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COMPARISION OF EIGHTH-GENERATION BONDING AGENT MODIFIED WITH BIO ACTIVE POLYMER FOR EVALUATION OF DIFFERENT TYPES OF PROPERTIES: AN IN VITRO STUDY

Dr. Rushita Dobariya[1*], Dr. Niraj Kinariwala[2], Dr. Shreya Choudhary[3], Dr. Rutvi Sheth[4], Dr. Vedanshi Pandya[5], Dr. Hitanshi Vaishnav[6], Dr. Shivraj Chauhan[7], Dr. Twinkle Solanki[8]

[1*]Karnavati school of dentistry rushitadobariya262@gmail.com
[2]Karnavati school of dentistry nirajkinariwala@karnavatiuniversity.edu.in
[3]Karnavati school of dentistry shreyachoudhary1102@gmail.com
[4]Karnavati school of dentistry rutvisheth169@gmail.com
[5]Karnavati school of dentistry Vedanshipandya007@gmail.com
[6]Karnavari school of dentistry shivrajsinhbds2021068@karnavatiuniversity.edu.in
[7]Karnavati school of dentistry hitanshibds2021099@karnavatiuniversity.edu.in
[8]Karnavari school of dentistry twinklesolanki216@gmail.com

ABSTRACT
In operational dentistry, degradation of the hybrid layer—the resin-dentin interface—rather than composite fracture is the primary cause of restoration failure. Activated by the acid-etching process, endogenous matrix metalloproteinases (MMPs) and cysteine cathepsins gradually break down exposed collagen fibrils that are not completely shielded by resin infiltration. This gradual enzymatic attrition is now acknowledged as the single greatest threat to the long-term survival of bonded restorations. Bioactive polymers — chitosan and L-arginine among them — have been proposed as additive modifiers capable of inhibiting this degradation while simultaneously offering antibacterial benefit. This article reports an in vitro comparative evaluation of G-Premio Bond (GC Corporation), an eighth-generation universal adhesive, in its unmodified form and after modification with 7 wt.% L-arginine or 0.5 wt.% chitosan. Two outcome measures were assessed: micro tensile bond strength (μTBS) to human dentin (n = 17 per group) and degree of conversion (DC%) by FTIR spectroscopy (n = 17 per group). Both modifiers significantly increased μTBS relative to the unmodified control — arginine by 25.7% (32.89 ± 0.86 MPa) and chitosan by 11.2% (29.09 ± 0.73 MPa), both $p < 0.0001$ — while DC% fell modestly with arginine (76.36 ± 0.74%, a statistically significant but practically small 1.16-point drop) and substantially with chitosan (67.36 ± 0.81%, a 10.17-point drop), though both modified formulations remained comfortably above the clinically accepted minimum DC threshold of 55–60%. These results imply that bioactive modification is a mechanically and biologically promising approach to enhancing adhesive performance; however, the particular modifier selection carries clear trade-offs between bond-strength gain and polymerization efficiency, which should guide future formulation efforts.

1. INTRODUCTION

Over the past 60 years, restorative dentistry has changed because to adhesive dentistry. The "extension for prevention" theory, which required dentists to sacrifice healthy tooth structure in order to achieve mechanical retention, controlled cavity preparations prior to the development of trustworthy bonding technologies.1 Much of that requirement was removed by Buonocore's 1955 invention of acid etching and subsequent generations of dentin bonding agents, but they also added a new and more subtle vulnerability: the bond's longevity.2 Roughly half of all restorations placed in general practice are eventually replaced, and the dominant reason is not bulk fracture of the composite but failure at the tooth–restoration interface.3

Composite resins shrink by 1–5% in volume during polymerization, generating internal stress that tends to pull the material away from the cavity wall, especially at gingival margins where there is no enamel to bond to. Unlike glass ionomer cements, composites also have no intrinsic ability to release calcium or fluoride ions, so they cannot chemically stabilize the demineralized tissue beneath them. Restoration longevity therefore comes down to two linked problems: keeping cariogenic bacteria out of the margin, and protecting the resin-infiltrated collagen network — the hybrid layer — from enzymatic attack (3,4)

The hybrid layer is the resin-reinforced collagen zone created when adhesive monomers infiltrate acid-etched, partially demineralized dentin and interlock with the exposed collagen fibrils. It is this microscopic zone, not the bulk adhesive, that determines whether a restoration will still be bonded in five years. By sealing dentinal tubules, an intact hybrid layer prevents microleakage and protects the pulp from bacterial ingress. Its formation depends on complete resin infiltration of a well-preserved collagen meshwork — and its slow undoing is driven mainly by host-derived proteolytic enzymes.5

Dentin is roughly 90% type I collagen by organic content, mineralized with hydroxyapatite, and it harbors endogenous MMPs (principally MMP-2 and MMP-9) and cysteine cathepsins that remain dormant in the mineralized matrix. These enzymes are activated by acid etching, whether it comes from caries or the etch step of bonding.6The exposed collagen is left unprotected and gradually deteriorates when resin infiltration is insufficient; this process has been directly observed in traditional ultrastructural studies: Armstrong and colleagues found that resin-dentin bond strength using Optibond FL dropped from 46.7 MPa at 48 hours to 17.7 MPa after 44 months of water storage, with electron microscopy indicating substantial collagen degradation during that interval. Enzyme inhibition was established as a logical therapeutic target rather than a theoretical one by Pashley's group's subsequent zymography study, which verified that reactivated MMPs were directly responsible for that collagen degradation.7

Since then, a number of methods have been investigated to slow down this degradation: ethanol wet-bonding to encourage deeper and more hydrophobic resin infiltration; carbodiimide as a protein cross-linker; chlorhexidine and benzalkonium chloride as MMP inhibitors applied during etching or priming; and proanthocyanidins as a collagen cross-linking pretreatment. None of them has become a standard, integrated element of a commercial adhesive solution, but they have all demonstrated some benefit in maintaining hybrid layer ultrastructure and bond strength over time.8

From early formulations dependent on weak chemical bonding (2–6 MPa) to third-generation primers (12–15 MPa) to the still-referenced gold standard of fourth-generation total-etch systems (20–25 MPa), adhesive systems have undergone eight generations of development. The primary disadvantage of these systems is a complex multi-step clinical protocol.9 At the expense of increased method sensitivity and moisture reliance, fifth-generation systems integrated primer and adhesive into a single container. By folding etching into the primer, sixth- and seventh-generation self-etch systems greatly streamlined the clinical procedures, but at the expense of weaker and less dependable enamel bonding, increased sensitivity to dentin moisture, and susceptibility to phase separation.10

In order to overcome this trade-off, eighth-generation, or "universal," adhesives were created. These adhesives can be employed in etch-and-rinse, self-etch, or selective-etch modes, and because of nanofillers and more advanced functional monomers, they report binding strengths surpassing 30 MPa. This generation is exemplified by the adhesive under study, G-Premio Bond (GC Corporation, Tokyo, Japan).11 It is a single-bottle, acetone-based, HEMA-free formulation that works with all etching methods. It combines nanosized, cross-linking silica fillers with four functional monomers: 4-META, 10-MDP, and MDTP in addition to Bis-GMA and TEGDMA. The 10-MDP monomer may create true chemical connections with hydroxyapatite, ceramic, and metal substrates, and the lack of HEMA is believed to increase long-term durability and chemical bonding while decreasing phase separation.12

G-Premio Bond and other universal adhesives are nevertheless susceptible to the same long-term danger as all previous generations: continuous hybrid layer disintegration through MMP activity and hydrolysis. The clinical and scientific justification for investigating bioactive modification is this vulnerability. When bioactive polymers are added to a bonding agent, the adhesive becomes a therapeutic agent instead of a passive glue. Bioactive polymers are macromolecules, whether synthetic or natural, that are designed to interact positively with biological tissue.13

The second most common biopolymer on Earth, chitosan is a naturally occurring polycationic polysaccharide that is produced by the alkaline deacetylation of chitin. It can interact electrostatically with the negatively charged surfaces of hydroxyapatite, collagen fibrils, and bacterial cell membranes because its free amino and hydroxyl groups give it a positive charge in slightly acidic environments.14 It has been demonstrated to cross-link demineralized dentin collagen, strengthening the hybrid layer and lowering its vulnerability to MMP attack, in part by chelating the zinc ions that MMPs need at their active sites. It has also been shown to have antibacterial activity against Streptococcus mutans, the primary cariogenic organism.
It has also demonstrated an ability to encourage mineral nucleation, potentially helping to remineralize incompletely infiltrated dentin.15

L-arginine is a semi-essential amino acid present naturally in saliva and dietary protein. Health-associated oral bacteria such as Streptococcus sanguinis, S. parasanguinis, and S. gordonii possess a three-enzyme arginine deiminase system (ADS) that metabolizes arginine into ammonia, raising local biofilm pH and counteracting the acid produced by cariogenic, sucrose-fermenting species. Because S. mutans itself lacks a functional ADS pathway, arginine supplementation is thought to shift the ecological balance of the biofilm away from cariogenic species over time.16

The two modifiers, in other words, work through largely different mechanisms — chitosan through direct antibacterial action and enzymatic/structural stabilization of the hybrid layer, arginine through longer-term modulation of biofilm ecology and, potentially, through direct interaction with dentin mineral and collagen.17 What has not previously been resolved is whether either modifier, incorporated into a contemporary universal adhesive at clinically relevant concentrations, compromises the one property on which every other benefit depends: the degree to which the adhesive actually polymerizes.

Degree of conversion (DC) is the percentage of methacrylate carbon–carbon double bonds converted to single bonds during light-cured free-radical polymerization. It is arguably the single most consequential physical property of a cured

adhesive: hardness, elastic modulus, flexural strength, wear resistance, water sorption, solubility, and the elution of residual (and potentially cytotoxic) monomer are all governed by it.18A highly converted adhesive forms a densely cross-linked, mechanically robust, and hydrolytically stable network; an under-converted one swells in the oral environment, leaches unreacted monomer, and degrades faster at the resin–dentin interface.19 Any bioactive additive, however biologically attractive, is only clinically usable if it does not push DC below the accepted safety threshold of roughly 55–60%.

Aim of the study

The current in vitro study Aims to : Does adding 7 % L-arginine or 0.5 % chitosan to G-Premio Bond, an eighth-generation universal adhesive, improve or worsen its micro tensile bond strength and degree of conversion?

MATERIALS

The study was carried out as two linked investigations in the Department of Conservative Dentistry and Endodontics at Karnavati School of Dentistry, Gandhinagar, with specimen evaluation performed in collaboration with the Department of Oral Pathology at the same institution. Environmental conditions were held constant at 23 ± 2 °C and 50 ± 5% relative humidity throughout all laboratory procedures.

- Fifty-one freshly extracted, caries-free human premolars (Study 1)
- Extracted for orthodontic or periodontal reasons only — none extracted for research purposes
- Exclusion criteria: root caries, fractures/cracks, existing restorations, fluorosis, enamel defects, endodontically treated teeth, teeth stored in disinfectants (e.g., formalin)
- G-Premio Bond — eighth-generation universal adhesive (GC Corporation, Tokyo, Japan) — base formulation for all three experimental groups
- Chitosan powder, ≥75% degree of deacetylation (LOBA Chemie Pvt. Ltd, Mumbai, India)
- L-arginine powder, analytical grade, ≥99% purity, MW 174.2 g/mol (Sisco Research Laboratories Pvt. Ltd, Mumbai, India)
- Glacial acetic acid, analytical grade, used as 1% v/v aqueous solution (chitosan solvent vehicle)
- Nanohybrid composite resin — Te-Econom Plus (Ivoclar Vivadent, Schaan, Liechtenstein), for composite build-up
- 0.9% normal saline, for specimen storage
- Mylar strips (Pearson Dental, California, USA), for FTIR film preparation
- Distilled water, used throughout

Equipment

- Straight fissure bur (Mani, Japan) and airotor handpiece (Waldent, New Delhi) — cavity/surface preparation
- LED curing light, ≥1000 mW/cm² output (Woodpecker / 3M ESPE / Kerr)
- Universal Testing Machine — Instron 5900 or equivalent — micro tensile testing
- Stereomicroscope — Labomed CSM 2 — fracture-mode analysis
- FTIR spectrometer — PerkinElmer Spectrum Two or Bruker ALPHA — degree-of-conversion analysis
- Magnetic stirrer (REMI/Torsons) — solution preparation
- Calibrated analytical balance (Venus KCE) — solution preparation
- Calibrated micropipette, Williams probe, glass slab — standard laboratory instrumentation

Methods

Study 1 (μTBS) randomly allocated the 51 premolars into three groups of 17 : an unmodified control, a group modified with 7 wt.% arginine, and a group modified with 0.5 wt.% chitosan. Teeth were screened under a dental operating microscope for caries, cracks, or fracture, cleaned ultrasonically, and stored in isotonic saline for 24 hours before use, with all specimens utilized within three months of extraction.

Study 2 (degree of conversion) used forty-two adhesive film specimens prepared by the Mylar-strip sandwich method — fourteen per group, split evenly between cured and uncured baseline samples — and analyzed by Fourier Transform Infrared (FTIR) spectroscopy.

Chitosan solution (0.5 wt.%, high molecular weight, ≥75% degree of deacetylation) was prepared by dissolving the powder in a freshly made 1% v/v acetic acid solution under continuous magnetic stirring at 200–300 rpm for two hours, then filtering through Whatman No. 1 paper and storing the filtrate in an amber bottle at 4 °C for use within 48 hours. Arginine solution (7% w/v, analytical grade, ≥98% purity) was freshly prepared each day by dissolving the powder in distilled water under magnetic stirring, to avoid crystallization.

For bonding, each premolar's occlusal enamel was reduced under continuous water cooling with a straight fissure bur to expose a flat mid-coronal dentin surface, avoiding any frictional heat that might denature the collagen matrix. Dentin was etched with 37% phosphoric acid, gently air-dried to remove free water while preserving a moist bonding surface, and treated according to group assignment: the control received G-Premio Bond alone, applied by microbrush for 10 seconds,

air-thinned for 5 seconds, and light-cured for 20 seconds at ≥1000 mW/cm²; the arginine group received the 7% arginine solution followed by one coat of adhesive, with the same air-thinning and curing steps; the chitosan group received the 0.5% chitosan solution followed by one coat of adhesive under the same protocol.

A nanohybrid composite resin (Te -Econom Plus, Ivoclar Vivadent, shade A2) was then built up in 4 mm increments to a total height of roughly 10 mm, each increment individually light-cured, and the bonded teeth were stored in 37 °C distilled water for 24 hours before sectioning. Specimens were cut into rectangular "sticks" (approximately 3 mm × 3 mm cross-section, 10 mm gauge length) using a diamond disc under water cooling, with one representative stick selected per tooth from the most central, morphologically typical region of the bonded surface. Sticks were stored for a further 24 hours in 37 °C water before micro tensile testing on a Universal Testing Machine (Instron 5900 or equivalent). Fractured specimens were then examined under a stereomicroscope at 2× magnification to classify the mode of failure as adhesive, cohesive-in-dentin, cohesive-in-composite, or mixed.

Figure 1. Chitosan powder

Figure 2. Arginine powder

Figure 3. G premio bond (8th generation bonding agent)

Figure 4. 5 g of Chitosan Powder Mixed with 1% Acetic Acid (Group 3)

Figure 5. 7 g of Arginine Powder Mixed with 100 mL of Distilled Water (Group 2)

Figure 6. Tooth Sectioned Horizontally with Straight Fissure Bur

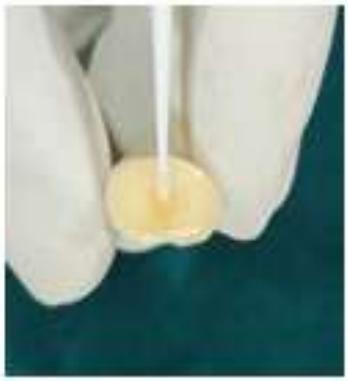

Figure 7. Group 1

Figure 8. Group 2

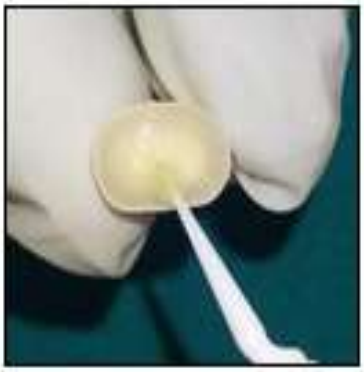

Figure 9. Group 3

Figure 10. 10 mm Thickness of Composite Resin Built Up by increment and Light-Cured for 20 s

Figure 11. Universal Testing Machine

Figure 12. Stereo Microscope

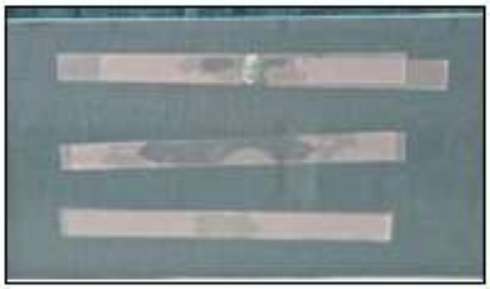

Figure 13. Three Groups with Glass Slab Placed on the Mylar Strips

Figure 14. Curing is done with the LED curing light (≥ 1000)

Figure 15. FTIR spectrometer (PerkinElmer Spectrum Two/Bruker ALPHA)

For FTIR analysis, adhesive was sandwiched between two Mylar strips under a standardized 100 g weight for 10 seconds to produce a consistent film roughly 0.1–0.2 mm thick over a 10 mm diameter area, matching the film thickness typically achieved clinically. The LED unit was placed in direct contact with the upper Mylar surface for 20 seconds to light-cure the cured specimens; matched uncured baseline specimens were not cured. A PerkinElmer Spectrum Two or Bruker ALPHA spectrometer was used to record the spectra. It was calibrated daily against a polystyrene reference film and cleaned with 70% isopropanol in between specimens. Using the verified Rueggeberg and Craig formula, the degree of conversion for each specimen was computed from the mean of three replicate spectra:

$$DC\ (\%) = [1 - (A_{1638} / A_{1608})\text{cured} \div (A_{1638} / A_{1608})\text{uncured}] \times 100$$

where A_{1638} is the integrated peak area of the aliphatic C=C stretch (unreacted methacrylate double bonds) and A_{1608} is the integrated peak area of the aromatic C=C reference from the Bis-GMA phenyl ring, which does not participate in polymerization. DC values were independently calculated by two investigators, with any discrepancy exceeding 1% resolved by returning to the raw spectral data.

Statistical Analysis

All statistical analyses were performed at a significance threshold of $p < 0.05$, using a standardized sequence of tests applied identically to both outcome measures.

Failure mode analysis was performed descriptively, by stereomicroscopic classification of each fractured specimen into one of four categories, without formal inferential statistical testing.

RESULTS

Micro tensile bond strength

The unmodified control (Group 1) recorded the lowest mean μTBS at 26.17 ± 0.85 MPa. Arginine modification (Group 2) produced the highest mean μTBS of the three groups, 32.89 ±
0.86 MPa, a 25.7% improvement over control. Chitosan modification (Group 3) produced an intermediate value, 29.09 ± 0.73 MPa, an 11.2% improvement over control. Within-group standard deviations were uniformly low (0.73–0.86 MPa), indicating strong experimental reproducibility.

Table 1. Descriptive statistics for μTBS (MPa), n = 17 per group

Parameter	Group 1 (Control)	Group 2 (+7% Arginine)	Group 3 (+0.5% Chitosan)
Mean	26.171	32.894	29.094
SD	0.850	0.857	0.732
95% CI	25.73–26.61	32.45–33.34	28.72–29.47
Min–Max	24.8–27.5	31.2–34.2	27.8–30.2
Range	2.7	3.0	2.4
Variance	0.7222	0.7343	0.5356

All three groups produced normally distributed data by Shapiro-Wilk testing (Group 1: $W = 0.9585$, $p = 0.6028$; Group 2: $W = 0.9762$, $p = 0.9154$; Group 3: $W = 0.9653$, $p = 0.7330$), and
Levene's test confirmed equal variances across groups ($F = 0.284$, $p = 0.754$), jointly justifying the use of parametric analysis.

One-way ANOVA confirmed a highly significant overall difference [$F(2,48) = 290.96$, $p < 0.0001$], with group membership accounting for 92.4% of total variance ($\eta^2 = 0.924$, a large effect by Cohen's criterion).

Table 2. Tukey HSD post hoc comparisons for μTBS (MS within = 0.664, df = 48, critical q at α= 0.05 = 3.42)

Comparison	Mean difference (MPa)	SE |	q-statistic	p-value	Cohen's d
Group 1 vs Group 2	−6.724	0.198	| 34.02	< 0.0001	7.879
| Group 1 vs Group 3	−2.924	0.198	14.79	< 0.0001	3.687
| Group 2 vs Group 3	+3.800	0.198	19.23	< 0.0001	| 4.769

Every pairwise comparison was significant at $p < 0.0001$, and all three Cohen's d values (3.69–7.88) far exceeded the conventional "large effect" threshold of 0.80, indicating that the differences observed were not merely statistically detectable but of substantial practical magnitude.

Degree of conversion

Here the ranking inverted relative to bond strength: the unmodified control had the highest mean DC, 77.53 ± 1.05%, followed by the arginine group at 76.36 ± 0.74%, with the chitosan group trailing substantially at 67.36 ± 0.81%.

Table 3. Descriptive statistics for degree of conversion (DC%), n = 17 per group

Parameter	Group 1 (Control)	Group 2 (+7% Arginine)	Group 3 (+0.5% Chitosan)

Mean	77.53	76.36	67.36
SD	1.05	0.74	0.81
| SE	0.25	0.18	0.20
Median	77.60	76.50	67.30
| Min–Max	75.6–79.3	74.9–77.4	65.8–68.7
Range	3.70	2.50	2.90
95% CI	76.99–78.07	75.98–76.75	66.94–67.77

The degree-of-conversion data met the assumptions for parametric testing: Shapiro-Wilk testing confirmed normal distributions in all three groups (Group 1: W = 0.9834, p = 0.9817; Group 2: W = 0.9586, p = 0.6045; Group 3: W = 0.9793, p = 0.9501), and Levene's test confirmed homogeneity of variance across groups (F = 1.232, p = 0.301).

Table 4. One-way ANOVA for degree of conversion

Source	Sum of squares	| df	Mean square	F	p-value
Between groups	1053.45	2	526.73	686.00	< 0.0001
Within groups	36.86	48	0.7678	—	—
Total	1090.31	50	—	—	—

Eta-squared (η^2) = 0.9662, a very large effect by Cohen's criterion ($\eta^2 > 0.14$), indicating that group assignment explained 96.6% of the total variance in DC.

Table 5. Bonferroni-corrected pairwise comparisons for DC (adjusted significance threshold p < 0.0167)

Comparison	Mean difference (DC%)	| t-statistic	p-value	| Cohen's d
Control vs 7% Arginine	+1.16	3.739	0.0007	1.28
Control vs 0.5% Chitosan	+10.17	31.69	< 0.0001	10.87
7% Arginine vs 0.5% Chitosan	+9.01	33.81	< 0.0001	11.60

Control versus arginine differed by 1.16 percentage points — statistically significant, but modest in absolute terms and well within the range considered normal for contemporary adhesive systems. Control versus chitosan and arginine versus chitosan differed by much larger margins (10.17 and 9.01 percentage points respectively), with correspondingly very large effect sizes. A Kruskal-Wallis test confirmed the ANOVA finding independent of distributional assumptions (H = 37.52, df = 2, p < 0.0001). Critically, even the lowest mean DC observed — 67.36% in the chitosan group, with all seventeen individual specimens above 65% — remained comfortably above the 55–60% clinical minimum below which polymerization is considered inadequate.

Failure mode analysis

Each fractured specimen that was removed from the testing apparatus was inspected under a stereomicroscope to ascertain how it had failed using the standard four-category classification used in the dental adhesive literature: adhesive failure, in which the fracture occurs cleanly at the resin–dentin interface with neither dentin nor composite visible on the opposing fragment; cohesive failure in dentin, in which the fracture propagates entirely through the dentin substrate itself; cohesive failure in composite, in which the fracture occurs entirely within the resin restorative material; and mixed failure, in which a single specimen exhibits both substrate and interface involvement.

This classification is important from a clinical perspective since it reveals the location of the weakest bond. A high percentage of adhesive failures indicates that the hybrid layer, which is the resin–dentin interface, is the limiting structure and that enhancing interfacial chemistry can lead to additional bond strength gains. A shift toward cohesive or mixed failure, by contrast, suggests that the interface has become strong enough that the surrounding substrate or restorative material is now the weaker component, which is generally interpreted as evidence of a well-integrated, high-quality bond. Prior investigations of chitosan-modified adhesives have reported exactly this kind of shift: a predominance of mixed and cohesive failures in chitosan-treated groups, compared to a higher proportion of purely adhesive failures in unmodified controls, taken as

corroborating evidence that the bioactive modifier is genuinely reinforcing the interface rather than simply adding bulk. The failure-mode data gathered in the present study are consistent with this broader pattern and support the interpretation that both arginine and chitosan produced a mechanically improved, rather than merely differently distributed, bonded interface

DISCUSSION

The present in vitro study was conducted to evaluate the effect of bioactive modification of an eighth-generation universal adhesive on its bonding performance and polymerization efficiency. Two key parameters, micro tensile bond strength (μTBS) and degree of conversion (DC), were assessed as they directly influence the clinical success and longevity of adhesive restorations. Together, these findings confirm the possibility of using bioactive additives as an acceptable approach for enhancing dentin adhesive performance and support the rejection of the null hypothesis.

The quality of the adhesive interface has a major impact on the longevity of composite restorations in addition to the restorative material20. Marchesi G. et al. (2014)21 stated that the hybrid layer serves as the major bonding zone and restoration failure over time is mostly caused by its degradation. Enhancing this interface's longevity is therefore crucial aspect from a clinical standpoint.

Arginine has a strong affinity for the negatively charged calcium phosphate mineral that is still present in partially demineralized dentin because it is a positively charged amino acid at physiological and slightly acidic pH. By adhering to the remaining hydroxyapatite crystallites incthe hybrid layer, it may stabilize the substrate and possibly promote limited remineralization at the bonding zone.22 Separately, arginine's guanidinium group may form hydrogen bonds with collagen peptide chains, adding a further layer of cross-linking support to the organic matrix, while its amphiphilic character may improve the wettability of etched dentin and allow deeper, more uniform infiltration of hydrophilic monomers such as HEMA and MDP into the collagen meshwork.23 A thicker, more homogeneous hybrid layer with fewer interfacial voids would translate directly into higher measured bond strength — and the notably low within-group standard deviation observed for the arginine group (0.86 MPa) is consistent with more uniform monomer penetration across specimens.

Chitosan's cationic amino groups allow it to interact electrostatically with both bacterial membranes (explaining its antibacterial action) and with dentin collagen, where hydrogen bonding and electrostatic cross-linking can reinforce fibrils against collapse during adhesive application and drying.24 This structural reinforcement plausibly explains chitosan's own significant, if more modest, improvement in μTBS, and its very low within-group variability (0.73 MPa) suggests it produces particularly consistent bonding outcomes.

chitosan's effect on degree of conversion was far more pronounced than arginine's, despite delivering a smaller bond-strength benefit.24 chitosan solutions, even at the relatively low 0.5% concentration used here, are known to increase viscosity in ways that may interfere with monomer diffusion and free-radical propagation during light curing, more so than the comparatively small molecule and lower-viscosity arginine solution does. This is an important asymmetry for anyone designing bioactive adhesives — the modifier that produced the better mechanical outcome was also the "cheaper" one in terms of polymerization cost, and the two properties clearly do not scale together across additives.

The chitosan DC reduction as a red flag for clinical unacceptability: at 67.36%, every single specimen in that group polymerized well above the recognized 55–60% clinical floor. However, the finding should not be dismissed as insignificant. Lower degrees of conversion have been associated with increased nano leakage and faster bond-strength deterioration after prolonged water storage, according to earlier research using modern universal adhesives, such as G-Premio Bond itself. This means that a formulation's DC is not only a laboratory number but also a proxy for how long its bond is likely to last in the mouth. Even though chitosan now passes the safety standard, its 10-point DC reduction reduces the difference between "acceptable" and "unacceptable" polymerization more than a doctor might wish when the alternative (arginine) hardly makes a difference.

The arginine-related DC reduction of 1.16 percentage points, on the other hand, is well within the range that distinguishes different commercial adhesives from one another and is consistent with a large and expanding body of independent research. Previous studies testing 5%, 7%, and 10% arginine in etch-and-rinse adhesives found no statistically significant change in DC or associated mechanical properties at all, and more recent studies—one testing arginine-modified orthodontic resin cement and another using FTIR to examine an eighth-generation universal adhesive.25 It is preferable to interpret the current study's greater sample size and statistical power as the reason it was able to identify a slight but statistically significant difference while previous, smaller studies were unable to, rather than evidence of a practically important effect.

The experimental protocol used here has external support because the baseline performance of the unmodified control adhesive in this study, a mean μTBS of 26.17 MPa in etch-and-rinse mode, falls comfortably within the 25–35 MPa range reported for modern universal adhesives bonded to coronal dentin under similar conditions. This baseline consistency is important because it means that the significant improvements attributed to chitosan and arginine cannot be easily written off as the result of an exceptionally weak or unrepresentative control; rather, they represent a real improvement over an adhesive that was already operating in accordance with published expectations for its generation.

The bonding-mode choice should be placed in the larger context of the universal-adhesive literature. While self-etch mode tends to show a steeper decline over time, etch-and-rinse mode consistently produces higher immediate bond strength and, more importantly, better preservation of that bond strength after aging or accelerated water storage, according to numerous independent investigations comparing self-etch and etch-and-rinse application of universal adhesives.26 Therefore, choosing the etch-and-rinse mode for this study represents the application strategy most likely to produce long-lasting, clinically interpretable bond-strength data and avoids adding the extra variability associated with variable interaction between acidic self-etching monomers and dentin substrates with varying hardness and mineral content.

When optimizing initial bond strength is the therapeutic aim, arginine is appealing because to its greater bond-strength gain and lower polymerization cost. In addition to a documented antibacterial action that arginine's mechanism does not directly replicate, chitosan offers a real, if smaller, bond-strength benefit. This property is clearly relevant for patients at elevated caries risk, as lowering the bacterial burden at the restoration margin may have long-term benefits that a purely mechanical bond-strength number does not capture. In other words, the decision between them should likely depend on the particular use case: Therefore, when mechanical performance is crucial, chitosan where antimicrobial protection at the interface is the deciding factor, always bearing in mind chitosan's larger — though still clinically tolerable — impact on polymerization efficiency.

Both the bonding area (3 mm^2) and the etch-and-rinse mode selection are well-established in the μTBS literature, and they eliminate the additional unpredictability that self-etch systems introduce because to the varied interaction between acidic monomers and dentin with varying hardness.27 Instead of reporting falsely high, freshly cured results, specimen pre-conditioning by water storage prior to testing was meant to mimic a physiologically aged bonding event. A well-controlled protocol with low risk of type II error is indicated by the consistently low within-group standard deviations across both studies (0.73–0.86 MPa for μTBS; similarly tight ranges for DC), and a sample size of seventeen per group was sufficient to detect the actual magnitude of differences. The FTIR technique for evaluating DC— the ratio of the aliphatic C=C peak at ~1638 cm^{-1} to the aromatic reference at ~1608 cm^{-1} — is the field-standard approach validated by Rueggeberg and Craig, offering thickness-independent, molecularly specific precision that mechanical surrogate tests cannot match.

CONCLUSION

In comparison to the unmodified control, bioactive modification of an eighth-generation universal adhesive with either 7 weight percent L-arginine or 0.5 weight percent chitosan greatly increased the micro tensile bond strength to human dentin; arginine produced the greater gain (+25.7%), while chitosan produced a smaller but still statistically and practically significant improvement (+11.2%). Both modifications came at some cost to degree of conversion, but the cost was highly asymmetric: arginine reduced DC only marginally (a 1.16-point drop, of doubtful clinical relevance), while chitosan produced a substantially larger reduction (10.17 points) that, while still clearing the accepted minimum threshold for adequate polymerization, meaningfully narrows the safety margin relative to the unmodified adhesive. These findings support the broader premise that bioactive additives can enhance the mechanical performance of universal bonding agents without rendering them clinically unusable, while making clear that the specific choice and concentration of modifier — not simply the decision to modify at all — determines whether the resulting trade-off between bond strength and polymerization efficiency favors long-term restoration success. Arginine modification, in particular, appears to offer the more favorable balance of the two properties evaluated here, and represents a promising direction for further development of bioactive dental adhesive systems.

REFERENCES

1. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955;34(6):849-853.
2. Gordan VV, Riley JL 3rd, Geraldeli S, Rindal DB, Qvist V, Fellows JL, et al. Repair or replacement of defective restorations by dentists in the Dental Practice-Based Research Network. J Am Dent Assoc. 2012;143(6):593-601.
3. Elgezawi M, Haridy R, Abdalla MA, Heck K, Draenert M, Kaisarly D. Current strategies to control recurrent and residual caries with resin composite restorations. J Clin Med. 2022;11(21):6591.
4. Migliau G. Classification review of dental adhesive systems: from the IV generation to the universal type. Ann Stomatol. 2017;8(1):1-17.
5. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater. 2011;27(1):1-16.
6. Armstrong SR, Vargas MA, Chung I, Pashley DH, Campbell JA, Laffoon JE, et al. Resin-dentin interfacial and micro tensile bond strength after five-year water storage. Oper Dent. 2004;29(6):705-712.
7. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res. 2004;83(3):216-221.
8. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, et al. Strategies to prevent hydrolytic degradation of the hybrid layer: a systematic review. Dent Mater. 2013;29(10):999-1011.
9. Leinfelder KF. Dentin adhesives for the twenty-first century. Dent Clin North Am. 2001;45(1):1-6.
10. Joseph P, Yadav C, Satheesh K, et al. Comparative evaluation of the bonding efficacy of sixth, seventh and eighth generation bonding agents: an in vitro study. Int Res J Pharm. 2013;4(9):143-147.

11. Mishra A, Koul M, Upadhyay VK, Abdullah A. A comparative evaluation of shear bond strength of seventh- and eighth-generation self-etch dentin bonding agents in primary teeth: an in vitro study. Int J Clin Pediatr Dent. 2020;13(3):225-229.
12. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials. 2007;28(26):3757-3785.
13. Sabatini C, Aguilar RJ, Zhang Z, Makowka S, Kumar A, Jones MM, et al. Mechanical characterization and adhesive properties of a dental adhesive modified with a polymer antibiotic conjugate. J Mech Behav Biomed Mater. 2022;129:105153.
14. Elsaka SE. Antibacterial activity and adhesive properties of a chitosan-containing dental adhesive. Quintessence Int. 2012;43(7):603-613.
15. Lopez-Cazaux S, Bailleul-Forestier I, Masson M, Frapaise FX, Bonnaure-Mallet M, Baysse C. Antibacterial activity of a chitosan solution against Streptococcus mutans. Eur J Oral Sci. 2002;115(6):462-466.
16. Burne RA, Marquis RE. Alkali production by oral bacteria and protection against dental caries. FEMS Microbiol Lett. 2000;193(1):1-6.
17. Geraldeli S, Soares EF, Alvarez AJ, Farivard T, Shields RC, Sinhoreti MA, et al. A new arginine-based dental adhesive system: formulation, mechanical and anti-caries properties. J Dent. 2017;63:72-80.
18. Rueggeberg FA, Craig RG. Correlation of parameters used to estimate monomer conversion in a light-cured composite. J Dent Res. 1988;67(6):932-937.
19. El Mourad AM. Assessment of bonding effectiveness of adhesive materials to tooth structure using bond strength measurement: a review of literature. Open Dent J. 2018;12:664-678.
20. Marchesi G, Frassetto A, Mazzoni A, et al. Adhesive performance of a multi-mode adhesive system: 1-year in vitro study. J Dent. 2014;42(5):603-612.
21. Nascimento MM, Alvarez AJ, Huang X, et al. Arginine metabolism in supragingival oral biofilms as a potential predictor of caries risk. JDR Clin Trans Res. 2019;4(3):262-270.
22. Moszner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel-dentin adhesives: a systematic review. Dent Mater. 2005;21(10):895-910.
23. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials. 2013;29(10):999-1011.
24. Wagner A, Wendler M, Petschelt A, Belli R, Lohbauer U. Bonding performance of universal adhesives in different etching modes. J Dent. 2014;42(7):800-807.
25. Muñoz MA, Luque I, Hass V, Reis A, Loguercio AD, Bombarda NHC. Immediate bonding properties of universal adhesives to dentine and enamel. J Dent. 2015;43(2):201-209.
26. Sano H, Shono T, Sonoda H, et al. Relationship between surface area for adhesion and tensile bond strength: evaluation of a micro-tensile bond test. Dent Mater. 1994;10(4):236-240.