Journal of Corrosion and Materials
www.corromat.com

J. Corros. Mater. (2026) 5(1), 113–127.

Concentration-Resolved Catechol–Lysine Methacrylate Priming for Hydrothermal Protection of Resin–Dentin Hybrid Layers

Ath.....[1,*]

Australian National University .....

*Corresponding author: .....

(Received: 3 March 2026. Received in revised form: 15 May 2026. Accepted: 27 May 2026. Published online: 30 June 2026.)

Abstract

The key components for successful resin-dentin bond involve a thin zone composed of polymerized methacrylate resin, demineralized type I collagen matrix, water, dentinal permeability, and enzymatic activity of endogenous enzymes. The aim is to identify a specific concentration of catechol-Lys-methacrylate (CLM) primer capable of improving the characteristics of this critical zone upon hydrothermal aging without interfering with adhesive cure. CLM preparation entailed the synthesis of a protected Lys–methacrylate intermediate followed by the incorporation of the catechol moiety and the removal of protecting groups using acidic conditions. Four different compounds including the final product CLM were synthesized in yields of 67.79%, 93%, 69%, and 89%, respectively. The presence of the methacrylate vinyl groups in CLM was confirmed by proton NMR in the range of 5.5–6.25 ppm. The disappearance of aromatic protons from the Fmoc protection group confirmed that acid cleavage occurred in the intermediate step prior to CLM synthesis. The presence of the final catechol and Lys protons was confirmed in the regions of 6.42–7.04 ppm and 2.73 ppm, respectively. Viability of human dental pulp cells following treatment with CLM, determined via median lethal dose, ranged between 140 µg mL^{-1} and 160 µg mL^{-1}. This value was consistent with UDMA and below that reported for Bis-GMA using the same exposure protocol. Dentin adhesion studies were performed using an etch-and-rinse procedure, utilizing Single Bond 2 after application of water or CLM solutions at a concentration of 1 mg mL^{-1}, 5 mg mL^{-1}, or 10 mg mL^{-1}. The parameters examined included adhesive conversion, microtensile bond strength in fresh and aged specimens exposed to 10,000 thermal cycles between 5 °C and 55 °C, scanning electron microscopy, silver nitrate nanoleakage test, in situ zymography, protein-ligand docking, attenuated total reflectance Fourier transform infrared (ATR–FTIR) spectroscopy, collagen mechanical testing, thermogravimetric analysis, dry mass loss, hydroxyproline leaching, and type IV collagenase inhibition.The primer concentration 10 mg mL^{-1} decreased degree of conversion, but the remaining 1 mg mL^{-1} and 5 mg mL^{-1} were both compatible with adhesive polymerization. With 5 mg mL^{-1}, the bond strength immediately and under thermal cycling was maximum. It also minimized the thermal-aging bond strength loss from 45.03% for the control group to 17.54%. The latter also maintained a relatively unbroken hybrid layer, generated the least amount of silver-tagged nanoleakages and lower collagenase enzyme activity within the bonding zone. The same phenomenon allowed for maintaining the integrity of the hybrid layer more consistently, generated fewer nanoleakages marked by the presence of silver, and had lower levels of collagenase activity in the region of bond. Docking studies of CLM with the collagen molecules 1QSU, 1CGD, and 4OY5 gave binding energy values of -3.83, -4.71, and -4.60 kcal mol^{-1} respectively. Studies using ATR–FTIR and the collagen stability assay suggested direct interaction of CLM with demineralized dentine collagen. Among the tested concentrations, 5 mg mL^{-1} CLM provided the clearest balance between wet-interface adhesion, collagen protection, and resin-network formation.

Keywords: resin–dentin adhesion; catechol; lysine; methacrylate primer; hybrid layer; nanoleakage; collagen stabilization; wet bonding; thermocycling; type I collagen.

1. Introduction

Hydroxyapatite-free collagen fiber matrices are required for durable composite retention since the resin-to-substrate interface must effectively dissipate stress from resin while retaining its resistance to the harsh conditions found inside the mouth. As such, dentin consists of a collagen matrix containing apatite crystals, dentinal fluid, and non-collagenous protein, as well as enzymes supplied by the host organism. The outermost collagen-rich layer needs to be de-mineralized with an etching procedure to open up access for adhesive molecules. Buonocore's work originally established acid conditioning as a route for improving adhesion to enamel surfaces [1]. Nakabayashi and co-workers later introduced the concept of adhesion through monomer infiltration into tooth substrates [2]. Contemporary adhesion literature then clarified that bonding to enamel and dentin involves different substrate conditions and future challenges [3]. In other words, the bonded layer in composite fillings is more than just an adhesive coating applied onto the surface of teeth. Long-term adhesion reviews show that durability depends on the stability of this resin–tooth interphase [4]. Dentin-bonding studies further emphasize that the interface is controlled by collagen, water, monomer infiltration, and enzymatic degradation [5]. Recent adhesive-dentistry reviews confirm that hydration state, solvent removal, polymerization, and controlled porosity remain central to the hybrid layer [6].

Wet bonding gains clinical significance due to the presence of an inherent conflict. Excessively dry collagen fibers are prone to collapse and reduce the availability of inter-fibrillar spaces, thereby preventing adhesive materials from entering. However, excessive moisture causes water accumulation that would affect the ability of adhesives to properly penetrate demineralized dentin. Nanoleakage analysis shows that residual water may compromise the resin–dentin interface [7]. Phase-separation studies indicate that excess moisture can interfere with the organization of adhesive components [8]. Adhesive-interface work further shows that water, solvents, and hydrophilic domains can reduce the long-term stability of the monomer–collagen region [9]. Therefore, contemporary etch-and-rinse adhesives have to operate within a limited range of moisture conditions. The latter means sufficient wetting capability to access demineralized dentin; sufficient polymerization to prevent water absorption and phase transitions; and sufficient contact of monomers with collagen for proper infiltration.

The aged hybrid layer undergoes degradation processes on both polymer and collagen sides. Hydrophilic areas can remain hydrated and ester-rich methacrylates may undergo hydrolysis [10]. Collagen exposure also increases susceptibility to endogenous proteolytic activity [11]. Matrix metalloproteinases and cysteine cathepsins have been identified as important contributors to degradation of dentin bonding [12]. Reviews of bonding durability further connect polymer degradation and collagen breakdown with the loss of interface integrity [13]. Nanoleakage gives one visible manifestation of this coupled deterioration process [14]. Resin–dentin interface studies show that degradation can proceed through the hybrid layer over time [15]. Mechanical-test reviews further connect interface deterioration with reduced bond strength [16]. Later work confirms that bond degradation cannot be inferred from initial strength alone [17]. In other words, a high initial bond strength is a poor indicator of hybrid layer longevity.

Several approaches to delay this process of degradation have been developed. Chlorhexidine treatment has been used to preserve the hybrid layer by inhibiting enzymatic activity [18]. In vivo studies showed that chlorhexidine could improve the durability of resin–dentin bonds [19]. Complementary work confirmed that chlorhexidine can slow collagen degradation within the bonded interface [20]. Later research also examined how MMP inhibition supports adhesive longevity [21]. Recent studies continued to connect MMP control with dentin-bond preservation [22]. Crosslinking agents provide another route for stabilizing demineralized dentin [23]. Proanthocyanidin-containing treatment can increase dentin stiffness and reduce enzymatic vulnerability [24]. Collagen crosslinking studies further show that the mechanical condition of the matrix can be improved before bonding [25]. Ethanol wet-bonding can improve resin infiltration through solvent exchange [26]. The clinical sensitivity of this technique nevertheless remains a practical limitation [27]. It is clear from these approaches that interface stability can be achieved but there are certain challenges that arise in the process. The presence of enzyme inhibitors does not necessarily result in better resin infiltration, crosslinking is unlikely to involve a modified collagen surface in the cured adhesive network, and solvent exchange cannot compensate for the need for proper interfacial chemistry.

It appears that the strategy of using catechol-based wet adhesion could be applied successfully to address this combined problem. Mussel-inspired adhesive chemistry demonstrates that catechol groups can support wet adhesion through multiple interactions [28]. Related work showed that dopamine and catechol-containing molecules can interact strongly with wet mineral and organic substrates [29]. Biological-adhesion reviews further connect catechol chemistry with hydrogen bonding, metal coordination, oxidative coupling, and redox activity [30]. The cooperative action of catechols and lysine is important for bonding with organic surfaces [31]. Hydration-layer disruption by cationic groups can help the adhesive molecule approach the wet substrate more closely [32]. The collagen–apatite–water mixture with ions creates a similar problem on dentin interfaces but on a different level.

Thus, CLM incorporates three functional groups into one molecule. The catechol moiety allows it to interact with the collagen fibers and residual mineral through hydrogen bonds, cation-π interaction, and oxidative coupling. Lysine residues allow it to disrupt the hydration layer on the wet interface and establish cationic interaction. Methacrylate is indispensable since CLM needs to participate in polymerization as part of the cured adhesive instead of being removed from the interface as a separate pretreatment molecule. The composition of CLM is unlike adding a separate modifier of collagen, which is undesirable for a primer used with dental adhesives as it is supposed to become an integral part of the interface. Monomer–collagen binding studies suggest that molecular structure controls the interaction with dentin collagen [33]. Further work on functionalized monomers supports the role of hydrophobicity and interfacial compatibility [34]. Bio-inspired dental monomers indicate that catechol-containing chemistry can be relevant for adhesive interfaces [35]. Dental-adhesive studies also show that such monomers must balance bonding performance with polymerization behavior [36]. Enzyme-interaction studies connect this molecular design with collagenase inhibition and matrix preservation [37].

However, a high concentration of molecules capable of forming catechol interactions could interfere with the free radical polymerization of methacrylates. Instead of eliminating one defect, it is possible to create another problem, as catechol groups will compete with methacrylates for reactive sites. The task is to find the concentration of CLM that improves the bonding and resistance of the aged interface while ensuring the proper polymerization and suppressing the activity of

MMPs. Four cases were compared: a deionized water control group and CLM primers with concentrations of 1 mg mL^{-1}, 5 mg mL^{-1}, and 10 mg mL^{-1}. Interpretation relies on the analysis of synthesis yields, NMR spectra, cell viability, adhesive conversion, aged bonding strength, interface morphology, leakage, zymography, collagen interaction, FTIR spectra, and enzyme stability tests.

The concentration-dependent approach also shows how a compound needs to be tested to be used in dental adhesives. While it is desirable for a potential additive to demonstrate high affinity to collagen, it is unacceptable to use a molecule that interferes with curing or creates a thin intermediate layer that could be penetrated by MMPs. On the contrary, a good curing reaction will be pointless if the hybrid layer becomes unstable and porous. Thus, several criteria needed to be examined: chemical composition, polymerization behavior, cellular toxicity, adhesive properties, aged bonding, leakage, enzymatic activity, collagen binding, and FTIR spectroscopy.

2. Materials and Methods

2.1 Synthesis and molecular confirmation of CLM

CLM was prepared via four successive chemical reactions such that methacrylate group was incorporated while catechol and lysine groups were also introduced. In the beginning, Fmoc-L-Lys(Boc) (5 g, 10.67 mmol) was dissolved in 25 mL dichloromethane. Subsequently, HOBt (1.59 g, 11.74 mmol) and EDC (2.25 g, 11.74 mmol) were added to the reaction flask followed by the stirring under nitrogen atmosphere with cooling at 0 °C for 30 min. Purification via silica-gel column using ethyl acetate/petroleum ether as eluent gave P1 (white solid) in 4.2 g with 67.79%

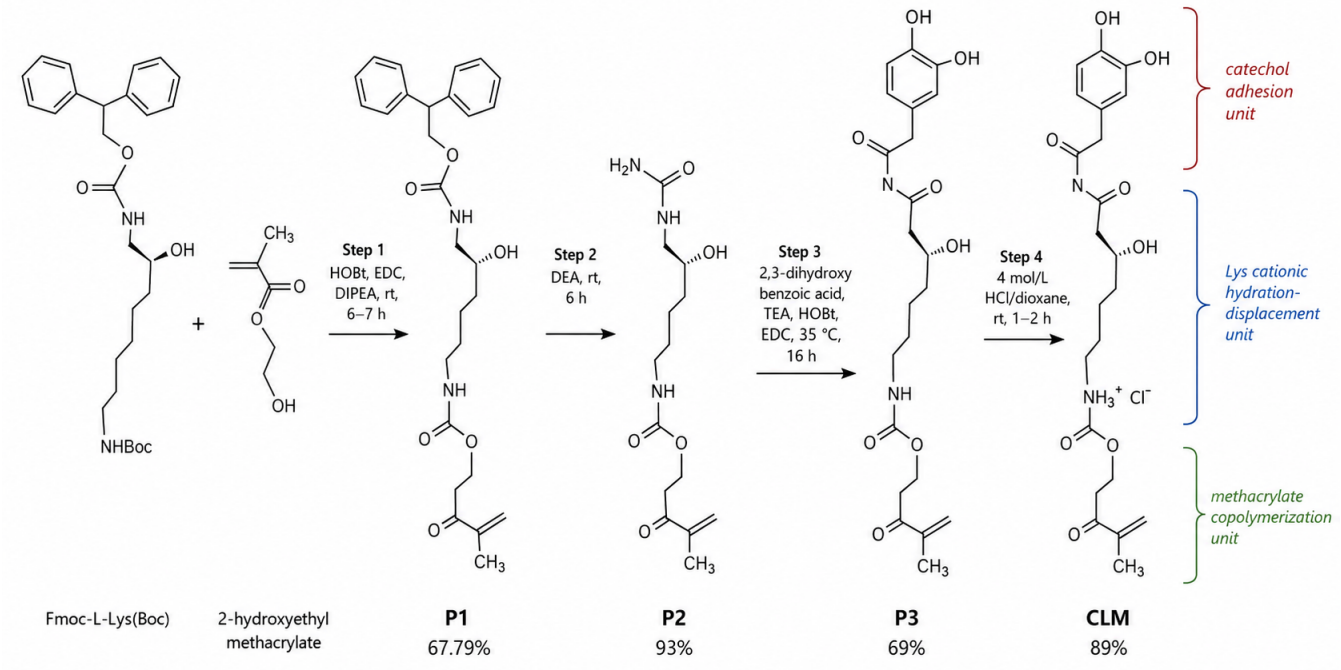

Figure 1: CLM synthesis and functional domains.

Reaction described by Figure 1 represents the chemical basis for performing bonding tests. Unlike reaction with three individual compounds, reaction path involves combination of catechol, Lys, and methacrylate in one monomer. This detail is crucial since physical mixture could lead to changing of wetting property without participating in formation of cured adhesive matrix while CLM remains the same with methacrylate capable of copolymerization with Single Bond 2. Furthermore, protecting groups are used not to initiate other reactions but to preserve Lys part responsible for interfacial properties.

P1 was dissolved in 70 mL of DCM/diethylamine in equal proportions and left stirring for 6 h at room temperature. Evaporation of solvent and column chromatography using ethyl acetate/petroleum ether and dichloromethane/methanol resulted in obtaining compound P2 with mass of 2.4 g and yield of 93%. Then, 6.71 mmol (2.4 g) P2, 3.36 mmol (0.518 g) 2,3-dihydroxybenzoic acid, and 3.36 mmol (0.454 g) of HOBt were mixed in 30 mL of tetrahydrofuran. TEA (10.08 mmol, 1.40 mL) and EDC–HCl (10.08 mmol, 1.93 g) were added, and the coupling reaction took place for 16 h at 35 °C. Further extraction, washing, evaporation, and column chromatography using dichloromethane/methanol gave compound P3 as yellow oily substance with mass of 1.15 g and yield of 69%. Reaction of P3 with 50 mL of 4 N HCl/dioxane during 1–2 h at room temperature produced desired CLM as brown oily liquid with mass of 0.81 g and yield of 89%.

Table 1: Chemical checkpoints.

Product	Principal observation	Meaning for the primer design
P1	67.79% yield; methacrylate signals retained	The protected Lys–methacrylate intermediate preserves the polymerizable unit required for integration with the adhesive resin.
P2	93% yield; Fmoc aromatic signals removed	Deprotection exposes the amine-containing Lys segment needed for cationic interaction at hydrated dentin.
P3	69% yield after catechol coupling	The catechol wet-adhesion unit is introduced without eliminating the methacrylate group.
CLM	89% yield; catechol, Lys, and methacrylate signals present	The final monomer contains all intended functional domains in a single molecule.

Table 1 explains why the chemical characterization must take place before the interface experiments. Deproteination efficiency was considered critical as any remaining protecting group would lower the cationic nature of Lys. Methacrylate peaks were similarly crucial in ensuring that a primer without methacrylate functionality would not be easily extracted to form a weak interface. The subsequent tests for bonding therefore depend on the successful detection of catechol, Lys derivative, and methacrylate peaks.

Proton NMR spectra were obtained using a Bruker Avance III HD 500 MHz spectrometer. Signals from methacrylate C=C protons between 5.5–6.25 ppm indicated retained functionality. Removal of the Fmoc aromatic group peaks around 7.25–8.0 ppm validated the deprotection in P2. The final CLM revealed catechol aromatic peaks between 6.42–7.04 ppm and the Lys-related peaks around 2.73 ppm.

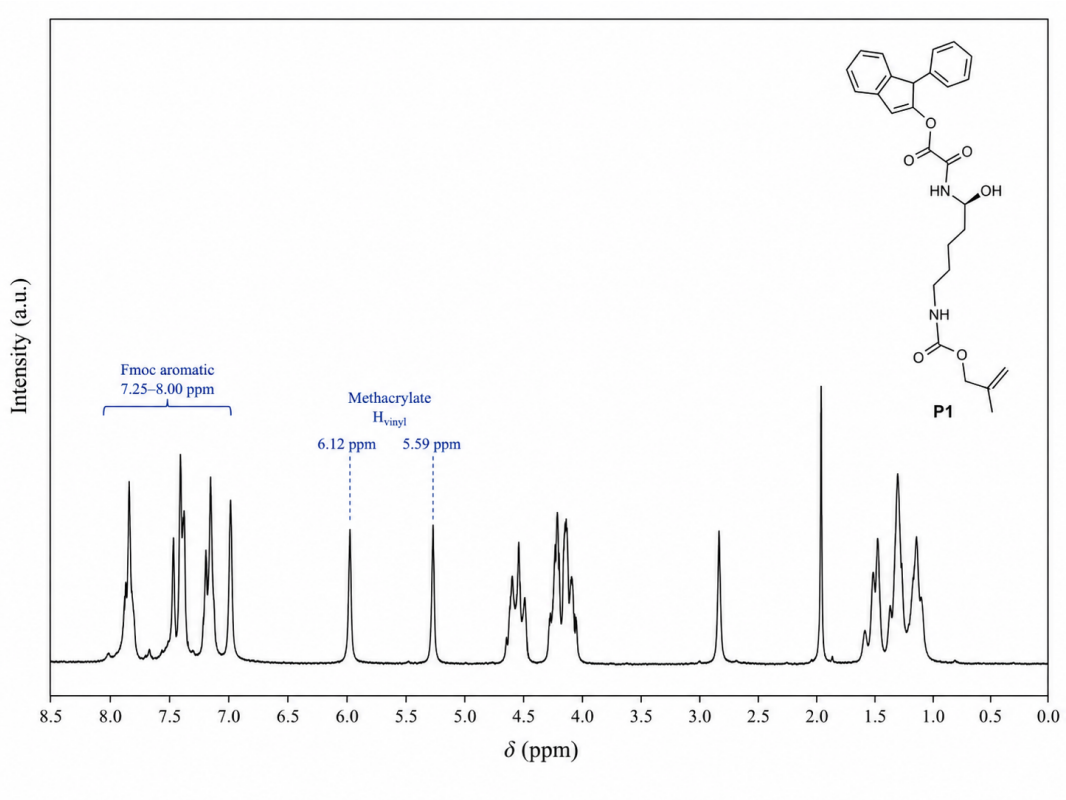

Figure 2: P1 ^{1}H NMR spectrum.

The presence of both the protected Lys structure resonances and the methacrylate vinyl resonances in the P1 spectrum shown in Figure 2 satisfies the first structural requirement, indicating that the coupling reaction did not use up the methacrylate function during the coupling reaction process, and further reactions with this methacrylate group were possible.

As shown in Figure 3, the removal of the Fmoc aromatic envelope without disappearance of methacrylate and aliphatic Lys-related signals indicates the successful deprotection, which resulted in exposure of the reactive amine containing precursor without degradation of the methacrylate moiety essential for bonding capability.

In Figure 4, the CLM molecule spectrum indicates the formation of a structure containing all three required functionalities: catechol, methacrylate, and Lys-related. In other words, this was no simple case of producing a compound, but an experimentally confirmed composition of a primer with three specific functional groups for conducting the tests of the three different interactions described.

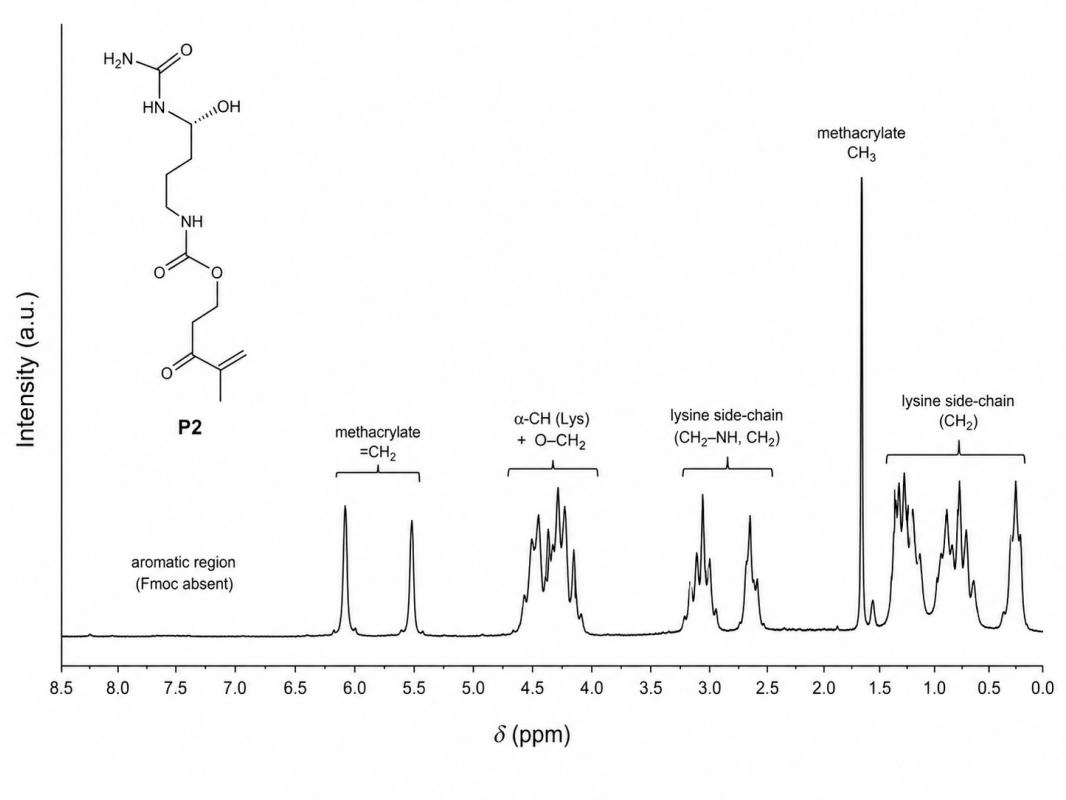

Figure 3: P2 ^{1}H NMR spectrum.

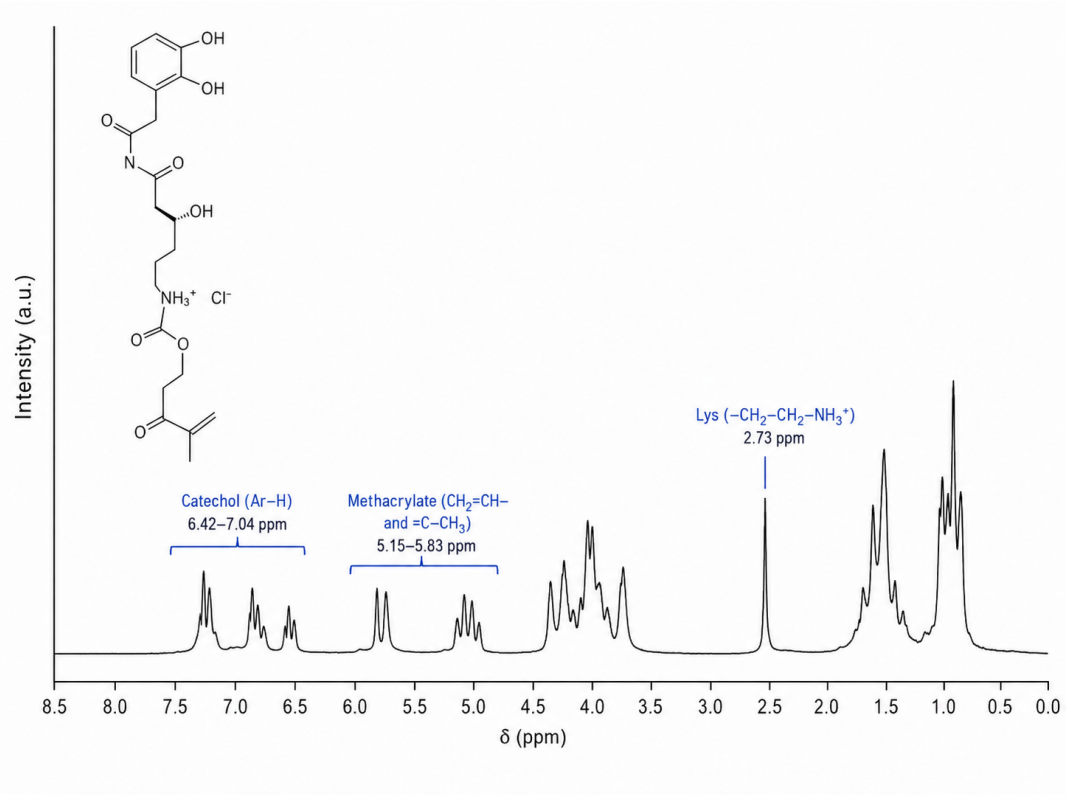

Figure 4: CLM ^{1}H NMR spectrum.

2.2 Cell-viability assay

CLM, compared to Bis-GMA, TEGDMA, HEMA, and UDMA, was tested for its cytotoxicity using human dental pulp stem cells. Monomers were dissolved in DMSO and further diluted in serum-free alpha-modified essential medium to concentrations of 20 µg mL^{-1}, 40 µg mL^{-1}, 60 µg mL^{-1}, 80 µg mL^{-1}, 100 µg mL^{-1}, 120 µg mL^{-1}, 140 µg mL^{-1}, 160 µg mL^{-1} and 180 µg mL^{-1}. Cells were seeded into wells at a density of 1×10^4 cells/well. After 24 hours in culture and subsequent starvation for 24 hours, cells were incubated in monomer-containing medium for 24 more hours. Thereafter, Cell Counting Kit-8 was applied, and absorption was measured at 450 nm after 4-hour incubation. It is worth mentioning that although it was a screen rather than clinical cytotoxicity test, excessive loading of monomers in order to increase primer concentration would be unacceptable in a clinically usable primer.

2.3 Dentin bonding, thermal aging, and bond testing

Non-carious intact human third molars were kept in 5% chloramine-T solution at 4 °C for up to a month. CLM primers were prepared in deionized water at concentrations of 1 mg mL^{-1}, 5 mg mL^{-1}, and 10 mg mL^{-1}; the control group included deionized water as a primer. In total, 128 teeth were equally divided into four groups. Each molar's occlusal one-third was removed, and exposed dentin was polished with 600-grit silicon carbide paper for 60 seconds while continuously washing it under water. Dentin was etched with 37% phosphoric acid for 15 seconds, rinsed, and treated with selected primer for 60 seconds. Further, Single Bond 2 was applied as per the manufacturer's instructions to the etched surface and a composite restoration consisting of Filtek Z250 was performed in 6-mm thick build-ups.

The samples were equally divided into immediate and thermally aged subgroups. Samples from the immediate subgroup were placed in distilled water for 24 hours; aged samples went through 10,000 cycles between 5 °C and 55 °C with 1-minute dwell time in each condition. Bonded sticks ($10 \times 1 \times 1$ mm) were cut perpendicularly to the long axis of the molar. Their microtensile bond strength was evaluated with EZ-TEST 500 N tester at 1 mm/min cross-head speed. Four sticks of each molar were averaged.

2.4 Conversion of Adhesives

A mixture of CLM and Single Bond 2 in the proportion of 1:9 was prepared to evaluate the effect of the primer on the degree of polymerization of the adhesive. A drop of 20 µL of this adhesive mixture was placed on the ATR–FTIR crystal, air dried for 5 s, covered with celluloid, and scanned 24 times in the range of 4000 cm^{-1} to 500 cm^{-1} with a resolution of 4 cm^{-1} before curing. After light curing for 20 s, FTIR analysis was performed at 40 s, 60 s, 5 min, 10 min, and 30 min after curing. Degree of conversion was determined using the decrease in intensity of aliphatic C=C vibrations at 1638 cm^{-1} normalized by the relatively constant aromatic C–C vibrations at 1608 cm^{-1} according to the following equation:

$$DC(\%) = \left[1 - \frac{(A_{1638}/A_{1608})_{\text{cured}}}{(A_{1638}/A_{1608})_{\text{uncured}}}\right] \times 100. \quad (1)$$

Equation 1 is a relationship between the spectroscopic measure and the functional requirement of the material. The primer that enhances collagen binding and simultaneously reduces the percentage of reacted methacrylate double bonds might lower the cohesive strength of the adhesive layer, leading to reduced load transfer. The conversion test, on this account, becomes indispensable relative to bond strength test.

2.5 Interface Morphology, Nanoleakage, and Enzymatic Activity

The bonded interfaces were polished, etched using 37% phosphoric acid, soaked in 5% sodium hypochlorite, sonicated, fixed, dehydrated, sputtered with gold and examined through field emission scanning electron microscopy. The nanoleakage samples were coated with nail varnish leaving aside an approximate 1 mm area around the bonding interface, soaked in 50 wt% ammoniacal silver nitrate for 24 hours, sonicated, immersed in photo-developing solution, irradiated with fluorescent light for eight hours, sputtered with gold, and then studied using backscattered imaging. The enzymatic activity of the aged bonded interface was analyzed using confocal laser scanning microscopy following in situ zymography using EnzChek collagenase assay kit. The above protocols were chosen as they measure different parameters related to the deterioration of interfaces namely structural integrity, water-filled conduits, and enzymatic collagen degradation.

2.6 Collagen Docking, ATR–FTIR, and Collagen Stability Assay

Molecular docking analysis was carried out using Molecular Operating Environment (MOE) software on collagen types I structures (4OY5, 1CGD, and 1QSU). The 2D structure of CLM was modeled into a 3D structure by minimizing its energy

content. The protonation and hydrogen position were optimized at pH 7 and temperature 300K. Docking was carried out using the AMBER10:EHT force field with R-field solvation model. Docked conformations were ranked using London dG scoring function and then refined by force-field minimization and rescored with GBVI/WSA dG.

ATR–FTIR interaction experiments utilized demineralized dentin slabs. These specimens were etched in 10% phosphoric acid to induce full demineralization and were then analyzed from 4000 to 500 cm^{-1}, subjected to CLM treatment for 5 min, ultrasonically cleaned, and rescanned. The mechanical stability of collagen was investigated using $1 \times 1 \times 6$ mm demineralized dentin beams pre-treated with distilled water, 1 mg mL^{-1} CLM, 5 mg mL^{-1} CLM, 10 mg mL^{-1} CLM, or 2.5% glutaraldehyde for 5 min. Twenty beams in each group were subject to determination of their ultimate tensile strength, while another 20 specimens in each category were additionally pre-treated with 0.1 mg mL^{-1} type IV collagenase for 24 h followed by tensile testing. TGA analysis of demineralized dentin powder was carried out between 20 °C and 200 °C at heating rate of 10 °C min^{-1}. Loss of dry mass and hydroxyproline release caused by collagenase were determined as well. Type IV collagenase inhibition was estimated using DQ gelatin substrate under excitation/emission wavelengths of 495/515 nm.

3. Results

3.1 Molecular synthesis produced a polymerizable catechol–Lys primer

All intermediates synthesized during the route were produced in amounts sufficient for the following biological and adhesive experiments. P1 was isolated as white solid in yield of 67.79% after coupling of Fmoc-L-Lys(Boc) with HEMA. Removal of Fmoc protection provided P2 in 93% yield, demonstrating adequate unblocking of Lys-derived amine residue. Coupling to catechol resulted in obtaining of P3 as light-yellow oil in 69% yield, followed by acid deprotection and isolation of CLM in brown oil form at 89%. Thus, yields were high enough to obtain the required amount of material for further cell testing, adhesive polymerization, adhesive bonding and testing, imaging and collagenase stability measurements.

These results gave evidence for the structural progression necessary to confirm CLM as a genuine interfacial monomer. The P1 spectrum exhibited methacrylate peaks from 5.5 to 6.25 ppm alongside Fmoc aromatic peaks, indicating that the initial coupling reaction preserved the reactive vinyl group. The P2 spectrum lacked Fmoc aromatics, confirming deprotection, whereas methacrylate and aliphatic Lys-related features were retained. Finally, in CLM, there were peaks associated with catechol aromatic (6.42 to 7.04 ppm), methacrylate, and Lys (2.73 ppm) together. Therefore, the data confirm the role of CLM in contributing both to the wet substrate interaction and formation of the methacrylate network.

This finding is crucial for understanding the general measurement strategy used throughout the rest of the research. It is not possible to attribute any future increase in either the bonding strength or enzyme stability without this NMR evidence since they could simply reflect non-specific effects of the added compound. Instead, the existence of the monomer allows us to explain observed changes as due to a chemically linked system, rather than the mixture of unrelated compounds that stabilize it.

3.2 Cell response identified CLM as belonging to the monomer concentration range

The cell viability decreased as the concentration of CLM increased, which proves that CLM behaves as a monomer rather than as a stabilizer. The lethal concentration was between 140 µg mL^{-1} and 160 µg mL^{-1}. Within identical exposure conditions, the cell toxicity level was comparable to UDMA and lower than that of Bis-GMA, whereas that of TEGDMA and HEMA was relatively high throughout the tested concentration range.

First, the cell viability dose-response graph (Figure 5) shows that CLM can be used further as an adhesive monomer, since its level of toxicity is not significantly greater than that of high-molecular-weight resins. Second, the decrease in cell viability at high concentration levels means that it is important to find the minimum effective concentration level for the primer. A compound that needs to be present in high quantities before becoming functional is undesirable, since it imposes unnecessary restrictions on the polymerization process and increases biological risk.

Therefore, the toxicity behavior relative to other monomers is relevant in terms of its use for further adhesive studies. Even though different dental resins vary in their hydrophobicity, molecular weight, leachability level, and toxicity, the data help to place CLM into the context of its potential application. It is evident that the concentration of CLM that gave the maximum bonding strength did not reach the threshold where cell viability became comparable to that of Bis-GMA.

3.3 Adhesive conversion gave a limit for CLM concentration

The conversion test showed a concentration-dependent effect. 1 mg mL^{-1} and 5 mg mL^{-1} primer solutions exhibited a close-to-control curing rate, but 10 mg mL^{-1} primer yielded noticeably lower values of conversion at all curing times. As a consequence, we have identified an upper boundary to CLM loading when used with Single Bond 2.

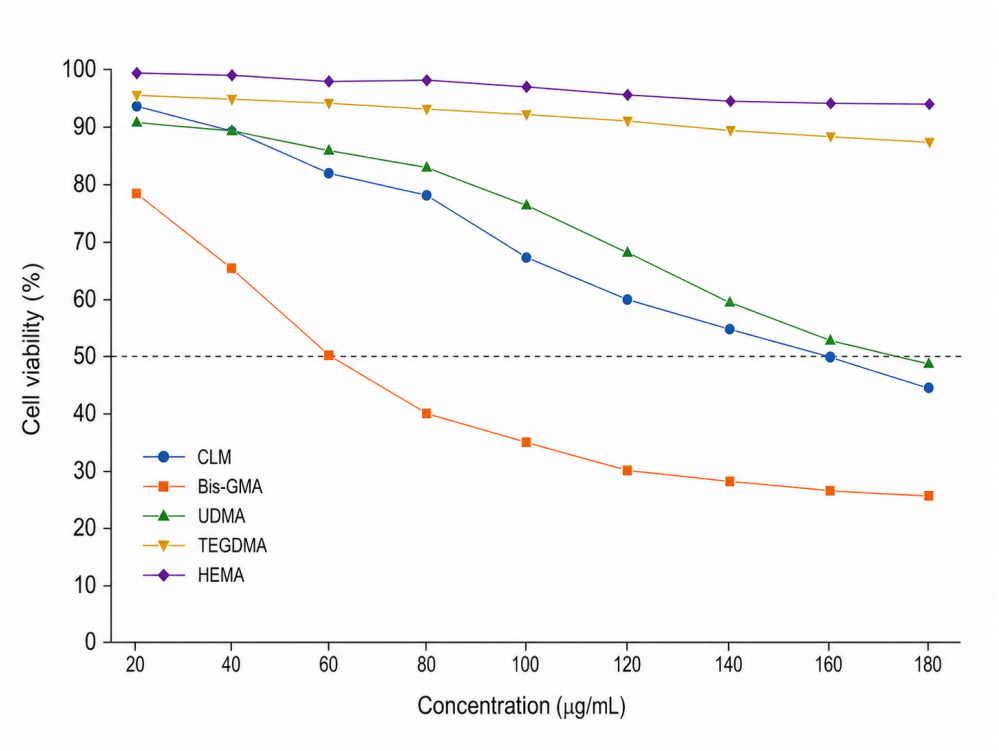

Figure 5: Dental-pulp stem-cell viability.

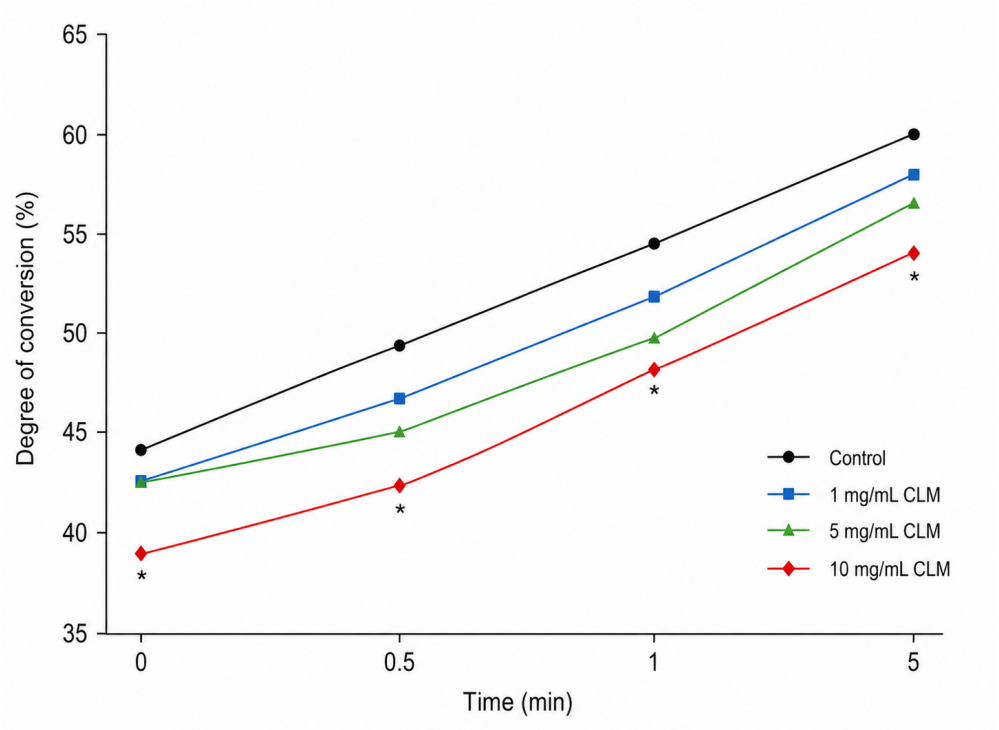

Figure 6: Adhesive degree of conversion.

However, the data from the conversion test (Figure 6) suggest that it is impossible to evaluate CLM using dose-response logic, since increasing the dose of catechol–Lys monomer is not always advantageous. Indeed, 10 mg mL^{-1} primer gives better wet-adhesion characteristics, but the negative impact on curing behavior becomes too large. This trade-off implies that there exists an optimal level of CLM loading, which needs to be determined based on balancing both parameters.

3.4 Thermal aging enabled separation of immediate and durable adhesion

Bond strength in microtensile test results depended on both concentration and aging procedures. Prior to any treatment, the 5 mg mL^{-1} group achieved a higher bond strength value at 32.2 MPa, while the water control group demonstrated 28.6 MPa, and the other two groups, 1 mg mL^{-1} and 10 mg mL^{-1} groups had bond strength values of 28.1 and 26.0 MPa, respectively. Upon subjecting all groups to 10,000 cycles at temperatures ranging from 5 °C to 55 °C, all groups experienced loss of bond strength; however, the extent of the reduction varied greatly among groups. In particular, the water control reduced to 15.7 MPa, which translates into a **45.03%** loss. The 5 mg mL^{-1} group retained 26.6 MPa, showing only **17.54%** loss. The 1 mg mL^{-1} group retained 22.0 MPa, while the 10 mg mL^{-1} group retained 19.2 MPa.

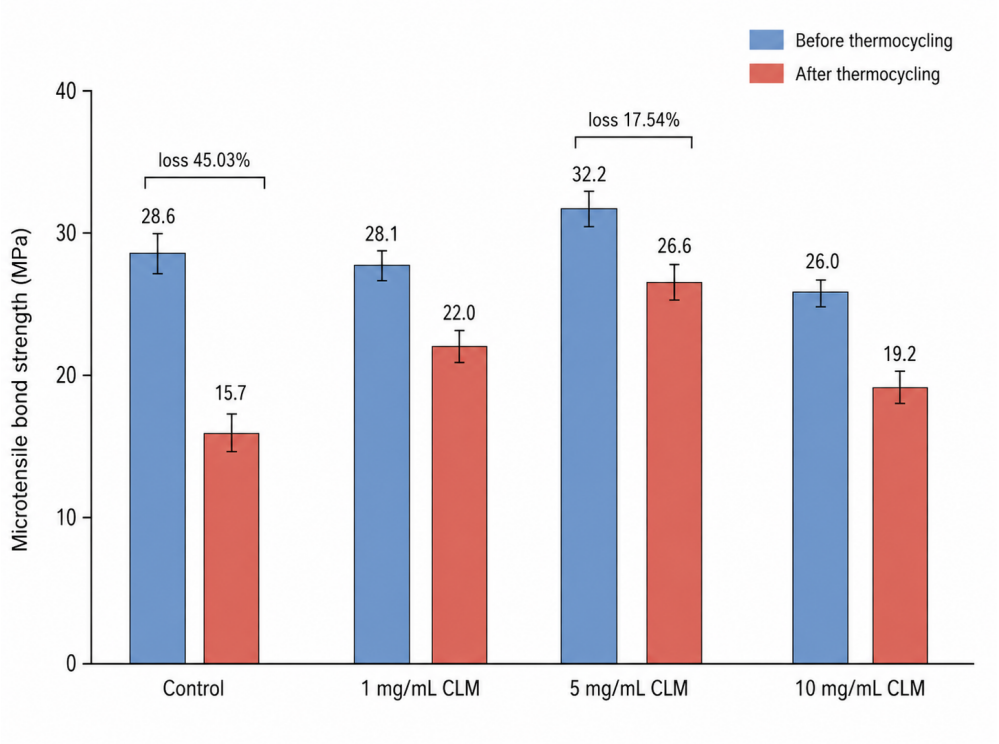

Figure 7: Microtensile bond retention.

In terms of bond strength in Figure 7, the outcome associated with aging retention becomes paramount. The initial increase in bond strength at 5 mg mL^{-1} is helpful, but what matters in scientific terms is the fact that this particular group held its own better against hydrothermal degradation compared to the control group. The difference in percentage losses of 45.03 % and 17.54 % is indicative of the reduction of mechanisms involved in aging processes such as moisture flow, thermal mismatches, collagen exposure, and enzyme breakdown, all of which were limited by CLM. The 10 mg mL^{-1} group did not outperform the 5 mg mL^{-1} group despite greater primer loading, consistent with the conversion penalty observed in Figure 6.

Values in Table 2 help explain the rationale for using 5 mg mL^{-1} concentration as an optimal one. It means that the response is not monotonic in nature. While the lowest concentration allows for curing, it is not very reinforcing, whereas the highest CLM concentration poses a challenge for curing. At the same time, the 5 mg mL^{-1} concentration offers enough interfacial stabilization without compromising on curing properties of the adhesive network.

According to the results of failure-mode analysis, the mode in which bond failure occurred was primarily mixed. Thermocycling and CLM concentration had no significant effect on the distribution of modes. The conclusion drawn based on these data is important, as they suggest that the observed strengthening response resulted from improvement in the material properties of the interface and not due to shifting the fracture location farther from the adhesive.

As for bond strength analysis, they reveal another reason why an aged absolute strength value needs to be supplemented by percentage loss data. According to the results obtained, water control sample displayed a moderate initial strength but failed to preserve enough strength even without CLM addition. In other words, the initial stability of the hybrid layer was

Table 2: Bond-strength response.

Condition	Immediate strength	Aged strength	Interpretation
Water control	28.6 MPa	15.7 MPa	Large strength loss indicates a thermally and aqueously vulnerable hybrid layer.
$1\,\mathrm{mg\,mL^{-1}}$ CLM	28.1 MPa	22.0 MPa	Low loading preserved conversion and improved aging response but did not maximize strengthening.
$5\,\mathrm{mg\,mL^{-1}}$ CLM	32.2 MPa	26.6 MPa	This condition gave the strongest immediate and aged interface with the smallest measured strength loss.
$10\,\mathrm{mg\,mL^{-1}}$ CLM	26.0 MPa	19.2 MPa	Excess loading reduced conversion and limited the potential benefit of additional catechol–Lys chemistry.

not good enough. Furthermore, $10\,\mathrm{mg\,mL^{-1}}$ concentration managed to preserve lower strength than $5\,\mathrm{mg\,mL^{-1}}$, although it used twice as much CLM. Thus, both properties discussed above were important and necessary. However, the $5\,\mathrm{mg\,mL^{-1}}$ condition improved the aged strength value and lowered relative loss rate.

3.5 Hybrid-layer morphology is supportive of mechanical result

Results obtained via field-emission scanning electron microscopy confirmed that in all experimental groups, a proper hybrid-layer interface with recognizable resin–dentin interface was formed using the chosen etch-and-rinse protocol. When comparing the aged interfaces, the control sample had a roughened hybrid layer, while the $5\,\mathrm{mg\,mL^{-1}}$ CLM interface had a more compact and continuous structure. Moreover, resin tags were broken in the former sample but more intact in the latter one.

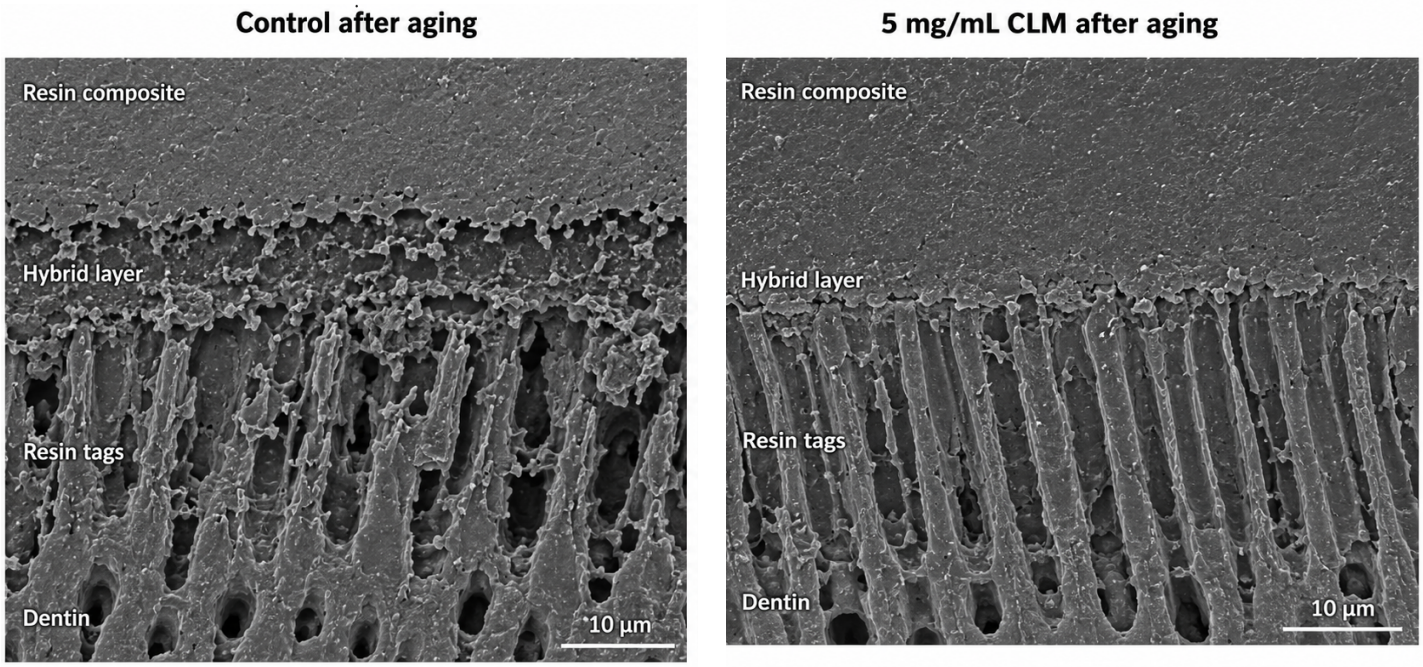

Figure 8: Aged hybrid-layer morphology.

The micrographs provided in Figure 8 support the results obtained when analyzing bond strength. First, fractures in the tags and roughness in the hybrid layer imply stresses generated due to aging. Second, a more integrated appearance of this layer in $5\,\mathrm{mg\,mL^{-1}}$ samples implies increased wetting ability and stabilization of collagen matrix within the transitional layer. This, in turn, is the explanation for the importance of the aged strength value, since the difference is becoming more apparent under aging conditions.

3.6 Less water-rich transport pathways were detected by nanoleakage test

According to the silver nitrate nanoleakage test, the differences in the degree of water-rich defects are evident. In the control group, linear deposits were observed long before aging, which speaks of the presence of a permeable channel within

or close to the hybrid layer. Aging by thermocycling led to the increase in silver nitrate deposition in the vicinity of the lower region of the hybrid layer in all groups, although the lowest deposit was recorded for the $5\,\mathrm{mg\,mL^{-1}}$ CLM group.

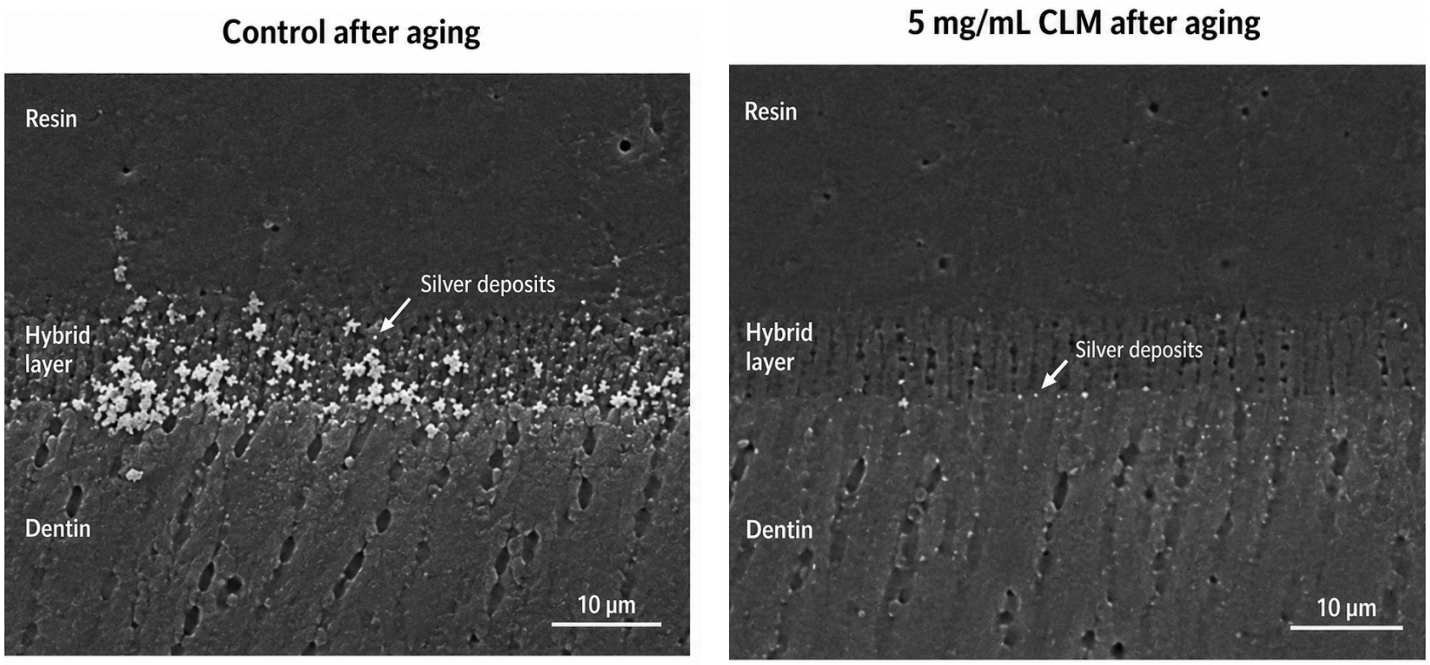

Figure 9: Silver-nitrate nanoleakage.

The above pattern in Figure 9 requires further consideration since deposits do not just form spots; they are indicative of the presence of microscopic channels capable of transmitting water molecules. Low deposits of the silver nitrate solution in the $5\,\mathrm{mg\,mL^{-1}}$ group imply that such channels are less common in the hybrid layer in that case. Indeed, this observation aligns with the results from the mechanical study, as water-filled pathways increase the process of plasticizing the bonding agent, accelerating its hydrolytic and collagen exposure reaction. The nanoleakage test provided an insight into one of the mechanisms of a decrease in adhesion after thermocycling.

3.7 In situ zymography indicated decreased collagenolytic activity in CLM-treated samples

In situ zymography demonstrated strong green coloration close to the bottom of the aged control sample, implying active collagenases in the region, where resins tend to be infiltrated insufficiently. Treatment of specimens with CLM caused the reduction in the green color intensity; in the case of the $5\,\mathrm{mg\,mL^{-1}}$ group, the difference was especially noticeable. Red fluorescence corresponded to the adhesive layer, while green fluorescence meant degradation of gelatin.

Zymography data in Figure 10 offers a biochemical rationale for previous observations on structure and mechanics. Low green fluorescence confirms both low permeability and low enzymatic activity of the bond. This is important because dentin-bond degradation typically starts from the bottom of the hybrid layer where water is retained and collagen is partially encapsulated. CLM seems to minimize the vulnerability of aged hybrids by interacting with collagen and by lowering collagenase-related activity in precisely that interfacial area where age-induced debonding is expected.

3.8 Collagen docking and ATR–FTIR confirmed direct molecular association

Docking studies indicated that CLM could interact with type I collagen models via multiple modes. In case of model structure 1QSU, the binding free energy was -3.83 kcal $\mathrm{mol^{-1}}$. CLM formed a hydrogen bond with the backbone nitrogen of Gly12, and a covalent interaction occurred with the side chain of Lys14. Model 1CGD showed binding energy -4.71 kcal $\mathrm{mol^{-1}}$. Hydrogen bonds formed between CLM and the backbone nitrogen atoms of Gly24 and Hyp26. Structure 4OY5 exhibited binding energy -4.60 kcal $\mathrm{mol^{-1}}$. CLM made a hydrogen bond interaction with the backbone nitrogen atom of Gly4 and a cation–π interaction between the benzene ring of CLM and a nitrogen-containing residue.

The binding energy values are moderate and suitable for a wet primer. A dental wet primer must be associated with collagen but able to permit adhesive infiltration and curing. Several moderate interactions may secure molecular residence on the collagen surface but do not lock the complex into a rigid, unreactive form. Diversity of potential interaction modes, such as hydrogen bonds, cation–π interactions, covalent bonding and van der Waals forces, corresponds well to the catechol–Lys wet adhesion chemistry.

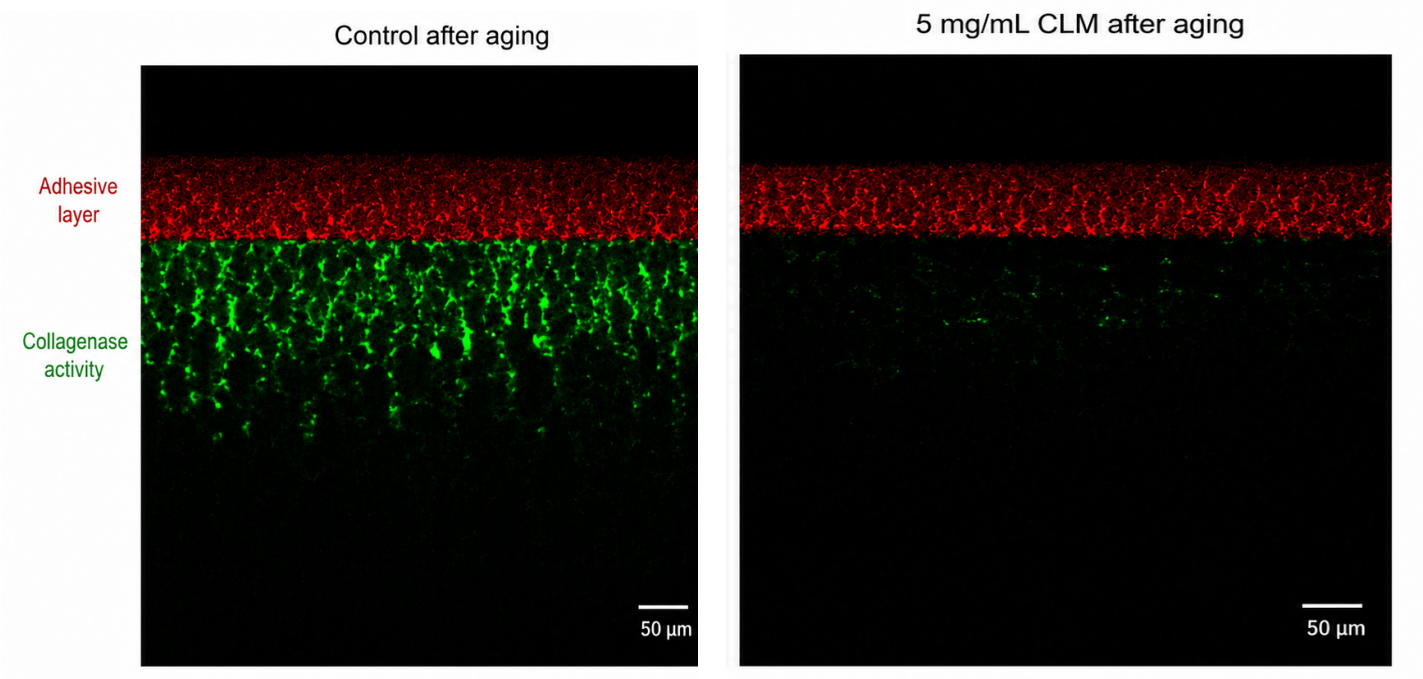

Figure 10: In situ zymography.

ATR–FTIR analysis demonstrated that CLM interacted with demineralized dentin collagen. Collagen alone featured typical Amide A, Amide I, Amide II and Amide III bands at 3297, 1632, 1551 and 1239 cm^{-1} respectively. Upon treatment with CLM followed by ultrasonic washing, there was a noticeable shift in the Amide I band to a lower wavenumber. In addition, new peaks appeared in the range of 1600 cm^{-1} to 670 cm^{-1} and corresponded to C=C, C=O and phenolic O-H absorption maxima, respectively. Preservation of CLM-associated bands during the washing procedure suggested that CLM was not simply rinsed off, but was incorporated into the demineralized collagen framework.

3.9 Collagen-stability tests provided insight into the mechanism of aged bond retention improvement

CLM contributed significantly to improved collagen stability, tested by mechanical, thermal and enzymatic approaches. The tensile strength of collagen before collagenase treatment increased due to CLM. Furthermore, treated collagen samples demonstrated higher tensile strength upon subsequent collagenase challenge than control collagen. There was also an increase in thermal stability, as evidenced by thermogravimetric analyses. Treated collagen showed reduced dry mass loss and hydroxyproline loss in comparison with untreated samples following type IV collagenase treatment. Zymography showed decreased enzyme activity in treated demineralized dentin. Direct analysis of type IV collagenase enzyme using DQ gelatin assay confirmed its concentration-dependent inhibition by CLM.

Collagen-level effects of CLM are important for interpretation of the results of bonded interfaces experiments. It is almost inevitable that in acid-etched dentin, there will be collagen fibrils that have been incompletely encapsulated by the adhesive. If these fibrils remain mechanically weak and prone to degradation by proteolytic enzymes, there is a risk of further degradation of the bonded interface even in case of apparently successful bonding. CLM minimized this risk by enhancing the stability of collagen framework through improvements in tensile strength, thermal stability, reduced mass loss, lowered hydroxyproline loss, and collagenase inhibition.

4. Discussion

The findings effectively address the study question by demonstrating that 5 $mg\,mL^{-1}$ CLM primer produces optimal resin–dentin bonding under hydrothermal aging conditions. This conclusion arises from convergence of multiple outcomes rather than one mechanical test result. The optimal primer preserved adhesive conversion, produced maximal immediate and aged bond strength, demonstrated lowest thermocycling-induced strength reduction, had more intact aged hybrid layer structure, showed lower silver nitrate penetration, had less collagenase activity at the bonded interface, and provided stability improvement of demineralized collagen. Agreement across multiple endpoints is crucial since resin–dentin bond degradation involves several factors. It is impossible for a single test to determine whether a primer decreases water transport, stabilizes collagen molecules, and is compatible with radical polymerization.

The most valuable feature of the results is non-monotonic concentration dependence. $1\,mg\,mL^{-1}$ CLM primer successfully preserved conversion but did not achieve maximum benefits of increased dose. $10\,mg\,mL^{-1}$ CLM primer contained more catechol-Lys chemical groups but demonstrated reduced conversion and lower bond strength compared with the intermediate $5\,mg\,mL^{-1}$ concentration. Hence, the middle group represented the right compromise rather than higher concentration of the same molecule. For dental adhesion, it is critical for an interfacial primer to enhance properties of the substrate without decreasing those of the adhesive polymer. Catechol compounds can disrupt the polymerization process via redox reactions and radical consumption. Hence, the observed decrease in conversion confirms this possibility experimentally.

Age-related bond strength maintenance provides more insights than immediate bond strength alone. The latter parameter mainly reflects formation of the coherent interface. Thermocycling is a more comprehensive test for assessing long-term performance of the resin–dentin adhesive. The control group showed 45.03% reduction in bond strength during aging, whereas the $5\,mg\,mL^{-1}$ group lost only 17.54%. This result proves that CLM did not improve only short-term surface contact and wetting but increased resistance of the interface to degradation during water/temperature aging cycles.

The results of the morphological analysis explain the difference between aging behaviors. The interface of the control group had irregular hybrid layer and fragmented resin tags, which indicate lack of cohesion at the bonded interface. In contrast, the $5\,mg\,mL^{-1}$ group had relatively more intact structure of the transition area. This observation proves that the primer successfully increased cohesive forces between the two materials during aging. Presumably, the cationic moiety reduced shielding effect of the bound water, and the catechol groups allowed for multiple interactions with collagen and minerals. Lastly, the methacrylate functionality participated in the adhesive polymer network formation.

The results of the nanoleakage analysis refine the information obtained through the SEM examination. Silver nitrate penetrated water-filled defects invisible using standard morphological techniques. Numerous deposits were found along the hybrid layer of the control interface, while there were fewer deposits in the $5\,mg\,mL^{-1}$ CLM group. This finding proves that CLM reduced quantity of channels available for water, ions, and enzymes. Practically, it means that treated dentin collagen received less external stress from the aging medium than control collagen.

The zymography results reveal another mechanism explaining increased bond strength. The enzyme activity appeared highly concentrated in the basal part of the hybrid layer in the control group. In contrast, the interface created by $5\,mg\,mL^{-1}$ CLM showed decreased enzyme activity, which was confirmed by weaker fluorescence. Presumably, reduced activity resulted from lower permeability of the bonded interface, direct interference of CLM molecules with enzymes, collagen stabilization against degradation, or all these processes occurring together. The experiments with enzymatic stability of demineralized collagen and isolated collagenase inhibition prove this hypothesis.

The docking results and ATR-FTIR analysis serve as a connection between microscopic image analysis and collagen level assays. The software detected possible association of CLM molecules with three collagen models. Additionally, ATR-FTIR revealed amide bands of CLM-treated samples, presence of C=C, C=O, and phenolic O-H groups, and absence of these signals after ultrasound cleaning. Therefore, CLM appears in a demineralized matrix and interacts with collagen via hydrogen bonds and π-π stacking. Such interactions were not considered strong covalent linkages but relatively strong weak interactions providing increased residence and stability under aqueous conditions. Catechol–lysine systems in natural adhesive proteins support this interpretation [31]. Hydration-layer studies further explain why these weak interactions can remain effective under aqueous conditions [32].

The results of the collagen-stability tests reveal the reason why the interface retained the benefits during aging. Treated demineralized collagen showed better tensile properties, higher 5% mass loss temperature, decreased dry weight loss, lower amount of hydroxyproline released from collagenase-challenged samples, and lower enzymatic activity. This information implies that the primer improved mechanical properties of collagen molecules and prevented their degradation even under aggressive conditions. Hence, the bond strength remained high in a sample with more stable collagen than in the control sample, where molecules became fragile and easily fragmented.

The relation between conversion rate and collagen stabilization defines the practical implications of the work. The $10\,mg\,mL^{-1}$ CLM primer probably had a more pronounced collagen-stabilizing effect but decreased the degree of conversion of the adhesive. In a clinical bonding procedure, the adhesive polymer should have high tensile strength to resist masticatory forces, fluid diffusion, and hydrolysis during prolonged exposure. A partially cured network absorbs water more easily, loses integrity, and provides additional degradation pathways. Thus, lower conversion leads to weaker interface, and higher conversion is crucial for obtaining durable bonding.

The research also makes a contribution to the field of material degradation not only related to dentistry. The resin–dentin interface can be viewed as a hydrated polymer-biological interface exposed to temperature changes, aging by thermal cycling and water transport, hydrolysis, and biological agents. Similar concepts are used to evaluate coatings, biomaterials, and hybrid materials. The primer serves as an example of a compound modifying the interface to reduce disruptive action of water, increase affinity toward collagen, contribute to the polymer layer, and decrease enzymatic activity. This combination

is much more insightful than considering the primer as a wetting or enzyme inhibitor.

Moreover, the selection of analytical methods demonstrates their complementary role in investigating interfacial modifications. Synthesis and nuclear magnetic resonance ensure the creation of the right compound. Viability testing prevents recommendations on use of excessive concentrations. Degree of conversion determines the limits of radical polymerization. Microtensile testing evaluates mechanical function of the sample and its degradation. SEM allows for evaluating structural continuity. Nanoleakage provides information about transport pathways. Zymography detects sites of enzymatic activity. Molecular modeling and spectroscopy establish relationships between the modifier and substrate at the molecular level. Stability studies of collagen determine its degradation state.

Such a range of methods proves the scientific value of the research because it deals with materials acting at biological interfaces. One particular assay can demonstrate the success of primer due to an erroneous reason. For example, improved wetting will temporarily increase mechanical strength without affecting the adhesive layer. Strong inhibition of enzymes will be beneficial when applied to purified collagen powder. Increased cross-linking will provide mechanical reinforcement but hinder monomer infiltration. However, in the presented work, the primer showed high performance under the conditions that allowed simultaneous satisfaction of several requirements.

It is also necessary to discuss limitations imposed by experimental parameters. Only human third molars were tested, and the aging occurred only using artificial cycles. Oral environment is complex, and thermocycling does not represent all environmental factors experienced by teeth. Discrete concentration range provided limited information about the optimal dose, which could be obtained using neighboring values. Only Single Bond 2 was tested, and adhesion to other types of adhesives, including universal primers and self-etch systems, requires verification. The studied adhesion remained stable in aqueous medium; other types of aging should also be examined, such as fatiguing, biofilm formation, or exposure to dietary acids.

In spite of the outlined limitations, the presented results prove that only $5\,\mathrm{mg\,mL^{-1}}$ CLM primer successfully addressed multiple concerns associated with dental adhesion and its long-term stability. The consistency of observations implies that CLM interacts with the biological substrate through multiple chemical and biological processes creating a stable modified interface. The unique value of the molecule consists in maintaining balance between interfacial adhesion and polymerization. For hydrated dentin, this approach seems to be the key strategy.

Another implication of these data is that adhesive primer development should also include information on cure compatibility and aged transport behavior, along with bond strength. Indeed, the measurements demonstrated that the most stable configuration is obtained not by increasing the complexity of functional chemistry, but by identifying a critical concentration at which such chemistry would still remain compatible with the overall composition of the adhesive. For hydrated dentin, the optimal target is an effective but restrained interfacial chemical reaction.

5. Conclusion

Polymerization of a catechol-Lys-methacrylate primer was essential for improving the durability of resins bonding to dentin, and only $5\,\mathrm{mg\,mL^{-1}}$ CLM demonstrated the most optimal conditions for this process. CLM achieved the highest levels of microtensile bond strength immediately after bonding and during aging, minimized bond-strength loss caused by thermocycling from 45.03% to 17.54%, improved the continuity of the hybrid layer, decreased silver nitrate nanoleakage, and inhibited collagenase activity in the bonded dentin.

This conclusion is supported by multiple types of evidence. Proton NMR verified that CLM possesses the appropriate chemical structure and molecular weight. Cytotoxicity analysis confirmed that CLM possessed an appropriate level of toxicity to be used within the dental-monomer concentration sensitivity range. Degree of conversion analysis revealed that $10\,\mathrm{mg\,mL^{-1}}$ of CLM surpassed the polymerization compatibility range for Single Bond 2, whereas $1\,\mathrm{mg\,mL^{-1}}$ and $5\,\mathrm{mg\,mL^{-1}}$ remained compatible. Finally, docking studies, ATR-FTIR spectroscopy, tensile tests, thermogravimetric analysis, dry mass loss, hydroxyproline release, and collagenase inhibition revealed CLM interaction and stabilization of demineralized dentin collagen.

As shown in the results, CLM is only beneficial within a particular range of concentrations. Too low primer concentration will provide inadequate interfacial reaction, whereas too high will interfere with curing. Thus, a $5\,\mathrm{mg\,mL^{-1}}$ concentration appears to represent the optimal balance of these factors. Adhesives designed for bonding to dentin should be optimized to balance both dentin collagen stability and adhesive resin network stability. Any of the factors could limit the performance of an aged restoration. Additional testing should involve long-term aging, fatigue cycles, fluid flow, pH cycling, biofilm exposure, and cross-compatibility with other adhesives.

Conflict of Interest

The author declares no competing interests.

References

[1] Buonocore, M. G. (1955). A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. Journal of dental research, 34(6), 849-853.
[2] 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.
[3] Van Meerbeek, B., De Munck, J., Yoshida, Y., Inoue, S., Vargas, M., Vijay, P., ... & Vanherle, G. (2003). Adhesion to enamel and dentin: current status and future challenges. OPERATIVE DENTISTRY-UNIVERSITY OF WASHINGTON-, 28(3), 215-235.
[4] De Munck, J. D., 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.
[5] Pashley, D. H., Tay, F. R., Breschi, L., Tjäderhane, L., Carvalho, R. M., Carrilho, M., & Tezvergil-Mutluay, A. (2011). State of the art etch-and-rinse adhesives. Dental materials, 27(1), 1-16.
[6] Van Meerbeek, B., Yoshihara, K., Van Landuyt, K., Yoshida, Y., & Peumans, M. (2020). From Buonocore's pioneering acid-etch technique to self-adhering restoratives. A status perspective of rapidly advancing dental adhesive technology. Journal of Adhesive Dentistry, 22(1), 7-34.
[7] Tay, F. R., & Pashley, D. H. (2003). Have dentin adhesives become too hydrophilic?. Journal-Canadian Dental Association, 69(11), 726-732.
[8] Yiu, C. K., Pashley, E. L., Hiraishi, N., King, N. M., Goracci, C., Ferrari, M., ... & Tay, F. R. (2005). Solvent and water retention in dental adhesive blends after evaporation. Biomaterials, 26(34), 6863-6872.
[9] Spencer, P., Ye, Q., Park, J., Topp, E. M., Misra, A., Marangos, O., ... & Katz, J. L. (2010). Adhesive/dentin interface: the weak link in the composite restoration. Annals of biomedical engineering, 38(6), 1989-2003.
[10] Breschi, L., Mazzoni, A., Ruggeri, A., Cadenaro, M., Di Lenarda, R., & Dorigo, E. D. S. (2008). Dental adhesion review: aging and stability of the bonded interface. Dental materials, 24(1), 90-101.
[11] Liu, Y., Tjäderhane, L., Breschi, L., Mazzoni, A., Li, N., Mao, J., ... & Tay, F. R. (2011). Limitations in bonding to dentin and experimental strategies to prevent bond degradation. Journal of dental research, 90(8), 953-968.
[12] Tjäderhane, L., Nascimento, F. D., Breschi, L., Mazzoni, A., Tersariol, I. L., Geraldeli, S., ... & Pashley, D. H. (2013). Strategies to prevent hydrolytic degradation of the hybrid layer—a review. Dental materials, 29(10), 999-1011.
[13] Frassetto, A., Breschi, L., Turco, G., Marchesi, G., Di Lenarda, R., Tay, F. R., ... & Cadenaro, M. (2016). Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability—A literature review. Dental Materials, 32(2), e41-e53.
[14] Sano, H., Takatsu, T., Ciucchi, B. J. A. H., Horner, J. A., Matthews, W. G., & Pashley, D. H. (1995). Nanoleakage: leakage within the hybrid layer. Operative dentistry, 20(1), 18-25.
[15] Hashimoto, M. (2010). A Review—Micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions. Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 92(1), 268-280.
[16] Armstrong, S., Geraldeli, S., Maia, R., Raposo, L. H. A., Soares, C. J., & Yamagawa, J. (2010). Adhesion to tooth structure: a critical review of "micro" bond strength test methods. Dental materials, 26(2), e50-e62.
[17] Sano, H., Chowdhury, A. F. M. A., Saikaew, P., Matsumoto, M., Hoshika, S., & Yamauti, M. (2020). The microtensile bond strength test: Its historical background and application to bond testing. Japanese Dental Science Review, 56(1), 24-31.
[18] Hebling, J., Pashley, D. H., Tjäderhane, L., & Tay, F. R. (2005). Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. Journal of dental research, 84(8), 741-746.
[19] Carrilho, M. R. O., Carvalho, R. M. D., De Goes, M. F., Di Hipolito, V., Geraldeli, S., Tay, F. R., ... & Tjäderhane, L. (2007). Chlorhexidine preserves dentin bond in vitro. Journal of dental research, 86(1), 90-94.
[20] Carrilho, M. R. O., Geraldeli, S., Tay, F., De Goes, M. F., Carvalho, R. M., Tjäderhane, L., ... & Pashley, D. (2007). In vivo preservation of the hybrid layer by chlorhexidine. Journal of dental research, 86(6), 529-533.
[21] Mazzoni, A., Tjäderhane, L., Checchi, V., Di Lenarda, R., Salo, T., Tay, F. R., ... & Breschi, L. O. R. E. N. Z. O. (2015). Role of dentin MMPs in caries progression and bond stability. Journal of dental research, 94(2), 241-251.
[22] Li, K., Tay, F. R., & Yiu, C. K. Y. (2020). The past, present and future perspectives of matrix metalloproteinase inhibitors. Pharmacology & therapeutics, 207, 107465.
[23] Bedran-Russo, A. K. B., Pashley, D. H., Agee, K., Drummond, J. L., & Miescke, K. J. (2008). Changes in stiffness of demineralized dentin following application of collagen crosslinkers. Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 86(2), 330-334.
[24] Al-Ammar, A., Drummond, J. L., & Bedran-Russo, A. K. (2009). The use of collagen cross-linking agents to enhance dentin bond strength. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 91(1), 419-424.
[25] Castellan, C. S., Pereira, P. N., Grande, R. H. M., & Bedran-Russo, A. K. (2010). Mechanical characterization of proanthocyanidin–dentin matrix interaction. Dental Materials, 26(10), 968-973.
[26] Kuhn, E., Farhat, P., Teitelbaum, A. P., Mena-Serrano, A., Loguercio, A. D., Reis, A., & Pashley, D. H. (2015). Ethanol-wet bonding technique: clinical versus laboratory findings. Dental Materials, 31(9), 1030-1037.
[27] Ayar, M. K. (2016). A review of ethanol wet-bonding: Principles and techniques. European journal of dentistry, 10(01), 155-159.
[28] Lee, H., Dellatore, S. M., Miller, W. M., & Messersmith, P. B. (2007). Mussel-inspired surface chemistry for multifunctional coatings. science, 318(5849), 426-430.
[29] Lee, B. P., Messersmith, P. B., Israelachvili, J. N., & Waite, J. H. (2011). Mussel-inspired adhesives and coatings. Annual review of materials research, 41(1), 99-132.
[30] Waite, J. H. (2017). Mussel adhesion–essential footwork. Journal of Experimental Biology, 220(4), 517-530.
[31] Maier, G. P., Rapp, M. V., Waite, J. H., Israelachvili, J. N., & Butler, A. (2015). Adaptive synergy between catechol and lysine promotes wet adhesion by surface salt displacement. Science, 349(6248), 628-632.
[32] Gebbie, M. A., Wei, W., Schrader, A. M., Cristiani, T. R., Dobbs, H. A., Idso, M., ... & Israelachvili, J. N. (2017). Tuning underwater adhesion with cation–π interactions. Nature chemistry, 9(5), 473-479.
[33] Hiraishi, N., Tochio, N., Kigawa, T., Otsuki, M., & Tagami, J. (2013). Monomer-collagen interactions studied by saturation transfer difference NMR. Journal of Dental Research, 92(3), 284-288.
[34] Hiraishi, N., Tochio, N., Kigawa, T., Otsuki, M., & Tagami, J. (2014). Role of 2-hydroxyethyl methacrylate in the interaction of dental monomers with collagen studied by saturation transfer difference NMR. Journal of Dentistry, 42(4), 484-489.
[35] Li, K., Yao, C., Sun, Y., Wang, K., Wang, X., Wang, Z., ... & Yiu, C. K. Y. (2021). Enhancing resin-dentin bond durability using a novel mussel-inspired monomer. Materials Today Bio, 12, 100174.
[36] Li, K., Zhang, Z., Sun, Y., Yang, H., Tsoi, J. K. H., Huang, C., & Yiu, C. K. Y. (2021). In vitro evaluation of the anti-proteolytic and cross-linking effect of mussel-inspired monomer on the demineralized dentin matrix. Journal of Dentistry, 111, 103720.
[37] Li, K., Ngo, F. M., Yau, A. Y. L., Tam, W. W. L., Tse, E. C. M., Tsoi, J. K. H., & Yiu, C. K. Y. (2022). Mussel-inspired monomer–a new selective protease inhibitor against dentine collagen degradation. Dental Materials, 38(7), 1149-1161.