean values and standard deviations (n = 10)/group. Different letters indicate significant differences ($p < 0.05$). *(From the supplementary data published in Study I)*

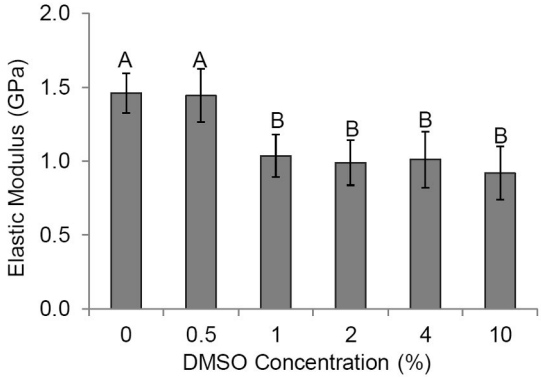


Figure 17. Elastic modulus of experimental dental adhesive resins with increasing DMSO concentrations. Mean values and standard deviations (n = 10)/group. Different letters indicate significant differences ($p < 0.05$). *(From the supplementary data published in Study I)*

5.8 Degree of conversion of experimental adhesives (*Study I*)

One-way ANOVA revealed that incorporation of DMSO into relatively hydrophilic resins significantly affected degree of conversion ($p < 0.0001$). While no significant differences occurred between resins with DMSO concentrations ranging from 0 % to 4 %, significantly higher conversion (13.2 %) ($p < 0.05$) was observed in resins containing 10 % DMSO compared to the resin without DMSO **(Figure 18)**.

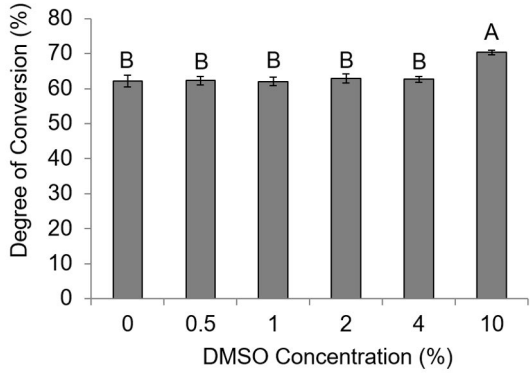


Figure 18. Degree of conversion of experimental dental adhesive resins with increasing DMSO concentrations. Mean values and standard deviations (n = 8)/group. Different letters indicate significant differences ($p < 0.05$). *(From the supplementary data published in Study I)*

5.9 Adhesive conversion in the hybrid layer (*Study IV*)

Two-way ANOVA revealed that the interaction between "dentin treatments" and "hybrid layer depth" ($p < 0.001$; $\eta_P^2 = 0.246$) significantly affected degree of conversion. The degree of conversion of Scotchbond Multi-Purpose Bond polymerized on a Mylar strip (71.25 % ±4.57) served as a reference value. Means and standard deviations for all groups are shown in **Figure 19**. Considering the top of hybrid layers, no significant differences between EtOH, DMSO/H_2O or DMSO/EtOH: conversion values ranged from 33 to 44 %. Monomer conversion was not uniform across the uppermost and lowest portions of hybrid layers: significant reductions were observed for the control group, roughly -75 %, but to lower extensions for DMSO/H_2O, roughly -34 %. Ethanol-wet bonding, DMSO/H_2O and DMSO/EtOH produced a ~2.5-fold increase in monomer conversion at the bottom of hybrid layers compared to untreated dentin. Degree of conversion for pretreatments containing ethanol (ethanol-wet bonding and DMSO/EtOH) were more uniform with no significant differences between the top and bottom portions of hybrid layers.

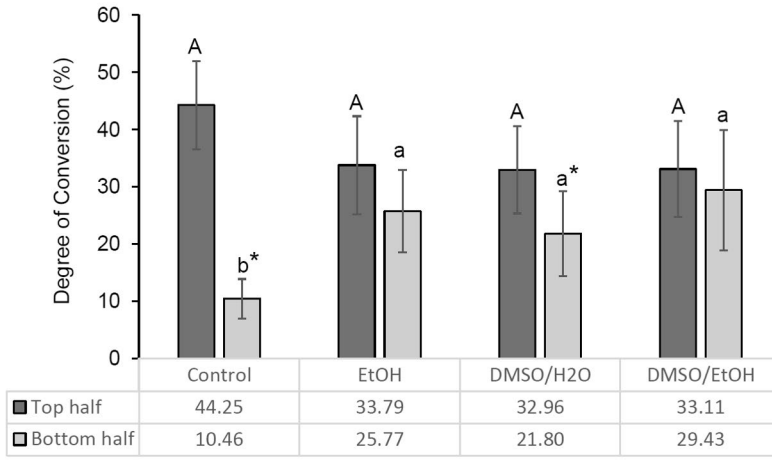


Figure 19. Degree of conversion (%) at the top and bottom halves of hybrid layers (n = 6) bonded (SBMP) with aqueous and ethanolic-DMSO solutions or following ethanol-wet bonding as dentin pretreatments. Different upper case letters indicate significant differences between groups at the top half of the hybrid layer. Different lower case letters indicate significant differences between groups at the bottom half of hybrid layer. * indicates significant differences between the top and bottom halves of hybrid layers within pretreatments. *(From the supplementary data published in Study IV)*

5.10 Water sorption and solubility of experimental adhesives (*Study I*)

Incorporation of DMSO into relatively hydrophilic resins significantly affected water sorption ($p < 0.001$) and solubility ($p < 0.001$) levels. Significantly higher water uptake (25 %) and solubility (82 %) ($p < 0.05$) were observed for 10 % DMSO compared to the control resin: no significant differences occurred in resins containing 4 % or less DMSO **(Figure 20)**.

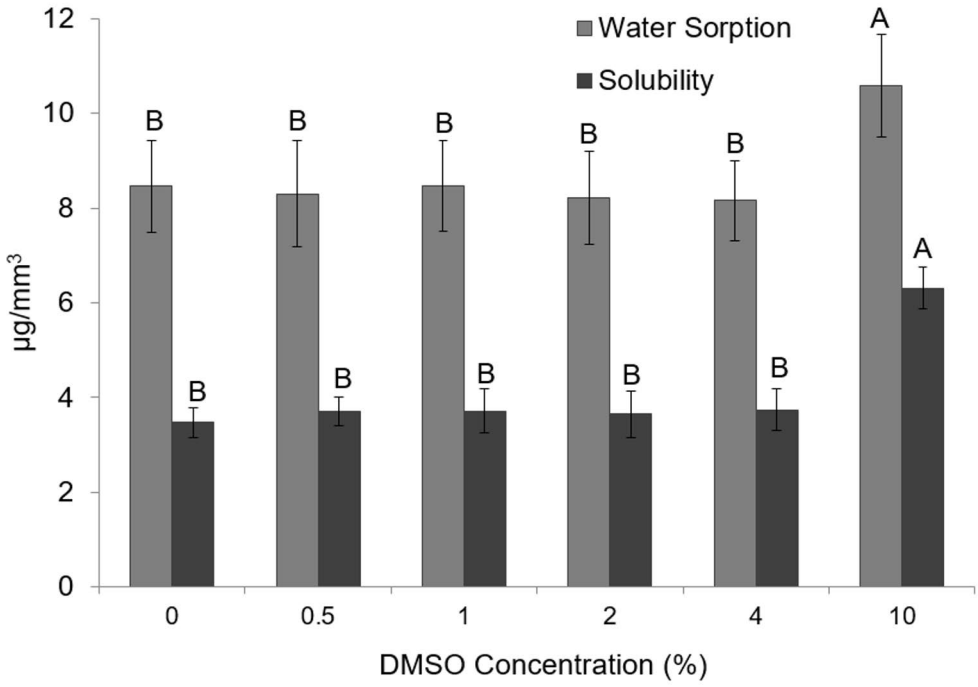


Figure 20. Water sorption and solubility of experimental dental adhesive resins containing increasing DMSO concentrations. Mean values and standard deviations (n = 10)/group. Different letters indicate significant differences ($p < 0.05$) for water sorption and solubility, respectively. *(From the supplementary data published in Study I)*

5.11 Loss of dry mass (*Study IV*)

"Dentin treatments" ($p < 0.001$) had significant effects on loss of dry mass from demineralized collagen beams after 30 days of storage at 37 ℃ in artificial saliva according to the Kruskal-Wallis test. Loss of dry mass reductions in % and standard deviations are shown in **Figure 21**. Untreated samples presented reductions in dry mass in the order of -58.7 %, which was not significantly different from EtOH (roughly -51.7 %). DMSO-containing treatments produced significantly lower reductions in dry mass compared to untreated and EtOH treated samples. Loss of dry mass of roughly -30 % were observed in DMSO/EtOH and DMSO/H_2O; pure DMSO resulted in -37 % reduction without significant differences between each other.

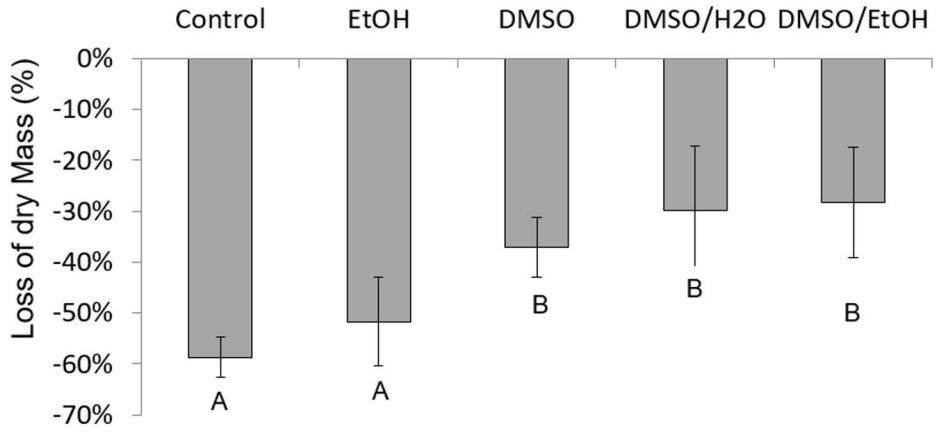


Figure 21. Loss of dry mass (%) means and standard deviations of completely demineralized dentin beams submitted to different dentin treatments after incubation in a calcium- and zinc-containing ageing medium for 30 days at 37 ℃. The loss of dry mass from each beam (n = 10) was calculated as a percentage of the dry mass of that beam at baseline. Groups with different upper case letters were statistically significant. *(From the supplementary data published in Study IV)*

5.12 Contact angle measurements (*Study V*)

A rapid decrease in contact angles for both bonding resins occurred during the initial 5 s, followed by a slower but still considerable decrease until 20 s. Contact angles then decreased slowly and reached a nearly constant value at approximately 180 s for the hydrophobic resin and 210 s for the hydrophilic resin. **Figure 22 and 23** illustrate variations in contact angles over time for hydrophilic and hydrophobic resins, respectively. Contact angles from each group followed a logarithmic decay model, allowing the determination of the kinetics parameters listed as the spreading rate constants during the initial 20 s in **Table 6**. Factorial ANOVA showed that the

study factors “initial collagen hydration” ($p < 0.001$; $\eta p^2 = 0.19$), “dentin pretreatment” ($p < 0.001$; $\eta p^2 = 0.601$), “collagen moisture prior to hybridization” ($p < 0.001$; $\eta p^2 = 0.152$), “resin” ($p < 0.001$; $\eta p^2 = 0.54$), “time” ($p < 0.001$; $\eta p^2 = 0.77$) and the interactions “dentin pretreatment * resin * time” ($p < 0.017$; $\eta p^2 = 0.77$), “dentin pretreatment * collagen moisture prior to hybridization * resin” ($p < 0.002$; $\eta p^2 = 0.27$) significantly affected contact angles. For the control groups, the hydrophilic resin produced significantly lower contact angles on wet than on dry dentin at both time periods (0.1 s and 20 s). In contrast, the hydrophobic resin presented no significant differences between wet-untreated or dry-untreated dentin at the same time periods. The hydrophobic resin produced significantly higher contact angles (roughly 90 %) than the hydrophilic resin when deposited on untreated dentin at 0.1 s. Similarly at 20 s, hydrophobic resins also produced higher contact angles than the hydrophilic resin (roughly 85 % higher) under wet conditions; however, no significant differences between resins occurred on air-dried dentin at 20 s. For the hydrophilic resin, DMSO pretreatments produced significantly lower contact angles than their respective dry-control group on both time periods irrespective of the initial collagen hydration (wet or dry) or moisture control (blot- or air-drying). Such contact angles were not statistically different from their respective wet-control groups. Unlike the hydrophilic resin, the hydrophobic resin produced significantly lower values on DMSO-pretreated collagen when compared to their respective dry- and wet-control groups. Reductions were in the order of 30 – 50 % at both time periods and occurred irrespective of initial dentin hydration (wet or dry) or moisture control (blot- or air-drying).

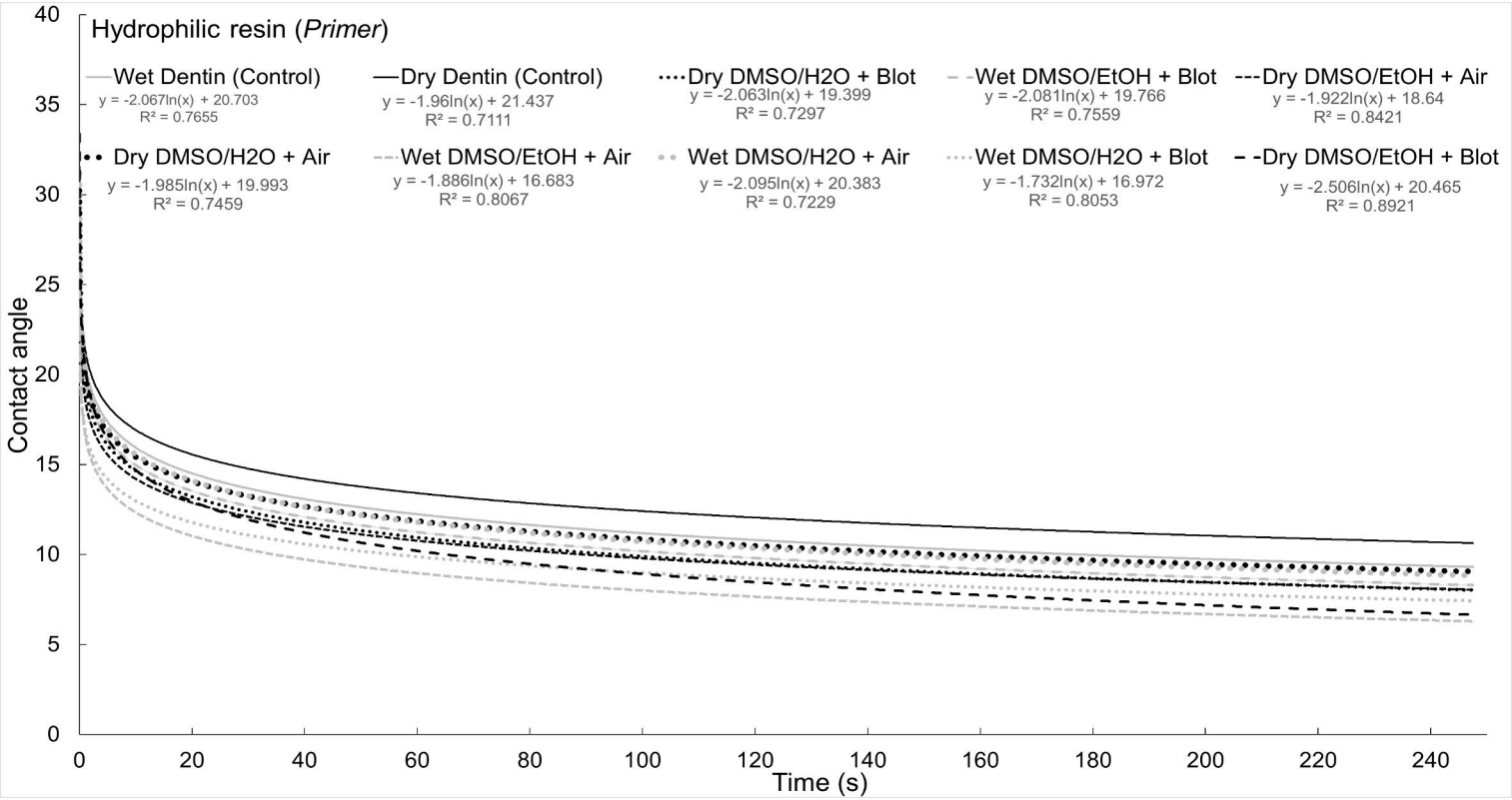


Figure 22. Contact angle evolution up to 240 s of hydrophilic (SBMP; Primer) deposited onto wet or dry H_3PO_4-etched dentin pretreated with aqueous- or ethanolic-DMSO solutions followed by blot- or air-drying. Trend lines for each group (n = 8 measurements/group) were determined by the logarithmic decay model. *(From the supplementary data published in Study V*

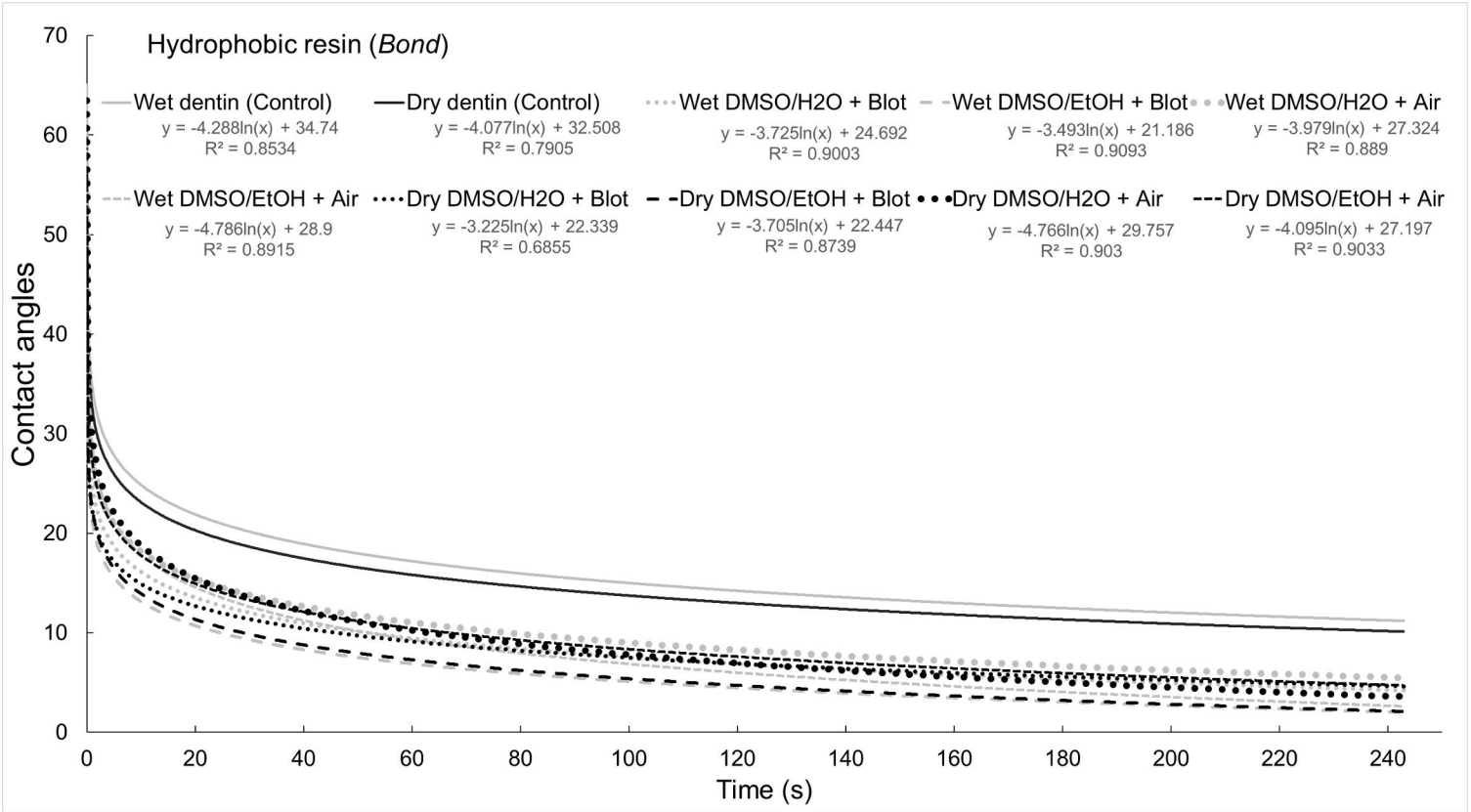


Figure 23. Contact angle evolution up to 240 s of hydrophobic (SBMP; Adhesive) deposited onto wet or dry H_3PO_4-etched dentin pretreated with aqueous- or ethanolic-DMSO solutions followed by blot- or air-drying. Trend lines for each group (n = 8 measurements/group) were determined by the logarithmic decay model. *(From the supplementary data published in Study V)*

Table 6. Wettability kinetics of hydrophilic (Primer) and hydrophobic (Bond) resins deposited onto DMSO-pretreated dentin with different moisture levels: contact angles at 0, 20 and 240 s, standard deviation and spreading rate constant (*k*) at the initial 20 s.

Resin	Initial dentin condition	Dentin pretreatment	Moisture control (drying)	0.1 s	20 s	*k*
Hydrophilic *(Primer)*	Wet	(control)	Blot	23.2 (3.3) ^{CD}	13.1 (2.1) ^{CD}	1.85
	Dry	(control)	Air	30.5 (3.4) ^{B}	20.9 (3.2) ^{B}	1.71
	Wet	DMSO/H_2O	Blot	16.9 (2.9) ^{D}	9.9 (1.9) ^{D}	2.98
			Air	21.6 (4.9) ^{D}	12.4 (2.7) ^{D}	3.29
		DMSO/EtOH	Blot	16.8 (3) ^{D}	10.6 (4.3) ^{D}	2.23
			Air	18.9 (2.9) ^{D}	12.6 (3.8) ^{D}	2.7
	Dry	DMSO/H_2O	Blot	18.3 (3.9) ^{D}	11.9 (3.6) ^{D}	2.34
			Air	22.1 (3.4) ^{D}	11.8 (3.7) ^{D}	2.93
		DMSO/EtOH	Blot	18.8 (3.7) ^{D}	11.3 (3.1) ^{D}	2.27
			Air	20.8 (3.3) ^{D}	11.2 (2.3) ^{D}	2.21
Hydrophobic *(Bond)*	Wet	(control)	Blot	44.1 (5.3) ^{A}	24.3 (3.6) ^{A}	3.62
	Dry	(control)	Air	45.5 (3.4) ^{A}	22.4 (3.1) ^{AB}	3.98
	Wet	DMSO/H_2O	Blot	28.3 (3.5) ^{BC}	15.5 (3.4) ^{C}	4.99
			Air	30.3 (6.7) ^{B}	16.1 (3.4) ^{C}	4.64
		DMSO/EtOH	Blot	22.4 (3.5) ^{CD}	10.2 (1.6) ^{D}	4.65
			Air	30.7 (3.5) ^{B}	11.1 (3.6) ^{D}	5.22
	Dry	DMSO/H_2O	Blot	28.3 (4.3) ^{BC}	14.7 (3.1) ^{C}	4.93
			Air	30.8 (3) ^{B}	16.4 (1.9) ^{C}	5.7
		DMSO/EtOH	Blot	24.1 (4.1) ^{C}	10.6 (1.8) ^{D}	4.5
			Air	30.3 (5.1) ^{B}	14 (1.5) ^{CD}	4.34

Contact angles with different superscript letters indicate significant difference according to Tukey test ($p < 0.05$) when analyzed per column.

6 Discussion

6.1 Optimal DMSO use in resin-dentin bonding

Dental adhesives require solvents to facilitate monomer diffusion into dentin. Considering the previously reported benefits of DMSO in resin-dentin bonding (Tjäderhane et al., 2013b), it seemed logical an attempt to incorporate DMSO into resin blends to improve bonding performance. Understanding the effects of DMSO incorporation into adhesive systems may guide resin formulations for optimal results. DMSO-containing adhesives could also simplify the application procedures compared to an additional DMSO-pretreatment application. *Study I* confirmed that DMSO incorporation into adhesive systems can indeed reduce resin-dentin bonding degradation in a dose-dependent manner. Regression analyses estimated the optimal percentage of DMSO incorporation into resin blends as approximately 1.6 % (w/w) for optimal long-term bond strengths. The interaction of DMSO with the studied methacrylate polymer composed by BisGMA and HEMA played an important role on the mechanical/physical polymer properties and thus on bond strengths.

Notably, hydrogen-bond interactions can reinforce the three-dimensional polymeric structure (Lemon et al., 2007). High DMSO content may have disrupted polymer interchain hydrogen bonding, altering the molecular structure and excessively increasing polymer chain mobility. Elastic modulus was more affected, starting as low as 1 % DMSO, while flexural strength presented significant reductions only with 4 % DMSO or higher concentrations. Similar findings corroborate the effect of DMSO-monomer ratios on the physical and mechanical properties of bonding resins (Salim Al-Ani et al., 2019). Due to DMSO's hygroscopic character, high DMSO incorporation also altered polymer-water interaction starting at 4 %. In spite of higher degree of conversion, solubility/sorption levels and flexural strengths were impaired for high DMSO incorporation. This may be explained by the fact that higher monomer conversion alone does not necessarily improve the mechanical and physical properties of polymers (Ye et al., 2007; Elliott et al., 2001). Crosslinking also plays an important role on the mechanical properties, water sorption and solubility of polymers. While high DMSO concentrations (10 %) improved degree of conversion, it possibly prevented the approximation between growing polymer chains during polymerization, thereby resulting in lower

crosslinking and invariably lower mechanical/physical properties. A similar effect was also observed on bond strengths. DMSO had no influence on immediate dentin bond strength when 4 % or lower concentrations were used; however, 2 % DMSO produced significantly higher values after aging. Contrary, incorporation of 10 % DMSO significantly impaired both immediate and especially long-term bond strengths. Increased water sorption and solubility, identified for resins with high DMSO incorporation, certainly played a major role on polymer degradation over time expediting bond strength loss. For simplified (*i.e.,* two-step etch-and-rinse) adhesives containing BisGMA and HEMA, it was evident that a threshold of 4 %-DMSO incorporation should be respected to prevent possible problems on polymer mechanical and physical properties.

Clearly, incorporation of 2 % DMSO can be easily adopted by manufactures to improve adhesive performance by reducing bond strength loss over time. Furthermore, 10 % DMSO (w/w) incorporation into commercial three-step etch-and-rinse systems, by partially replacing the primer's water content with DMSO, can prevent reduction in bond strengths caused by air-drying etched dentin. Under such circumstances, bond strengths were similar to the wet-bonding protocol (*Study III*). DMSO incorporation into adhesives at low concentrations can benefit resin-dentin bonding; however, the positive effect of separate dentin pretreatments with higher DMSO content (50 % v/v) was inarguably more profound (*Study II, III, IV and V*). Bond strengths of DMSO-pretreated dentin were not only higher at 24 h (*i.e.,* up to 40 %), but they remained stable after long-term aging for both wet- and dry-bonding protocols. Therefore, it is evident that dentin pretreatments with DMSO, albeit more time consuming, produced the most favorable long-term outcomes. The possibility of bonding methacrylate resins to air-dried-etched dentin with DMSO pretreatments challenges the current paradigm of wet-bonding requirement for the etch-and-rinse approach. The use of DMSO-dry bonding creates new possibilities to enhance the longevity of resin-dentin interfaces.

6.2 Hybrid layer formation and stability following DMSO-dry bonding

One of the main problems with conventional resin-dentin bonding lies on how to remove excess water without compromising resin-dentin interaction (Manso et al., 2008). Previous attempts to remove excess residual water using dry-bonding protocols have generally produced inadequate resin-dentin interfaces. Imperfections in the hybrid layer caused by inadequate management of collagen moisture were minimized with the proposed DMSO-bonding protocols in both wet and dry conditions (*Study II, III, IV and V*). The presence of excess water during dentin hybridization increases the formation of hydrogels at the HEMA-rich sites (Zou et

al., 2010a, 2010b). As a result, resin-dentin interfaces become more permeable. Silver (Tay et al., 2002) and fluorescein (Sauro et al., 2012, 2009) deposition within hybrid layers reflected the presence of poorly infiltrated water-rich zones. While nanoleakage analyses under SEM permits a precise interpretation with higher resolution images of silver deposits, micropermeability provides an indication of the relative sealing of the bonded interface (D'Alpino et al., 2006; Sidhu & Watson, 1998). Using a water-based fluorescein tracer solution under simulated pulpar pressure and CLSM for micropermeability assessment allow direct fluid movement visualization with minimal specimen preparation reducing the formation of possible artifacts (D'Alpino et al., 2006). Both techniques are effective to identify flaws within the bonded interface; however, micropermeability analyses characterizes a more dynamic approach representing the ability of resin-dentin interfaces to resist the steady dentinal fluid flow under pulpar pressure. Hence, nanoleakage analyses can be considered a more static approach, which may not readily account for the effects of dentinal fluid flow within the hybrid layer. This may underestimate flaws within the bonded interface. Nonetheless, both methods were in accordance to each other showing that DMSO-dry bonding greatly reduce flaws within the hybrid layer. The cosolvent (*i.e.,* water or ethanol) used for the formulation of DMSO pretreatments also had an impact on micropermeability and nanoleakage levels: DMSO/EtOH produced lower nanoleakage and micropermeability levels irrespective of the adhesive used (*i.e.,* SBMP or SU), especially when associated to dry bonding (*Study II*). Therefore, the combination of residual water evaporation by air-drying followed by the DMSO/EtOH pretreatment seems to be highly promising to reduce microporosities within the hybrid layer, irrespective of dentin moisture. This was further confirmed in *Study V* after long-term aging. In *Study V*, not only DMSO-dry bonded samples were aged for 2.5 years to assess long-term performance of DMSO-dried bonding on hybrid layer quality, but also an extra step of dentin air-drying after DMSO pretreatments was added before hybridization. The rationale for the extra air-drying step is due to the fact that water removal by evaporation is more efficient when performed before the primer/adhesive application (Yiu et al., 2005). Air-drying may potentialize water removal compared to routinely performed bonding protocols that rely exclusively on adhesive solvents to chase water molecules within demineralized collagen.

6.3 Dry- vs. DMSO-dry bonding

The findings presented in *Study II and V* reinforce the concept that etch-and-rinse adhesives must be preferably bonded to moist dentin to minimize issues related to collagen collapse (Pashley et al., 2007). Nonetheless, the underperformance of dry bonding was surmounted by pretreating demineralized collagen with aqueous- or

ethanolic-DMSO solutions. *Study V* demonstrated that the degree of collagen moisture prior to hybridization, ranging from partially wet to air-dried, had no effect on bonding performance of the water-based etch-and-rinse system when DMSO pretreatments were performed. Improved collagen wetting, reported in *Study V*, produced by DMSO pretreatments certainly contributed to better bonding outcomes. Furthermore, the ability of DMSO-containing pretreatments and bonding resins to expand collapsed collagen was a determinant factor of whether bonding protocols were successful or not.

Solvents may produce complete (*i.e.,* water) or partial (*i.e.,* ethanol, propanol, and acetone) re-expansion of collapsed collagen, depending on their hydrogen bonding solubility parameters (Pashley et al., 2007). Methacrylate-based bonding agents (*i.e.,* HEMA) do not always promote adequate re-expansion of dried collagen (Pashley et al., 2007). As a result, diffusion of methacrylate monomers through such densely packed collagen meshes is inefficient (Pashley et al., 2007). As a result, resin-dentin bonding is greatly compromised (Sebold et al., 2019; Reis et al., 2007; Nakajima et al., 2000). In addition, when combined with HEMA, the re-expansion potential of solvents tends to drop, except for HEMA-water mixtures (Pashley et al., 2007; Eddleston et al., 2003; Nakaoki et al., 2000). HEMA-water mixtures may re-expand dried collagen up to 92 %; however, subsequent solvent evaporation substantially shrinks the matrix again (Eddleston et al., 2003). This generally results in interpeptide hydrogen bonding, thereby expelling HEMA from within the collagen matrix (Carvalho et al., 2003). Such instability of the collagen matrix prevents optimal resin-dentin bonding.

Although HEMA-water mixtures may re-expand dried collagen quite effectively (Pashley et al., 2007; Eddleston et al., 2003; Nakaoki et al., 2000), additional measures, such as vigorous adhesive application, are necessary to produce equivalent outcomes to wet bonding (Reis et al., 2007). *In vitro*, vigorous application of water-based adhesives to air-dried collagen was effective to reestablish bond strengths. However, relying exclusively on this approach during clinical applications may be impractical considering the complex geometry of large cavity preparations. Application of vigorous pressure throughout the entire dentin-surface area would certainly be difficult, invariably resulting in areas of compromised hybrid layer formation. Such vigorous active adhesive applications should indeed be employed whenever possible, regardless of the bonding protocol used. However, simplification of dry-bonding procedures with DMSO pretreatments surpassed the mere bond strength equiparation to wet bonding. Added protective mechanisms were identified in DMSO-dry bonding, which could further potentialize hybrid layer preservation over longer periods.

6.4 Ethanol-wet vs. DMSO-dry bonding

DMSO-dry bonding protocols differ conceptually from the classic ethanol-wet bonding. The latter approach relies on chemical dehydration to replace residual water within the demineralized collagen network with ethanol, while DMSO-dry bonding additionally focuses on removing water by evaporation and disrupting residual-water conglomerates. This was possible due to water removal before and after DMSO pretreatments by air-drying. Water displacement is produced by the interactions between residual DMSO molecules and water (Vishnyakov et al., 2001), which are 1.3-fold stronger than DMSO-DMSO interactions. As a result, water self-association is broken (Vishnyakov et al., 2001). DMSO-water interactions rearrange DMSO molecules by exposing DMSO's methyl groups (CH_3) outwards, which facilitates hydrophobic interactions with methacrylate monomers **(Figure 2)**. Furthermore, both ethanol-wet bonding (Li et al., 2012) and DMSO-dry bonding (Mehtälä et al., 2017) improve dentin wetting, increase the infiltration of methacrylate monomers (Pashley et al., 2007; Tay et al., 2007) and reduce phase separation of hydrophobic-hydrophilic comonomers within the intrinsically wet dentin substrate (Hosaka et al., 2009). Hence, ethanol-wet bonding (Pashley et al., 2011; 2007) and DMSO-dry bonding contributed to improved resin-dentin bonding *in vitro*.

Another crucial aspect in resin-dentin bonding is the morphology of collagen after etching regarding interfibrillar spacing (Carvalho et al., 2003). Increase in collagen interfibrillar spacing occurs in both scenarios either by shrinkage of collagen fibrils, as a result of water removal by ethanol (Tay et al., 2007), or by direct modifications in collagen structure produced by DMSO (Zimmerley et al., 2009). Naturally, higher resin-dentin bond strengths have been reported for ethanol-wet bonding (Hosaka et al., 2009; Nishitani et al., 2006), albeit increase in bond strengths is adhesive dependent (Li et al., 2012). In *Study IV*, ethanol-wet bonding was unable to significantly increase immediate bond strengths of the tested three-step etch-and-rinse commercial adhesive; however, bond strengths were stable over the 2.5-year aging period. This reinforces the principle that ethanol-wet bonding can indeed prevent long-term degradation of resin-dentin bonds *in vitro* (Pashley et al., 2011; Sadek et al., 2010a, 2010b). When tested *in vivo*, however, ethanol-wet bonding was unable to replicate the immediate benefits obtained *in vitro* (Kuhn et al., 2015). Ethanol-wet bonding can be compromised by water contamination. As little as 5 % water can reduce bond strengths by 25 % (Sadek et al., 2007). Similar water detrimental effects were not verified for dentin pretreatments containing DMSO, which were not sensitive to water "contamination" under normal wet-bonding conditions. For instance, the ability of DMSO/H_2O to increase immediate bond strengths and maintain it over time was not affected by water used as a cosolvent (*i.e.,* total 50 % v/v) in DMSO-containing pretreatments. The absence of additional water in DMSO/EtOH invariably contributed to higher stability of resin-dentin bond

strengths over the storage period. Therefore, DMSO-bonding protocols (wet or dry) are not only less technique sensitive, but they are also easier to implement in comparison to ethanol-wet bonding. It is important to note that water displacement produced by DMSO additionally creates a protective effect that may last for as long as residual DMSO molecules are entrapped within the hybrid layer.

6.5 Long-term effect of DMSO-dry bonding

The protective mechanisms involved in resin-dentin bonding using DMSO pretreatments contributed to improved long-term performance. DMSO-dry bonding not only increased immediate bond strengths, but also stabilized them for over 2 years of accelerated aging in artificial saliva. *Study V* was the first report to demonstrate that DMSO-dry bonding, with DMSO/H_2O or DMSO/EtOH, can prevent resin-dentin degradation after long-term aging. Differently, conventional dry bonding resulted in substantial bond strength reductions, which is in accordance with previous reports (Sebold et al., 2019; Manso et al., 2008; Reis et al., 2007; Nakajima et al., 2000).

6.5.1 Inactivation of endogenous enzymes and reduction in collagen degradation

Endogenous proteases gradually lower the mechanical properties of collagen as peptides are slowly degraded (Tezvergil-Mutluay et al., 2011a, 2010a, 2010b). Consequently, hydrolysis of exposed collagen by endogenous proteases accounts for significant impairment of resin-dentin interfaces (Van Meerbeek et al., 2020; Breschi et al., 2018). To evaluate the potential effect of DMSO on dentin enzymatic activity, several methods were employed in this study series. Unspecific-indirect evaluations were performed by three-point bending to determine the mechanical stability of collagen (*Study IV*), loss of dry mass (*Study IV*) and hydroxyproline quantification (*Study III*) to determine collagen solubilization and *in situ* zymography (*Study III*) to visualize and to qualitatively analyze collagenolytic activity within the hybrid layer. These methods were in accordance with each other showing reduced collagenolytic activity for DMSO pretreatments. Loss of dry mass serves as an index of matrix degradation (Tezvergil-Mutluay et al., 2011a). Untreated collagen presented 50 % reduction in elastic modulus, which was not statistically different from ethanol-treated samples. Together with a large increase in dry mass loss, lower elastic modulus indicates that the inferior mechanical properties of untreated and ethanol-treated collagen were due to modifications in the collagen structure. Peptide solubilization contributed to the reduction of mechanical properties of collagen. DMSO solutions (50 % v/v) were not only effective to preserve the mechanical

properties of collagen, but they also reduced collagen solubilization, regardless of the cosolvent used (*i.e.,* water or ethanol). Similar findings were also observed for the hydroxyproline quantification supporting lower collagen solubilization after DMSO pretreatments. DMSO's ability to bind to enzyme's hydrophobic moieties leading to protein unfolding can result in protein denaturation (Arakawa et al., 2007). Reduction in metalloproteinase activity has been previously reported for DMSO (Tjäderhane et al., 2013b). Preferential protein binding of DMSO is affected by its concentration, substrate hydration and protein polarity (Arakawa et al., 2007). Less polar proteins tend to bind to DMSO more effectively as the solvent concentration increases and as the substrate hydration decreases (Arakawa et al., 2007). Reduced water availability in DMSO-dry bonding with DMSO/EtOH most likely maximized DMSO binding to endogenous enzymes explaining the highest enzymatic inactivation levels for the *in situ* and gel zymography. Specific interactions between DMSO and endogenous dentin enzymes must be further evaluated. The presented findings corroborate a partial enzymatic inactivation by DMSO. Lower enzymatic activity certainly contributed to preserve collagen integrity over time resulting in no significant changes in elastic modulus. Notably, pure DMSO was unable to prevent collagen plasticization, albeit lower levels of collagen solubilization were detected. In such conditions, it is possible to assume that the reduction in the mechanical properties of collagen was not entirely caused by peptide solubilization. Since DMSO can bind to proteins (Arakawa et al., 2007; Zheng & Ornstein, 1996), collagen treated by pure DMSO may gradually uptake water resulting in a plasticizing effect on the peptide structure. This may be aggravated over time. Such high DMSO concentrations should thereby not be employed in resin-dentin bonding. Direct evaluation of the effect of DMSO on MMP-2 and -9 was obtained with gel zymography. Partial inactivation of MMP-2 and -9 was also observed, with the lowest enzymatic activity for DMSO/EtOH under dry conditions. It is important to note that even though DMSO was not able to inhibit dentin proteases, the reduction of enzymatic activity characterized by partial MMP inhibition may slow down collagen degradation. Hence, lower collagen degradation invariably contributed to the prolonged resin-dentin bonding stability observed for DMSO-dry bonding.

6.5.2 Collagen-structure stabilization prior to hybridization

The main problem related to dry bonding resides on the active and rapid development of hydrogen bonds between adjacent collagen peptides, which decreases interfibrillar spaces resulting in collapse of the collagen matrix (Pashley et al., 2007). Diffusion of methacrylate monomers through such densely packed collagen mesh is inefficient (Pashley et al., 2007), greatly compromising resin-dentin bonding (Sebold et al., 2019; Reis et al., 2007; Nakajima et al., 2000). Re-expansion of collapsed collagen

by bonding resins containing solvents is thereby necessary; however, combination of methacrylate monomers (*i.e.,* HEMA) and solvents dramatically drops solvent efficiency to re-expand collagen (Pashley et al., 2007; Eddleston et al., 2003; Nakaoki et al., 2000). The incorporation of water in resin blends mitigates issues related to collagen re-expansion. Nonetheless, posterior water removal by evaporation becomes another problem. To address such challenges, collagen stabilization before hybridization can greatly contribute to more efficient resin-dentin bonding. DMSO-pretreated collagen may present a sufficient increase in stiffness to prevent collagen collapse (*Study IV*). This was possible without compromises in hybrid layer formation (*Study II, V and IV*). DMSO alters the structure of collagen forming larger discontinuous interfibrillar spacing (Zimmerley et al., 2009); however, increase in collagen stiffening was observed for both ethanol- and aqueous-DMSO solutions. DMSO/EtOH produced a 12.3-fold increase in elastic modulus compared to a 3.8-fold increase for DMSO/H_2O. Although rehydration reduced elastic modulus to baseline values, collagen dimensional stability may be maintained long enough for proper hybridization. Considering the improvements in hybrid layer formation, it is possible to speculate that the resultant collagen stiffening produced by DMSO contributes to improved hybridization by maintaining interfibrillar spaces for resin infiltration and simultaneously preventing collagen collapse. Hence, dentin pretreatments containing DMSO, especially in combination with ethanol, constitute a viable alternative to reduce the technique sensitivity of etch-and-rinse bonding.

6.5.3 Improvements in dentin wettability

One of the first requirements for good adhesion is adequate wettability of the bonding surface. Intimate contact between the bonding agent and the surface is thereby of great importance to produce reliable bonding. In resin-dentin bonding, better wettability allows more efficient spreading of the bonding agent onto the dentin surface. A positive correlation exists between dentin wetting and bond strengths (Rosales-Leal et al., 2001). Contact angles of the hydrophilic and hydrophobic resins (*i.e.,* primer and adhesive of SBMP) were measured to determine the specific effects of the DMSO-bonding protocols on their wettability on demineralized dentin. Dentin hydrophobicity increases with air-drying (Hitmi et al., 2002), which reduces the wettability of demineralized collagen (Hitmi et al., 2002; Rosales-Leal et al., 2001; Rosales et al., 1999) and further complicates resin-dentin bonding. This was corroborated by the bond strength studies (*Study II, III, IV and V*). The water content in the hydrophilic resin did not compensate for the reduced collagen moisture produced by dry bonding, resulting in higher contact angles. Even for conventional wet bonding, adhesive procedures usually fall short of adequate

resin spreading times (Grégoire et al., 2011; Hitmi et al., 2002; Rosales-Leal et al., 2001). This was verified by the tested hydrophobic and hydrophilic resins, which achieved near-equilibrium contact angles only at 180 and 210 s, respectively. Altogether, DMSO pretreatments accelerated resin spreading. Spreading rate constants (*k*) were 20 – 60 % higher for DMSO-pretreated dentin. DMSO increased the wettability of the hydrophilic resin under dry conditions to levels similar to those of wet dentin. The negative impact of air-drying on collagen wetting was counteracted by DMSO pretreatments. A more profound effect on the wettability of the hydrophobic resin was observed when ethanol was used as a cosolvent instead of water. Improvements in collagen wettability produce by DMSO-dry bonding invariably contributed to better outcomes in resin-dentin bonding.

6.5.4 Monomer conversion in the hybrid layer

Monomer conversion is a key factor to successful resin-dentin bonding (Hass et al., 2013; Pashley et al., 2011). At lower portions of the hybrid layer, hybridization efficiency accounts for no more than 25 % considering BisGMA/HEMA infiltration under wet-bonding conditions (Wang & Spencer, 2003). Reduced availability and increased spacing between monomers contributes to lower conversion further compromising adequate polymer formation. DMSO-containing pretreatments produced significantly higher monomer conversion at the lower half of the hybrid layer. Higher monomer infiltration produced by DMSO (Stape et al., 2015) most likely facilitated conversion at the lower half of the hybrid layer. Moreover, DMSO lowers the termination rates in poly-methacrylate free radical polymerization (Gupta & Nandi, 1970) producing longer chains, which might have also facilitated monomer conversion. Therefore, the more uniform degree of conversion across the hybrid layer produced by the DMSO pretreatments, with fewer areas presenting lower monomer conversion, contributed to improved resin-dentin bonding.

6.6 Prospects for DMSO-dry bonding

Improvements in immediate and long-term resin-dentin bonding, higher stability of hybrid layers, reduction of collagenolytic activity, lower collagen solubilization with better dimensional stability, enhanced dentin wettability, more uniform monomer conversion at the hybrid layer, broader moisture spectrum and reduced technique sensitivity *per se* characterize DMSO-dry bonding as an outstanding-viable alternative to extend the longevity of resin-dentin interfaces. Further work is necessary to assess the possibility of reducing application times of DMSO pretreatments to facilitate their wider acceptance by clinicians. Bonding more hydrophobic resins to etched dentin treated with DMSO under dry conditions is

likely possible considering the reduced water content in demineralized dentin and higher dentin wettability. This could reduce the high hydrophilic character of commercial adhesives, potentially solving a nearly two-decade-old criticism to resin-dentin bonding (Tay & Pashley, 2003b). Nonetheless, the applicability of DMSO-dry bonding extends much further than "simply" producing higher resin-dentin bond strengths with reduced water content that can resist long-term aging. Considering that DMSO is one of the most versatile solvents, DMSO-containing pretreatments may simultaneously act as vehicles to deliver active substances to improve resin-dentin bonding. For instance, antimicrobial and mineralizing agents could be easily introduced to produce "bioactive" dentin pretreatments together with all the benefits produced by DMSO-dry bonding. Therefore, DMSO-dry bonding enables a myriad number of possibilities to further revolutionize resin-dentin bonding.

7 Conclusions

Based on the studies included in this thesis, the following conclusions were drawn:

I. The effect of DMSO incorporation into methacrylate-based bonding resins was concentration dependent. Use of 2 % DMSO as a cosolvent improved resin-dentin bond durability under wet bonding. DMSO incorporation should be carefully performed since high DMSO incorporation had a detrimental effect on both polymer formation and resin-dentin bond strength;

II. Bonding hydrophilic resins to air-dried-etched dentin was achieved by using aqueous- or ethanolic-DMSO solutions. DMSO pretreatments allowed better removal of residual water from resin-dentin interfaces via air-drying, contributing to more consistent dentin hybridization with lower water entrapment, reduced hybrid layer porosity, improved interfacial sealing and higher bond strengths;

III. DMSO incorporation into water-containing adhesives is a viable alternative to bond hydrophilic resins to air-dried-etched dentin. DMSO pretreatments inactivated endogenous MMP at the hybrid layer, reducing collagen degradation especially under dry conditions;

IV. DMSO-dry bonding conferred enhanced interfacial stability after long-term aging. Stiffening of demineralized collagen by DMSO pretreatments allowed not only proper hybridization under dry conditions, but higher uniformity in monomer conversion across the hybrid layer along with lower peptide degradation;

V. DMSO-dry bonding minimized overdrying-related issues allowing extensive air-drying of demineralized collagen immediately before hybridization. Broadening the moisture spectrum of demineralized dentin to drier levels occurred without any compromise in collagen wettability.

Acknowledgements

This doctoral thesis was performed in the Department of Cariology and Restorative Dentistry, Institute of Dentistry, Faculty of Medicine, University of Turku between 2017 and 2021.

To my supervisors Professor Arzu Tezvergil-Mutluay and Professor Leo Tjäderhane I express my deepest and most sincere gratitude for not only granting the exceptional opportunity to join your research group, but also for the encouragement to pursue a PhD in Finland. Your expertise was invaluable in formulating the right research questions and selecting the proper methodologies. This study series would not be possible without your experience, knowledge or support. Thank you for the countless insightful feedbacks, for inspiring and motivating me and for all the guidance extending beyond just academic work.

I am grateful to Professor Yoav Finer for agreeing to serve as an opponent and for Professor Roberto Ruggiero Braga and Professor Julian Leprince for carefully reviewing the manuscript, for the invaluable comments and constructive suggestions.

To Professor Mustafa Murat Mutluay for all your kindness and patience especially during the endless lab technical assistances, methodology discussions, statistical support and for the help regarding everyday life in Finland.

To Docent Merja Anneli Laine and Dr. Teemu Tirri for the constructive discussions regarding clinical treatments and for the great team work at the pre-clinical teaching. I also kindly thank Mrs. Katri Kuismanen for the everlasting positive attitude and for all assistance with the dental materials used in the studies.

I would like to express my gratitude for the support of my friends Dr. Roda Seseogullari-Dirihan, Dr. Anas Aaqel Salim Al-Ani, Dr. Ikram Aqel Salim Al-Ani, Dr. Merve Uctasli, Dr. Ana Sezinando, Dr. Jaana Sippus and Dr. Omar Ismail who provided stimulating discussions as well as happy distractions outside the work environment.

To the co-authors who diligently contributed helping me conduct the experiments.

The financial support provided by CIMO Fellowship Program for students in Finland, Turku University Foundation and Finish Dental Society Apollonia were greatly appreciated.

I have great appreciation for the help from Dr. Vuokko Loimaranta and the assistance from our lab staff, Oona Hällforks, Katja Sampalahti and Aija Koivusaari, regarding lab-related issues.

My appreciation to the Finnish Student Health Service (Ylioppilaiden terveydenhoitosäätiö, YTHS) in Turku and Helsinki for providing extracted teeth for the experiments.

To the dental companies who kindly donated the materials used in the studies.

Last but not least, to my family and friends who supported me every step of the way during this journey.

5.11.2021

Thiago Henrique Scarabello Stape

References

Açil Y, Mobasseri AE, Warnke PH, Terheyden H, Wiltfang J, & Springer I. (2005). Detection of mature collagen in human dental enamel. *Calcified Tissue International*, *76*(2), 121–126. https://doi.org/10.1007/s00223-004-0122-0

Agee KA, Becker TD, Joyce AP, Rueggeberg FA, Borke JL, Waller JL, Tay FR, & Pashley DH. (2006). Net expansion of dried demineralized dentin matrix produced by monomer/alcohol saturation and solvent evaporation. *Journal of Biomedical Materials Research - Part A*, *79*(2), 349–358. https://doi.org/10.1002/jbm.a.30752

Agee KA, Prakki A, Abu-Haimed T, Naguib GH, Nawareg MA, Tezvergil-Mutluay A, Scheffel DLS, Chen C, Jang SS, Hwang H, Brackett M, Grégoire G, Tay FR, Breschi L, & Pashley DH. (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

Arakawa T, Kita Y, & Timasheff SN. (2007). Protein precipitation and denaturation by dimethyl sulfoxide. *Biophysical Chemistry*, *131*(1–3), 62–70. https://doi.org/10.1016/j.bpc.2007.09.004

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

Bachmann L, Gomes ASL, & Zezell DM. (2004). Bound energy of water in hard dental tissues. *Spectroscopy Letters*, *37*(6), 565–579. https://doi.org/10.1081/SL-200036395

Baum BJ, & Mooney DJ. (2000). The impact of tissue engineering on dentistry. *Journal of the American Dental Association*, *131*(3), 309–318. https://doi.org/10.14219/jada.archive.2000.0174

Becker TD, Agee KA, Joyce AP, Rueggeberg FA, Borke JL, Waller JL, Tay FR, & Pashley DH. (2007). Infiltration/evaporation-induced shrinkage of demineralized dentin by solvated model adhesives. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *80*(1), 156–165. https://doi.org/10.1002/jbm.b.30580

Bedran-Russo AK, Pauli GF, Chen SN, McAlpine J, Castellan CS, Phansalkar RS, Aguiar TR, Vidal CMP, Napotilano JG, Nam JW, & Leme AA. (2014). Dentin biomodification: Strategies, renewable resources and clinical applications. *Dental Materials*, *30*(1), 62–76. https://doi.org/10.1016/j.dental.2013.10.012

Bedran-Russo AKB, Pashley DH, Agee K, Drummond JL, & Miescke KJ. (2008). Changes in stiffness of demineralized dentin following application of collagen crosslinkers. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *86*(2), 330–334. https://doi.org/10.1002/jbm.b.31022

Bedran-Russo AKB, Vidal CMP, Dos Santos PH, & Castellan CS. (2010). Long-term effect of carbodiimide on dentin matrix and resin-dentin bonds. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *94*(1), 250–255. https://doi.org/10.1002/jbm.b.31649

Bertassoni LE, Orgel JPR, Antipova O, & Swain M V. (2012a). The dentin organic matrix - Limitations of restorative dentistry hidden on the nanometer scale. *Acta Biomaterialia*, *8*(7), 2419–2433. https://doi.org/10.1016/j.actbio.2012.02.022

Bertassoni LE, Stankoska K, & Swain MV. (2012b). Insights into the structure and composition of the peritubular dentin organic matrix and the lamina limitans. *Micron*, *43*(2–3), 229–236. https://doi.org/10.1016/j.micron.2011.08.003

Boyde A, Switsur V, & Steward A. (1963). An assessment of two new physical methods applied to the study of dental tissues. *In: Advances in Fluorine Research and Dental Caries Prevention. Vol 1. Oxford: Pergamon Press, Ltd*, 185–193.

Breschi L, Gobbi P, Lopes M, Prati C, Falconi M, Teti G, & Mazzotti G. (2003a). Immunocytochemical analysis of dentin: A double-labeling technique. *Journal of Biomedical Materials Research - Part A*, *67*(1), 11–17. https://doi.org/10.1002/jbm.a.10048

Breschi L, Maravic T, Comba A, Cunha SR, Loguercio AD, Reis A, Hass V, Cadenaro M, Mancuso E, Mayer-Santos E, Niu L, Pashley DH, Tay FR, & Mazzoni A. (2020). Chlorhexidine preserves the hybrid layer in vitro after 10-years aging. *Dental Materials*, *36*(5), 672–680. https://doi.org/10.1016/j.dental.2020.03.009

Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L, Pashley DH, Tay FR, & 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, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjäderhane L, Ruggeri A, Tay FR, Dorigo EDS, & Pashley DH. (2010a). Chlorhexidine stabilizes the adhesive interface: A 2-year in vitro study. *Dental Materials*, *26*(4), 320–325. https://doi.org/10.1016/j.dental.2009.11.153

Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjäderhane L, Visintini E, Cadenaro M, Tay FR, Dorigo EDS, & Pashley DH. (2010b). Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer. *Dental Materials*, *26*(6), 571–578. https://doi.org/10.1016/j.dental.2010.02.007

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

Breschi L, Perdigão J, Gobbi P, Mazzotti G, Falconi M, & Lopes M. (2003b). Immunocytochemical identification of Type I collagen in acid-etched dentin. *Journal of Biomedical Materials Research - Part A*, *66*(4), 764–769. https://doi.org/10.1002/jbm.a.10073

Cadenaro M, Antoniolli F, Codan B, Agee K, Tay FR, Dorigo EDS, Pashley DH, & Breschi L. (2010). Influence of different initiators on the degree of conversion of experimental adhesive blends in relation to their hydrophilicity and solvent content. *Dental Materials*, *26*(4), 288–294. https://doi.org/10.1016/j.dental.2009.11.078

Cadenaro M, Breschi L, Antoniolli F, Navarra CO, Mazzoni A, Tay FR, Di Lenarda R, & Pashley DH. (2008). Degree of conversion of resin blends in relation to ethanol content and hydrophilicity. *Dental Materials*, *24*(9), 1194–1200. https://doi.org/10.1016/j.dental.2008.01.012

Cadenaro M, Breschi L, Rueggeberg FA, Suchko M, Grodin E, Agee K, Di Lenarda R, Tay FR, & Pashley DH. (2009). Effects of residual ethanol on the rate and degree of conversion of five experimental resins. *Dental Materials*, *25*(5), 621–628. https://doi.org/10.1016/j.dental.2008.11.005

Capriotti K, & Capriotti JA. (2012). Dimethyl Sulfoxide. *Clinical and Aesthethic Dermatology*, *5*(9), 24–26. https://doi.org/10.1001/jama.1967.03120240128036

Carrilho MR, Tay FR, Donnelly AM, Agee KA, Tjäderhane L, Mazzoni A, Breschi L, Foulger S, & Pashley DH. (2009). Host-derived loss of dentin matrix stiffness associated with solubilization of collagen. *Journal of Biomedical Materials Research. Part B, Applied Biomaterials*, *90*(1), 373–380. https://doi.org/10.1002/jbm.b.31295

Carrilho MRO, Carvalho RM, Goes MF de, Hipólito V di, Geraldeli S, Tay FR, Pashley DH, & Tjäderhane L. (2007). Chlorhexidine Preserves Dentin Bond in vitro. *Journal of Dental Research*, *86*(1), 90. https://doi.org/10.1177/154405910708600115

Carrilho MRO, Carvalho RM, Tay FR, Yiu C, & Pashley DH. (2005). Durability of resin-dentin bonds related to water and oil storage. *American Journal of Dentistry*, *18*(6), 315–319.

Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, & Tay FR. (2005). A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. *Biomaterials*, *26*(9), 1035–1042. https://doi.org/10.1016/j.biomaterials.2004.04.003

Carvalho RM, Mendonça JS, Santiago SL, Silveira RR, Garcia FCP, Tay FR, & Pashley DH. (2003). Effects of HEMA/solvent combinations on bond strength to dentin. *Journal of Dental Research*, *82*(8), 597–601. https://doi.org/10.1177/154405910308200805

Catalán J, Díaz C, & García-Blanco F. (2001). Characterization of binary solvent mixtures of DMSO with water and other cosolvents. *Journal of Organic Chemistry*, *66*(17), 5846–5852. https://doi.org/10.1021/jo010415i

Colthup NB. (1950). Spectra-Structure Correlations in the Infra-Red Region. *Journal of the Optical Society of America*, *40*(6), 397. https://doi.org/10.1364/josa.40.000397

Comper W. (1996). *Extracellular matrix Volume 2: Molecular components and interactions*. Amsterdam, NL: Overseas Publishers Association, B. V. https://www.worldcat.org/title/extracellular-matrix-vol-2-molecular-components-and-interactions/oclc/770443652

Cova A, Breschi L, Nato F, Ruggeri A, Carrilho M, Tjäderhane L, Prati C, Di Lenarda R, Tay FR, Pashley DH, & Mazzoni A. (2011). Effect of UVA-activated riboflavin on dentin bonding. *Journal of Dental Research*, *90*(12), 1439–1445. https://doi.org/10.1177/0022034511423397

D'Alpino PHP, Pereira JC, Svizero NR, Rueggeberg FA, & Pashley DH. (2006). Use of fluorescent compounds in assessing bonded resin-based restorations: A literature review. *Journal of Dentistry*, *34*(9), 623–634. https://doi.org/10.1016/j.jdent.2005.12.004

Dal-Bianco K, Pellizzaro A, Patzlaft R, de Oliveira Bauer JR, Loguercio AD, & Reis A. (2006). Effects of moisture degree and rubbing action on the immediate resin-dentin bond strength. *Dental Materials*, *22*(12), 1150–1156. https://doi.org/10.1016/j.dental.2005.10.010

Delaviz Y, Finer Y, & Santerre JP. (2014). Biodegradation of resin composites and adhesives by oral bacteria and saliva: A rationale for new material designs that consider the clinical environment and treatment challenges. *Dental Materials*, *30*(1), 16–32. https://doi.org/10.1016/j.dental.2013.08.201

De Munck J, Mine A, Poitevin A, Van Ende A, Cardoso MV, Van Landuyt KL, Peumans M, & Van Meerbeek B. (2012). Meta-analytical review of parameters involved in dentin bonding. *Journal of Dental Research*, *91*(4), 351–357. https://doi.org/10.1177/0022034511431251

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

Di Lullo GA, Sweeney SM, Körkkö J, Ala-Kokko L, & San Antonio JD. (2002). Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen. *Journal of Biological Chemistry*, *277*(6), 4223–4231. https://doi.org/10.1074/jbc.M110709200

Dickinson DP. (2002). Cysteine peptidases of mammals: Their biological roles and potential effects in the oral cavity and other tissues in health and disease. *Critical Reviews in Oral Biology and Medicine*, *13*(3), 238–275. https://doi.org/10.1177/154411130201300304

Eddleston CL, Hindle AR, Agee KA, Carvalho RM, Tay FR, Rueggeberg FA, & Pashley DH. (2003). Dimensional changes in acid-demineralized dentin matrices following the use of HEMA-water versus HEMA-alcohol primers. *Journal of Biomedical Materials Research - Part A*, *67*(3), 900–907. https://doi.org/10.1002/jbm.a.10151

Eick J, Wilko R, Anderson C, & Sorensen S. (1970). Scanning electron microscopy of cut tooth surfaces and identification of debris by use of the electron microprobe. *Journal of Dental Research*, *49*(6), 1359–1368.

Ekambaram M, Yiu CKY, & Matinlinna JP. (2015). An overview of solvents in resin-dentin bonding. *International Journal of Adhesion and Adhesives*, *57*, 22–33. https://doi.org/10.1016/j.ijadhadh.2014.09.007

Elliott JE, Lovell LG, & Bowman CN. (2001). Primary cyclization in the polymerization of bis-GMa and TEGDMA: A modeling approach to understanding the cure of dental resins. *Dental Materials*, *17*(3), 221–229. https://doi.org/10.1016/S0109-5641(00)00075-0

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

Fawzy AS, Nitisusanta LI, Iqbal K, Daood U, & Neo J. (2012). Riboflavin as a dentin crosslinking agent: Ultraviolet A versus blue light. *Dental Materials*, *28*(12), 1284–1291. https://doi.org/10.1016/j.dental.2012.09.009

Feitosa VP, Bazzocchi MG, Putignano A, Orsini G, Luzi AL, Sinhoreti MAC, Watson TF, & Sauro S. (2013). Dicalcium phosphate ($CaHPO_4 \cdot 2H_2O$) precipitation through ortho- or meta-phosphoric acid-etching: Effects on the durability and nanoleakage/ultra-morphology of resin-dentine interfaces. *Journal of Dentistry*, *41*(11), 1068–1080. https://doi.org/10.1016/j.jdent.2013.08.014

Ferracane JL. (2006). Hygroscopic and hydrolytic effects in dental polymer networks. *Dental Materials*, *22*(3), 211–222. https://doi.org/10.1016/j.dental.2005.05.005

Finer Y, Jaffer F, & Santerre JP. (2004a). Mutual influence of cholesterol esterase and pseudocholinesterase on the biodegradation of dental composites. *Biomaterials*, *25*(10), 1787–1793. https://doi.org/10.1016/j.biomaterials.2003.08.029

Finer Y, & Santerre JP. (2003). Biodegradation of a dental composite by esterases: Dependence on enzyme concentration and specificity. *Journal of Biomaterials Science, Polymer Edition*, *14*(8), 837–849. https://doi.org/10.1163/156856203768366558

Finer Y, & Santerre JP. (2004b). Salivary esterase activity and its association with the biodegradation of dental composites. *Journal of Dental Research*, *83*(1), 22–26. https://doi.org/10.1177/154405910408300105

Fusayama T, Nakamura M, Kurosaki N, & Iwaku M. (1979). Non-Pressure Adhesion of a New Adhesive Restorative Resin. *Journal of Dental Research*, *58*(4), 1364–1370. https://doi.org/10.1177/00220345790580041101

Garnero P, Borel O, Byrjalsen I, Ferreras M, Drake FH, McQueney MS, Foged NT, Delmas PD, & Delaissé JM. (1998). The collagenolytic activity of cathepsin K is unique among mammalian proteinases. *Journal of Biological Chemistry*, *273*(48), 32347–32352. https://doi.org/10.1074/jbc.273.48.32347

Garnero P, Ferreras M, Karsdal MA, Nicamhlaoibh R, Risteli J, Borel O, Qvist P, Delmas PD, Foged NT, & Delaissé JM. (2003). The Type I Collagen Fragments ICTP and CTX Reveal Distinct Enzymatic Pathways of Bone Collagen Degradation. *Journal of Bone and Mineral Research*, *18*(5), 859–867. https://doi.org/10.1359/jbmr.2003.18.5.859

Germann H -P, & Heidemann E. (1988). A synthetic model of collagen: An experimental investigation of the triple-helix stability. *Biopolymers*, *27*(1), 157–163. https://doi.org/10.1002/bip.360270112

Geurtsen W, Lehmann F, Spahl W, & Leyhausen G. (1998). Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. *Journal of Biomedical Materials Research*, *41*(3), 474–480. https://doi.org/10.1002/(SICI)1097-4636(19980905)41:3<474::AID-JBM18>3.0.CO;2-I

Goldberg B, & Sherr CJ. (1973). Secretion and extracellular processing of procollagen by cultured human fibroblasts. *Proc Natl Acad Sci USA*, *70*(2), 361–365. https://doi.org/10.1073/pnas.70.2.361

Grégoire G, Dabsie F, Dieng-Sarr F, Akon B, & Sharrock P. (2011). Solvent composition of one-step self-etch adhesives and dentine wettability. *Journal of Dentistry*, *39*(1), 30–39. https://doi.org/10.1016/j.jdent.2010.09.008

Gu LS, Cai X, Guo JM, Pashley DH, Breschi L, Xu HHK, Wang XY, Tay FR, & Niu LN. (2019). Chitosan-Based Extrafibrillar Demineralization for Dentin Bonding. *Journal of Dental Research*, *98*(2), 186–193. https://doi.org/10.1177/0022034518805419

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

Gupta SN, & Nandi US. (1970). Role of dimethyl sulfoxide as a solvent for vinyl polymerization. *Journal of Polymer Science Part A-1: Polymer Chemistry*, *8*(6), 1493–1501. https://doi.org/10.1002/pol.1970.150080614

Gwinnett A. (1984). Smear layer: morphological considerations. *Oper Dent Suppl*, *2*, 2–12.

Gwinnett AJ. (1992a). Moist versus dry dentin: its effect on shear bond strength. *American Journal of Dentistry*, *5*(3), 127–129.

Gwinnett AJ, & Kanca JA. (1992b). Micromorphology of the bonded dentin interface and its relationship to bond strength. *American Journal of Dentistry*, *5*(2), 73–77. http://www.ncbi.nlm.nih.gov/pubmed/1524750

Hass V, Dobrovolski M, Zander-Grande C, Martins GC, Gordillo LAA, Rodrigues Accorinte MDL, Gomes OMM, Loguercio AD, & Reis A. (2013). Correlation between degree of conversion, resin-dentin bond strength and nanoleakage of simplified etch-and-rinse adhesives. *Dental Materials*, *29*(9), 921–928. https://doi.org/10.1016/j.dental.2013.05.001

He LH, & Swain M V. (2007). Contact induced deformation of enamel. *Applied Physics Letters*, *90*(17), 1–5. https://doi.org/10.1063/1.2450649

He LH, & Swain M V. (2008). Understanding the mechanical behaviour of human enamel from its structural and compositional characteristics. *Journal of the Mechanical Behavior of Biomedical Materials*, *1*(1), 18–29. https://doi.org/10.1016/j.jmbbm.2007.05.001

Hebling J, Bianchi L, Basso FG, Scheffel DL, Soares DG, Carrilho MRO, Pashley DH, Tjäderhane L, & De Souza Costa CA. (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

Hebling J, Pashley DH, Tjäderhane L, & Tay FR. (2005). Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. *Journal of Dental Research*, *84*(8), 741–746. https://doi.org/10.1177/154405910508400811

Hitmi L, Bouter D, & Degrange M. (2002). Influence of drying and HEMA treatment on dentin wettability. *Dental Materials*, *18*(7), 503–511. https://doi.org/10.1016/S0109-5641(01)00075-6

Hosaka K, Nishitani Y, Tagami J, Yoshiyama M, Brackett WW, Agee KA, Tay FR, & Pashley DH. (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

Huang B, Cvitkovitch DG, Santerre JP, & Finer Y. (2018a). Biodegradation of resin–dentin interfaces is dependent on the restorative material, mode of adhesion, esterase or MMP inhibition. *Dental Materials*, *34*(9), 1253–1262. https://doi.org/10.1016/j.dental.2018.05.008

Huang B, Siqueira WL, Cvitkovitch DG, & Finer Y. (2018b). Esterase from a cariogenic bacterium hydrolyzes dental resins. *Acta Biomaterialia*, *71*, 330–338. https://doi.org/10.1016/j.actbio.2018.02.020

Imbeni V, Kruzic JJ, Marshall GW, Marshall SJ, & Ritchie RO. (2005). The dentin-enamel junction and the fracture of human teeth. *Nature Materials*, *4*(3), 229–232. https://doi.org/10.1038/nmat1323

Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, Rueggeberg FA, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR, & Pashley DH. (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

Jaffer F, Finer Y, & Santerre JP. (2002). Interactions between resin monomers and commercial composite resins with human saliva derived esterases. *Biomaterials*, *23*(7), 1707–1719. https://doi.org/10.1016/S0142-9612(01)00298-8

Kanca 3rd J. (1992). Resin bonding to wet substrate. 1. Bonding to dentin. *Quintessence Int*, *23*(1), 39–41.

Kenshima S, Francci C, Reis A, Loguercio AD, & Filho LER. (2006). Conditioning effect on dentin, resin tags and hybrid layer of different acidity self-etch adhesives applied to thick and thin smear layer. Journal of Dentistry, 34(10), 775–783. https://doi.org/10.1016/j.jdent.2006.03.001

Kim J, Uchiyama T, Carrilho M, Agee KA, Mazzoni A, Breschi L, Carvalho RM, Tjäderhane L, Looney S, Wimmer C, Tezvergil-Mutluay A, Tay FR, & Pashley DH. (2010a). Chlorhexidine binding to mineralized versus demineralized dentin powder. *Dental Materials*, *26*(8), 771–778. https://doi.org/10.1016/j.dental.2010.04.001

Kim J, Arola DD, Gu L, Kim YK, Mai S, Liu Y, Pashley DH, & Tay FR. (2010b). Functional biomimetic analogs help remineralize apatite-depleted demineralized resin-infiltrated dentin via a bottom-up approach. *Acta Biomaterialia*, *6*(7), 2740–2750. https://doi.org/10.1016/j.actbio.2009.12.052

Kinney JH, Marshall SJ, & Marshall GW. (2003). The mechanical properties of human dentin: A critical review and re-evaluation of the dental literature. *Critical Reviews in Oral Biology and Medicine*, *14*(1), 13–29. https://doi.org/10.1177/154411130301400103

Kirchner B, & Reiher M. (2002). The secret of dimethyl sulfoxide-water mixtures. A quantum chemical study of 1DMSO-nwater clusters. *Journal of the American Chemical Society*, *124*(21), 6206–6215. https://doi.org/10.1021/ja017703g

Kiuru O, Sinervo J, Vähänikkilä H, Anttonen V, & Tjäderhane L. (2021). MMP Inhibitors and Dentin Bonding: Systematic Review and Meta-Analysis. In *International Journal of Dentistry* (Vol. 27). Hindawi Limited. https://doi.org/10.1155/2021/9949699

Kleverlaan CJ, & Feilzer AJ. (2005). Polymerization shrinkage and contraction stress of dental resin composites. *Dental Materials*, *21*(12), 1150–1157. https://doi.org/10.1016/j.dental.2005.02.004

Kostoryz EL, Dharmala K, Ye Q, Wang Y, Huber J, Park JG, Snider G, Katz JL, & Spencer P. (2009). Enzymatic biodegradation of HEMA/BisGMA adhesives formulated with different water content. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *88*(2), 394–401. https://doi.org/10.1002/jbm.b.31095

Kuhn E, Farhat P, Teitelbaum AP, Mena-Serrano A, Loguercio AD, Reis A, & Pashley DH. (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

Isolan CP, Sarkis-Onofre R, Lima GS, Moraes RR. (2018). Bonding to Sound and Caries-Affected Dentin: A Systematic Review and Meta-Analysis. *J Adhes Dent,* 20(1):7-18. doi: 10.3290/j.jad.a39775. PMID: 29399679

Landis WJ, & Jacquet R. (2013). Association of calcium and phosphate ions with collagen in the mineralization of vertebrate tissues. *Calcified Tissue International*, *93*(4), 329–337. https://doi.org/10.1007/s00223-013-9725-7

Leme-Kraus AA, Aydin B, Vidal CMP, Phansalkar RM, Nam JW, McAlpine J, Pauli GF, Chen S, & Bedran-Russo AK. (2017). Biostability of the Proanthocyanidins-Dentin Complex and Adhesion Studies. *Journal of Dental Research*, *96*(4), 406–412. https://doi.org/10.1177/0022034516680586

Lemon MT, Jones MS, & Stansbury JW. (2007). Hydrogen bonding interactions in methacrylate monomers and polymers. *Journal of Biomedical Materials Research - Part A*, *83*(3), 734–746. https://doi.org/10.1002/jbm.a.31448

Lenz RW. (1993). Biodegradable polymers. *Advances in Polymer Science*, *107*, 1–40. https://doi.org/10.1007/bfb0027550

Li F, Liu X-Y, Zhang L, Kang J-J, & Chen J-H. (2012). Ethanol-wet bonding technique may enhance the bonding performance of contemporary etch-and-rinse dental adhesives. *The Journal of Adhesive Dentistry*, *14*(2), 113–120. https://doi.org/10.3290/j.jad.a21853

Liu Y, Chen M, Yao X, Xu C, Zhang Y, & Wang Y. (2013). Enhancement in dentin collagen's biological stability after proanthocyanidins treatment in clinically relevant time periods. *Dental Materials*, *29*(4), 485–492. https://doi.org/10.1016/j.dental.2013.01.013

Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, & Tay FR. (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

Lu S, Zhao SJ, Gao Y, Sun Y, Li X, & Chen JH. (2014). Proteoglycans affect monomer infiltration in the etch-and-rinse bonding technique. *Dental Materials*, *30*(11), e289–e299. https://doi.org/10.1016/j.dental.2014.05.015

Luzar A, & Chandler D. (1993). Structure and hydrogen bond dynamics of water-dimethyl sulfoxide mixtures by computer simulations. *The Journal of Chemical Physics*, *98*(10), 8160–8173. https://doi.org/10.1063/1.464521

MacGregor WS. (1967). the Chemical and Physical Properties of Dmso. *Annals of the New York Academy of Sciences*, *141*(1), 3–12. https://doi.org/10.1111/j.1749-6632.1967.tb34860.x

Mader CL, Baumgartner JC, & Peters DD. (1984). Scanning electron microscopic investigation of the smeared layer on root canal walls. *Journal of Endodontics*, *10*(10), 477–483. https://doi.org/10.1016/S0099-2399(84)80204-6

Mai S, Wei CC, Gu L sha, Tian F cong, Arola DD, Chen J hua, Jiao Y, Pashley DH, Niu L na, & Tay FR. (2017). Extrafibrillar collagen demineralization-based chelate-and-rinse technique bridges the gap between wet and dry dentin bonding. *Acta Biomaterialia*, *57*, 435–448. https://doi.org/10.1016/j.actbio.2017.05.017

Malacarne-Zanon J, Pashley DH, Agee KA, Foulger S, Alves MC, Breschi L, Cadenaro M, Garcia FP, & Carrilho MR. (2009). Effects of ethanol addition on the water sorption/solubility and percent conversion of comonomers in model dental adhesives. *Dental Materials*, *25*(10), 1275–1284. https://doi.org/10.1016/j.dental.2009.03.015

Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, Yiu CK, & Carrilho MR 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

Manso AP, Marquezini L, Silva SMA, Pashley DH, Tay FR, & Carvalho RM. (2008). Stability of wet versus dry bonding with different solvent-based adhesives. *Dental Materials*, *24*(4), 476–482. https://doi.org/10.1016/j.dental.2007.04.009

Martin-De Las Heras S, Valenzuela A, & Overall CM. (2000). The matrix metalloproteinase gelatinase A in human dentine. *Archives of Oral Biology*, *45*(9), 757–765. https://doi.org/10.1016/S0003-9969(00)00052-2

Martin D, Weise A, & Niclas H-J. (1967). The Solvent Dimethyl Sulfoxide. *Angewandte Chemie International Edition in English*, *6*(4), 318–334. https://doi.org/10.1002/anie.196703181

Mazzoni A, Carrilho M, Papa V, Tjäderhane L, Gobbi P, Nucci C, Di Lenarda R, Mazzotti G, Tay FR, Pashley DH, & Breschi L. (2011a). MMP-2 assay within the hybrid layer created by a two-step etch-and-rinse adhesive: Biochemical and immunohistochemical analysis. *Journal of Dentistry*, *39*(7), 470–477. https://doi.org/10.1016/j.jdent.2011.04.004

Mazzoni A, Mannello F, Tay FR, Tonti GAM, Papa S, Mazzotti G, Di Lenarda R, Pashley DH, & Breschi L. (2007). Zymographic analysis and characterization of MMP-2 and -9 forms in human sound dentin. *Journal of Dental Research*, *86*(5), 436–440. https://doi.org/10.1177/154405910708600509

Mazzoni A, Papa V, Nato F, Carrilho M, Tjäderhane L, Ruggeri A, Gobbi P, Mazzotti G, Tay FR, Pashley DH, & Breschi L. (2011b). Immunohistochemical and biochemical assay of MMP-3 in human dentine. *Journal of Dentistry*, *39*(3), 231–237. https://doi.org/10.1016/j.jdent.2011.01.001

Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjäderhane L, Toledano M, Pashley EL, & Tay FR. (2006). Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. *Biomaterials*, *27*(25), 4470–4476. https://doi.org/10.1016/j.biomaterials.2006.01.040

Mehtälä P, Pashley DH, & 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

Miura J, Nishikawa K, Kubo M, Fukushima S, Hashimoto M, Takeshige F, & Araki T. (2014). Accumulation of advanced glycation end-products in human dentine. *Archives of Oral Biology*, *59*(2), 119–124. https://doi.org/10.1016/j.archoralbio.2013.10.012

Mjör IA, & Nordahl I. (1996). The density and branching of dentinal tubules in human teeth. *Archives of Oral Biology*, *41*(5), 401–412. https://doi.org/10.1016/0003-9969(96)00008-8

Montagner AF, Sarkis-Onofre R, Pereira-Cenci T, & Cenci MS. (2014). MMP inhibitors on dentin stability: A systematic review and meta-analysis. In *Journal of Dental Research* (Vol. 93, Issue 8, pp. 733–743). J Dent Res. https://doi.org/10.1177/0022034514538046

Nagase H. (1997). Activation mechanisms of matrix metalloproteinases. In *Biological Chemistry* (Vol. 378, Issues 3–4, pp. 151–160).

Nakabayashi N, Kojima K, & Masuhara E. (1982). The promotion of adhesion by the infiltration of monomers into tooth substrates. *Journal of Biomedical Materials Research*, *16*(3), 265–273. https://doi.org/10.1002/jbm.820160307

Nakabayashi N. (1998). Hybridization of dental hard tissues. *Chicago: Quintessence Publishing Co*; 1st edition, August 15, 1998.

Nakajima M, Kanemura N, Pereira PNR, Tagami J, & Pashley DH. (2000). Comparative microtensile bond strength and SEM analysis of bonding to wet and dry dentin. *American Journal of Dentistry*, *13*(6), 324–328.

Nakaoki Y, Nikaido T, Pereira PNR, Inokoshi S, & Tagami J. (2000). Dimensional changes of demineralized dentin treated with HEMA primers. *Dental Materials*, *16*(6), 441–446. https://doi.org/10.1016/S0109-5641(00)00042-7

Nishitani Y, Yoshiyama M, Donnelly AMM, Agee KAA, Sword J, Tay FRR, & Pashley DHH. (2006). Effects of resin hydrophilicity on dentin bond strength. *J Dent Res*, *85*(11), 1016–1021. https://doi.org/10.1177/154405910608501108

Niu LN, Zhang W, Pashley DH, Breschi L, Mao J, Chen JH, & Tay FR. (2014). Biomimetic remineralization of dentin. *Dental Materials*, *30*(1), 77–96. https://doi.org/10.1016/j.dental.2013.07.013

Obermajer N, Jevnikar Z, Doljak B, & Kos J. (2008). Role of cysteine cathepsins in matrix degradation and cell signalling. *Connective Tissue Research*, *49*(3–4), 193–196. https://doi.org/10.1080/03008200802143158

Ogata M, Harada N, Yamaguchi S, Nakajima M, Pereira P, & Tagami J. (2001). Effects of different burs on dentin bond strengths of self-etching primer bonding systems. *Oper Dent*, *26*(4), 375–382.

Okuyama K, Takayanagi M, Ashida T, & Kakudo M. (1977). A new structural model for collagen. *Polymer Journal*, *9*(3), 341–343. https://doi.org/10.1295/polymj.9.341

Pashley DH. (1992). The effects of acid etching on the pulpodentin complex. In *Operative dentistry* (Vol. 17, Issue 6, pp. 229–242). Oper Dent. https://pubmed.ncbi.nlm.nih.gov/1303516/

Pashley DH. (1984). Smear layer: physiological considerations. *Oper Dent Suppl*, *3*, 13–29. http://www.ncbi.nlm.nih.gov/pubmed/6396586

Pashley DH. (1996). Dynamics of the pulpo-dentin complex. *Crit Rev Oral Biol Med, 7*(2):104-33. https://doi.org/10.1177/10454411960070020101

Pashley DH, Agee KA, Nakajima FR Tay M, Carvalho RM, Terada RSS, Harmon FJ, Lee WK, & Rueggeberg FA. (2001). Solvent-induced dimensional changes in EDTA-demineralized dentin matrix. *Journal of Biomedical Materials Research*, *56*(2):273-81. https://doi.org/10.1002/1097-4636(200108)56:2<273::AID-JBM1095>3.0.CO;2-A

Pashley DH, Carvalho RM, Tay FR, Agee KA, & Lee KW. (2002). Solvation of dried dentin matrix by water and other polar solvents. *American Journal of Dentistry*, *15*(2), 97–102.

Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, & Tezvergil-Mutluay A. (2011). State of the art etch-and-rinse adhesives. *Dental Materials*, *27*(1), 1–16. https://doi.org/10.1016/j.dental.2010.10.016

Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, 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 DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, & Ito S. (2004). Collagen degradation by host-derived enzymes during aging. *Journal of Dental Research*, *83*(3), 216–221. https://doi.org/10.1177/154405910408300306

Perdigão J, Carmo ARP, & Geraldeli S. (2005). Eighteen-month clinical evaluation of two dentin adhesives applied on dry vs moist dentin. *The Journal of Adhesive Dentistry*, *7*(3), 253–258.

Peumans M, De Munck J, Mine A, & Van Meerbeek B. (2014). Clinical effectiveness of contemporary adhesives for the restoration of non-carious cervical lesions. A systematic review. *Dental Materials*, *30*(10), 1089–1103. https://doi.org/10.1016/j.dental.2014.07.007

Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, & Van Meerbeek B. (2012). A 13-year clinical evaluation of two three-step etch-and-rinse adhesives in non-carious class-V lesions. *Clinical Oral Investigations*, *16*(1), 129–137. https://doi.org/10.1007/s00784-010-0481-z

Pidaparti RMV, Chandran A, Takano Y, & Turner CH. (1996). Bone mineral lies mainly outside collagen fibrils: Predictions of a composite model for osteonal bone. *Journal of Biomechanics*, *29*(7), 909–916. https://doi.org/10.1016/0021-9290(95)00147-6

Scaffa PM, Vidal CM, Barros N, Gesteira TF, Carmona AK, Breschi L, Pashley DH, Tjäderhane L, Tersariol IL, Nascimento FD, & Carrilho MR. (2012). Chlorhexidine inhibits the activity of dental cysteine cathepsins. *Journal of Dental Research*, *91*(4), 420–425. https://doi.org/10.1177/0022034511435329

Pucci CR, Gu LS, Zhang H yun, Song Q, Xia VW, Davis LB, de Souza Andrade D, Mazzoni A, Breschi L, Pashley DH, Tay FR, & Niu LN. (2018). Water-associated attributes in the contemporary dentin bonding milieu. *Journal of Dentistry*, *74*, 79–89. https://doi.org/10.1016/j.jdent.2018.04.016

Rainey JK, & Goh MC. (2009). A statistically derived parameterization for the collagen triple-helix. *Protein Science*, *11*(11), 2748–2754. https://doi.org/10.1110/ps.0218502

Reddy GK, & Enwemeka CS. (1996). A simplified method for the analysis of hydroxyproline in biological tissues. *Clinical Biochemistry*, *29*(3), 225–229. https://doi.org/10.1016/0009-9120(96)00003-6

Reis A, Chibinski AC, Stanislawczuk R, Wambier DS, Grande RHM, & Loguercio AD. (2012). The role of dentin moisture in the degradation of resin-dentin interfaces under clinical and laboratory conditions. *Journal of the American Dental Association*, *143*(7), e29–e36. https://doi.org/10.14219/jada.archive.2012.0274

Reis A, Pellizzaro A, Dal-Bianco K, Gomes OM, Patzlaff R, & Loguercio AD. (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

Ricard-Blum S. (2011). The Collagen Family. *Cold Spring Harbor Perspectives in Biology*, *3*(1), 1–19. https://doi.org/10.1101/cshperspect.a004978

Rosales-Leal JI, Osorio R, Holgado-Terriza JA, Cabrerizo-Vílchez MA, & Toledano M. (2001). Dentin wetting by four adhesive systems. *Dental Materials*, *17*(6), 526–532. https://doi.org/10.1016/S0109-5641(01)00014-8

Rosales JI, Marshall GW, Marshall SJ, Watanabe LG, Toledano M, Cabrerizo MA, & Osorio R. (1999). Acid-etching and hydration influence on dentin roughness and wettability. *Journal of Dental Research*, *78*(9), 1554–1559. https://doi.org/10.1177/00220345990780091001

Rueggeberg FA, Hashinger DT, & Fairhurst CW. (1990). Calibration of FTIR conversion analysis of contemporary dental resin composites. *Dental Materials*, *6*(4), 241–249. https://doi.org/10.1016/S0109-5641(05)80005-3

Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, & Tay FR. (2010a). Ethanol wet-bonding challenges current anti-degradation strategy. *Journal of Dental Research*, *89*(12), 1499–1504. https://doi.org/10.1177/0022034510385240

Sadek FT, Castellan CS, Braga RR, Mai S, Tjäderhane L, Pashley DH, & Tay FR. (2010b). One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. *Dental Materials*, *26*(4), 380–386. https://doi.org/10.1016/j.dental.2009.12.009

Sadek FT, Mazzoni A, Breschi L, Tay FR, & Braga RR. (2010c). 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 FT, Pashley DH, Nishitani Y, Carrilho MR, Donnelly A, Ferrari M, & Tay FR. (2007). Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique. *Journal of Biomedical Materials Research Part A*, *84A*(1), 19–29. https://doi.org/10.1002/jbm.a

Salim Al-Ani AA, Stape THS, 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*(5), 143–150. https://doi.org/10.1016/j.jmbbm.2019.02.015

Salim Al-Ani AA, Mutluay M, Stape THS, 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

Sauro S, Toledano M, Aguilera FS, Mannocci F, Pashley DH, Tay FR, Watson TF, & Osorio R. (2010). Resin–dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. *Dent Mater*, *26*(4), 1–7. https://doi.org/10.1038/jid.2014.371

Sauro S, Osorio R, Watson TF, & Toledano M. (2012). Assessment of the quality of resin-dentin bonded interfaces: An AFM nano-indentation, μtBS and confocal ultramorphology study. *Dental Materials*, *28*(6), 622–631. https://doi.org/10.1016/j.dental.2012.02.005

Sauro S, Toledano M, Aguilera FS, Mannocci F, Pashley DH, Tay FR, Watson TF, & Osorio R. (2011). Resin-dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. Part II: Effects of mechanical cycling load on microtensile bond strengths. *Dental Materials*, *27*(6), 563–572. https://doi.org/10.1016/j.dental.2011.02.010

Sauro S, Watson TF, Mannocci F, Miyake K, Huffman BP, Tay FR, & Pashley DH. (2009). Two-photon laser confocal microscopy of micropermeability of resin-dentin bonds made with water or ethanol wet bonding. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *90 B*(1), 327–337. https://doi.org/10.1002/jbm.b.31290

Schlueter RJ, Veis A, & Schlueter RJ. (1964). The Macromolecular Organization of Dentine Matrix Collagen. I. Characterization of Dentine Collagen. *Biochemistry*, *6405867*(11), 1650–1657. https://doi.org/10.1021/bi00899a009

Sebold M, André CB, Carvalho RM, & Giannini M. (2019). Dry-bonding to dentin using alternative conditioners based on iron-containing solutions or nitric acid. *Journal of the Mechanical Behavior of Biomedical Materials*, *94*(September 2018), 238–248. https://doi.org/10.1016/j.jmbbm.2019.03.015

Shokati B, Tam LE, Santerre JP, & Finer Y. (2010). Effect of salivary esterase on the integrity and fracture toughness of the dentin-resin interface. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *94*(1), 230–237. https://doi.org/10.1002/jbm.b.31645

Sidhu SK, & Watson TF. (1998). Interfacial characteristics of resin-modified glass-ionomer materials: A study on fluid permeability using confocal fluorescence microscopy. *Journal of Dental Research*, *77*(9), 1749–1759. https://doi.org/10.1177/00220345980770091101

Sorsa T, Tjäderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, Golub LM, Brown DL, & Mäntylä P. (2006). Matrix metalloproteinases: Contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. In *Annals of Medicine* (Vol. 38, Issue 5, pp. 306–321). Ann Med. https://doi.org/10.1080/07853890600800103

Spencer P, & Swafford JR. (1999). Unprotected protein at the dentin-adhesive interface. *Quintessence International 30*(7), 501–507.

Spencer P, & Wang Y. (2002). Adhesive phase separation at the dentin interface under wet bonding conditions. *Journal of Biomedical Materials Research*, *62*(3), 447–456. https://doi.org/10.1002/jbm.10364

Spencer P, Wang Y, & Katz JL. (2004). Identification of collagen encapsulation at the dentin/adhesive interface. *The Journal of Adhesive Dentistry*, *6*(2), 91–95.

Spencer P, Wang Y, Walker MP, Wieliczka DM, & Swafford JR. (2000). Interfacial chemistry of the dentin/adhesive bond. *Journal of Dental Research*, *79*(7), 1458–1463. https://doi.org/10.1177/00220345000790070501

Spencer P, Ye Q, Misra A, Goncalves SEP, & Laurence JS. (2014). Proteins, pathogens, and failure at the composite-tooth interface. *Journal of Dental Research*, *93*(12), 1243–1249. https://doi.org/10.1177/0022034514550039

Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, Wang Y, Bohaty BS, Singh V, Sene F, Eslick J, Camarda K, & Katz JL. (2010). Adhesive/Dentin interface: the weak link in the composite restoration. *Annals of Biomedical Engineering*, *38*(6), 1989–2003. https://doi.org/10.1007/s10439-010-9969-6

Stape THS, Tjäderhane L, Marques MR, Aguiar FHB, & Martins LRM. (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 THS, Tjäderhane L, Tezvergil-Mutluay A, Yanikian CRF, Szesz AL, Loguercio AD, & Martins LRM. (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 THS, Wik P, Mutluay MM, Al-Ani AAS, & Tezvergil-Mutluay A. (2018). Selective dentin etching: A potential method to improve bonding effectiveness of universal adhesives. *Journal of the Mechanical Behavior of Biomedical Materials*, *86*(February), 14–22. https://doi.org/10.1016/j.jmbbm.2018.06.015

Sulkala M, Larmas M, Sorsa T, Salo T, & Tjäderhane L. (2002). The localization of matrix metalloproteinase-20 (MMP-20, enamelysin) in mature human teeth. *Journal of Dental Research*, *81*(9), 603–607. https://doi.org/10.1177/154405910208100905

Sulkala M, Tervahartiala T, Sorsa T, Larmas M, Salo T, & Tjäderhane L. (2007). Matrix metalloproteinase-8 (MMP-8) is the major collagenase in human dentin. *Archives of Oral Biology*, *52*(2), 121–127. https://doi.org/10.1016/j.archoralbio.2006.08.009

Sulkala M, Wahlgren J, Larmas M, Sorsa T, Teronen O, Salo T, & Tjäderhane L. (2001). The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats. *Journal of Dental Research*, *80*(6), 1545–1549. https://doi.org/10.1177/00220345010800061301

Suyamaa Y, Lührsb AK, De Munckc J, Mined A, Poitevine A, Yamadaf T, Van Meerbeekg B, & Cardosoh MV. (2013). Potential smear layer interference with bonding of self-etching adhesives to dentin. *The Journal of Adhesive Dentistry*, *15*(4), 317–324. https://doi.org/10.3290/j.jad.a29554

Szesz AL, Pereira G de MA, Siqueira FSF de, Cardenas AFM, Bandeca MC, Armas-Vega A, Reis A, & Loguercio AD. (2021). Effect of Addition of Dimethyl Sulfoxide to Simplified Adhesives on Dentin Bond Durability after Three Years of Water Storage. *The Journal of Adhesive Dentistry*, *23*(2), 159–165. https://doi.org/10.3290/j.jad.b1079585

Takahashi M, Nakajima M, Tagami J, Scheffel DL, Carvalho RM, Mazzoni A, Cadenaro M, Tezvergil-Mutluay A, Breschi L, Tjäderhane L, Jang SS, Tay FR, Agee KA, Pashley DH. (2013). The importance of size-exclusion characteristics of type I collagen in bonding to dentin matrices. *Acta Biomater, 9*(12), 9522-8. https://doi.org/10.1016/j.actbio.2013.07.037

Takamizawa T, Barkmeier WW, Sai K, Tsujimoto A, Imai A, Erickson RL, Latta MA, & Miyazaki M. (2018). Influence of different smear layers on bond durability of self-etch adhesives. *Dental Materials, 34*(2), 246–259. https://doi.org/10.1016/j.dental.2017.11.002

Tallant C, Marrero A, & Gomis-Rüth FX. (2010). Matrix metalloproteinases: Fold and function of their catalytic domains. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1803*(1), 20–28. Biochim Biophys Acta. https://doi.org/10.1016/j.bbamcr.2009.04.003

Tay FR, Carvalho R, Sano H, & Pashley DH. (2000). *Effect of smear layers on the bonding of a self-etching primer to dentin*. The Journal of Adhesive Dentistry.

Tay FR, Gwinnett JA, & Wei SHY. (1996a). Micromorphological spectrum from overdrying to overwetting acid-conditioned dentin in water-free, acetone-based, single-bottle primer/adhesives. *Dental Materials*, *12*(4), 236–244. https://doi.org/10.1016/S0109-5641(96)80029-7

Tay FR, Gwinnett JA, Pang P, & Wei S. (1996b). Resin permeation into acid-conditioned, moist, and dry dentin: a paradigm using water-free adhesive primers. *Journal of Dental Research*, *75*(4), 1034–1044. https://doi.org/10.1177/00220345960750040601

Tay FR, Hashimoto M, Pashley DH, Peters MC, Lai SCN, Yiu CKY, & Cheong C. (2003a). Aging affects two modes of nanoleakage expression in bonded dentin. *Journal of Dental Research*, *82*(7), 537–541. https://doi.org/10.1177/154405910308200710

Tay FR, Osorio E, Osorio R, Aguilera FS, & Toledano M. (2010). Ethanol Wet-bonding Technique Sensitivity Assessed by AFM. *Journal of Dental Research*, *89*(11), 1264–1269. https://doi.org/10.1177/0022034510376403

Tay FR, & Pashley DH. (2003b). Have dentin adhesives become too hydrophilic? *Journal (Canadian Dental Association)*, *69*(11), 726–731. https://doi.org/10.1016/S0109-5641(03)00110-6

Tay FR, & Pashley DH. (2009). Biomimetic remineralization of resin-bonded acid-etched dentin. *Journal of Dental Research*, *88*(8), 719–724. https://doi.org/10.1177/0022034509341826

Tay FR, Pashley DH, Kapur RR, Carrilho MRO, Hur YB, Garrett L V., & Tay KCY. (2007). Bonding BisGMA to dentin - A proof of concept for hydrophobic dentin bonding. *Journal of Dental Research*, *86*(11), 1034–1039. https://doi.org/10.1177/154405910708601103

Tay FR, Pashley DH, & Yoshiyama M. (2002). Two modes of nanoleakage expression in single-step adhesives. *Journal of Dental Research*, *81*(7), 472–476. https://doi.org/10.1177/154405910208100708

Tersariol IL, Geraldeli S, Minciotti CL, Nascimento FD, Pääkkönen V, Martins MT, Carrilho MR, Pashley DH, Tay FR, Salo T, & Tjäderhane L. (2010). Cysteine Cathepsins in Human Dentin-Pulp Complex. *Journal of Endodontics*, *36*(3), 475–481. https://doi.org/10.1016/j.joen.2009.12.034

Tezvergil-Mutluay A, Agee KA, Hoshika T, Carrilho M, Breschi L, Tjäderhane L, Nishitani Y, Carvalho RM, Looney S, Tay FR, & Pashley DH. (2010a). The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. *Dental Materials*, *26*(11), 1059–1067. https://doi.org/10.1016/j.dental.2010.07.006

Tezvergil-Mutluay A, Agee KA, Hoshika T, Tay FR, & Pashley DH. (2010b). The inhibitory effect of polyvinylphosphonic acid on functional matrix metalloproteinase activities in human demineralized dentin. *Acta Biomaterialia*, *6*(10), 4136–4142. https://doi.org/10.1016/j.actbio.2010.05.017

Tezvergil-Mutluay A, Agee KA, Hoshika T, Uchiyama T, Tjäderhane L, Breschi L, Mazzoni A, Thompson JM, McCracken CE, Looney SW, Tay FR, & Pashley DH. (2011a). Inhibition of MMPs by alcohols. *Dental Materials*, *27*(9), 926–933. https://doi.org/10.1016/j.dental.2011.05.004

Tezvergil-Mutluay A, Mutluay MM, Gu LS, Zhang K, Agee KA, Carvalho RM, Manso A, Carrilho M, Tay FR, Breschi L, Suh BI, & Pashley DH. (2011b). The anti-MMP activity of benzalkonium chloride. *Journal of Dentistry*, *39*(1), 57–64. https://doi.org/10.1016/j.jdent.2010.10.003

Tezvergil-Mutluay A, Agee KA, Uchiyama T, Imazato S, Mutluay MM, Cadenaro M, Breschi L, Nishitani Y, Tay FR, & Pashley DH. (2011c). The inhibitory effects of quaternary ammonium methacrylates on soluble and matrix-bound MMPs. *Journal of Dental Research*, *90*(4), 535–540. https://doi.org/10.1177/0022034510389472

Tezvergil-Mutluay A, Mutluay M, Seseogullari-Dirihan R, Agee KA, Key WO, Scheffel DLS, Breschi L, Mazzoni A, Tjäderhane L, Nishitani Y, Tay FR, & Pashley DH. (2013). Effect of phosphoric acid on the degradation of human dentin matrix. *Journal of Dental Research*, *92*(1), 87–91. https://doi.org/10.1177/0022034512466264

Tezvergil-Mutluay A, Mutluay MM, Agee KA, Seseogullari-Dirihan R, Hoshika T, Cadenaro M, Breschi L, Vallittu P, Tay FR, & Pashley DH. (2012). Carbodiimide cross-linking inactivates soluble and matrix-bound MMPs, in vitro. *Journal of Dental Research*, *91*(2), 192–196. https://doi.org/10.1177/0022034511427705

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, Carrilho M, Breschi L, Tay FR, & Pashley DH. (2012). Dentin basic structure and composition-an overview. *Endodontic Topics*, *20*(1) 3–29. https://doi.org/10.1111/j.1601-1546.2012.00269.x

Tjäderhane L, & Haapasalo M. (2009). The dentin-pulp border: a dynamic interface between hard and soft tissues. *Endodontic Topics*, *20*(1), 52–84. https://doi.org/10.1111/j.1601-1546.2012.00266.x

Tjäderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, & Salo T. (1998). The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. *Journal of Dental Research*, *77*(8), 1622–1629. https://doi.org/10.1177/00220345980770081001

Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, Tezvergil-Mutluay A, Carrilho M, Carvalho RM, Tay FR, & Pashley DH. (2013a). 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, Mehtälä P, Scaffa P, Vidal C, Pääkkönen V, Breschi L, Hebling J, Tay FR, Nascimento FD, Pashley DH, & Carrilho MR. (2013b). 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 FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, Tezvergil-Mutluay A, Carrilho MR, Carvalho RM, Tay FR, & Pashley DH. (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

Tyas MJ, Burns GA, Byrne PF, Cunningham PJ, Dobson BC, & Widdop FT. (1989). Clinical evaluation of Scotchbond™ three-year results. *Australian Dental Journal*, *34*(3), 277–279. https://doi.org/10.1111/j.1834-7819.1989.tb00683.x

Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P, & Van Meerbeek B. (2007). Systematic review of the chemical composition of contemporary dental adhesives. *Biomaterials*, *28*(26), 3757–3785. https://doi.org/10.1016/j.biomaterials.2007.04.044

Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, & Vanherle G. (2003). Adhesion to enamel and dentin: Current status and future challenges. *Operative Dentistry*, *28*(3), 215–235.

Van Meerbeek B, Peumans M, Verschueren M, Gladys S, Lambrechts P, Vanherle G, & Braem M. (1994). Clinical status of ten dentin adhesive systems. *Journal of Dental Research*, *73*(11), 1690–1702. https://doi.org/10.1177/00220345940730110401

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

Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, & Van Landuyt KL. (2011). State of the art of self-etch adhesives. *Dental Materials*, *27*(1), 17–28. https://doi.org/10.1016/j.dental.2010.10.023

Van Wart HE, & Birkedal-Hansen H. (1990). The cysteine switch: A principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(14), 5578–5582. https://doi.org/10.1073/pnas.87.14.5578

Vanherle G, Lambrechts P, & Braem M. (1991). An evaluation of different adhesive restorations in cervical lesions. *The Journal of Prosthetic Dentistry*, *65*, 341–347. https://doi.org/10.1016/0022-3913(91)90222-i

Veis A, & Schlueter RJ. (1964). The Macromolecular Organization of Dentine Matrix Collagen. I. Characterization of Dentine Collagen. In *Biochemistry* (Vol. 3, Issue 11, pp. 1650–1657). https://doi.org/10.1021/bi00899a009

Versluis A, Tantbirojn D, Pintado MR, DeLong R, & Douglas WH. (2004). Residual shrinkage stress distributions in molars after composite restoration. *Dental Materials*, *20*(6), 554–564. https://doi.org/10.1016/j.dental.2003.05.007

Verzár F. (1964). Aging of the collagen fiber. In *International review of connective tissue research* (Vol. 2, p. 244). Elsevier. https://doi.org/10.1016/b978-1-4831-6751-0.50012-4

Vishnyakov A, Lyubartsev AP, & 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

Visse R, & Nagase H. (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. In *Circulation Research* (Vol. 92, Issue 8, pp. 827–839). Circ Res. https://doi.org/10.1161/01.RES.0000070112.80711.3D

Wang Y, & Spencer P. (2002). Quantifying adhesive penetration in adhesive/dentin interface using confocal Raman microspectroscopy. *Journal of Biomedical Materials Research*, *59*(1), 46–55. https://doi.org/10.1002/jbm.1215

Wang Y, & Spencer P. (2003). Hybridization efficiency of the adhesive/dentin interface with wet bonding. *Journal of Dental Research*, *82*(2), 141–145. https://doi.org/10.1177/154405910308200213

Wang Y, Spencer P, & Walker MP. (2007). Chemical profile of adhesive/caries-affected dentin interfaces using Raman microspectroscopy. *Journal of Biomedical Materials Research - Part A*, *81*(2), 279–286. https://doi.org/10.1002/jbm.a.30981

Wang Y, Spencer P, & Yao X. (2006). Micro-Raman imaging analysis of monomer/mineral distribution in intertubular region of adhesive/dentin interfaces. *Journal of Biomedical Optics*, *11*(2), 024005. https://doi.org/10.1117/1.2187992

Yamauchi M, & Shiiba M. (2008). Lysine Hydroxylation and Cross-linking of Collagen. In *Post-translational Modifi cations of Proteins* (Vol. 446, pp. 95–108). Humana Press. https://doi.org/10.1007/978-1-60327-084-7_7

Ye Q, Wang Y, & Spencer P. (2009a). Nanophase separation of polymers exposed to simulated bonding conditions. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *88*(2), 339–348. https://doi.org/10.1002/jbm.b.31047

Ye Q, Park J, Topp E, & Spencer P. (2009b). Effect of photoinitiators on the in vitro performance of a dentin adhesive exposed to simulated oral environment. *Dental Materials*, *25*(4), 452–458. https://doi.org/10.1016/j.dental.2008.09.011

Ye Q, Spencer P, Wang Y, & Misra A. (2007). Relationship of solvent to the photopolymerization process, properties, structure in model dentin adhesives. *Journal of Biomedical Materials Research - Part A*, *80*(2), 342–350. https://doi.org/10.1002/jbm.a.30890

Yiu CKY, Pashley EL, Hiraishi N, King NM, Goracci C, Ferrari M, Carvalho RM, Pashley DH, & Tay FR. (2005). Solvent and water retention in dental adhesive blends after evaporation. *Biomaterials*, *26*(34), 6863–6872. https://doi.org/10.1016/j.biomaterials.2005.05.011

Yoshida Y, VanMeerbeek B, Nakayama Y, Snauwaert J, Hellemans L, Lambrechts P, Vannerle G, & Wakasa K. (2000). Evidence of chemical bonding at biomaterial-hard tissue interfaces. *Journal of Dental Research*, *79*(2), 709–714. https://doi.org/10.1177/00220345000790020301

Yu F, Luo ML, Xu RC, Huang L, Yu HH, Meng M, Jia JQ, Hu ZH, Wu WZ, Tay FR, Xiao YH, Niu LN, & Chen JH. (2021). A novel dentin bonding scheme based on extrafibrillar demineralization combined with covalent adhesion using a dry-bonding technique. *Bioactive Materials*, *6*(10), 3557–3567. https://doi.org/10.1016/j.bioactmat.2021.03.024

Zander-Grande C, Ferreira SQ, da Costa TRF, Loguercio AD, & Reis A. (2011). Application of etch-and-rinse adhesives on dry and rewet dentin under rubbing action a 24-month clinical evaluation. *J Am Dent Assoc*, *142*(7), 828–835. https://doi.org/142/7/828 [pii]

Zhang Z yi, Tian F cong, Niu L na, Ochala K, Chen C, Fu B ping, Wang X yan, Pashley DH, & Tay FR. (2016). Defying ageing: An expectation for dentine bonding with universal adhesives? *Journal of Dentistry*, *45*, 43–52. https://doi.org/10.1016/j.jdent.2015.11.008

Zheng YJ, & Ornstein RL. (1996). A molecular dynamics and quantum mechanics analysis of the effect of DMSO on enzyme structure and dynamics: Subtilisin. *Journal of the American Chemical Society*, *118*(17), 4175–4180. https://doi.org/10.1021/ja9539195

Zhou J, Chiba A, Scheffel DLS, Hebling J, Agee K, Tagami J, Tan J, Abuelenain D, Nawareg MA, Hassan AH, Breschi L, Tay FR, & Pashley DH. (2016). Cross-linked dry bonding: A new etch-and-rinse technique. *Dental Materials*, *32*(9), 1124–1132. https://doi.org/10.1016/j.dental.2016.06.014

Zimmerley M, McClure RA, Choi B, & Potma EO. (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

Zou Y, Armstrong SR, & Jessop JLP. (2010a). Quantitative analysis of adhesive resin in the hybrid layer using raman spectroscopy. *Journal of Biomedical Materials Research - Part A*, *94*(1), 288–297. https://doi.org/10.1002/jbm.a.32692

Zou Y, Jessop JLP, & Armstrong SR. (2010b). In vitro enzymatic biodegradation of adhesive resin in the hybrid layer. *Journal of Biomedical Materials Research - Part A*, *94*(1), 187–192. https://doi.org/10.1002/jbm.a.32689

Zou Y, Jessop JLP, Armstrong SR, & Jessop JLP. (2009). Apparent conversion of adhesive resin in the hybrid layer, Part II: In situ studies of the resin-dentin bond. *Journal of Biomedical Materials Research - Part A*, *89*(2), 355–362. https://doi.org/10.1002/jbm.a.31954

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama, Turku, Finland 2021

ISBN 978-951-29-8734-4 (PRINT)
ISBN 978-951-29-8735-1 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)