

# Enamel Microhardness Preservation Under a Cariogenic Challenge and Shear Bond Strength of a Universal Adhesive Loaded with L-arginine-Containing Mesoporous Silica Nanoparticles (ArgC18@MSNs)

**Daniel Jiménez-Díez**

Universidad Rey Juan Carlos

**Camila Falconí-Páez**

Universidade Estadual de Ponta Grossa

**Gabriel Cochinski**

Universidade Estadual de Ponta Grossa

**Byron Carpio-Salvatierra**

Universidade Estadual de Ponta Grossa

**Francisco Navas**

Universidad Rey Juan Carlos

**Victoria Morales**

Universidad Rey Juan Carlos

**Raúl Sanz**

Universidad Rey Juan Carlos

**Rafael García-Muñoz**

Universidad Rey Juan Carlos

**Andrei C. Ionescu**

University of Milan

**Alessandro D. Loguercio**

Universidade Estadual de Ponta Grossa

**Victoria Fuentes**

Universidad Rey Juan Carlos

**Laura Ceballos**

[laura.cebaldos@urjc.es](mailto:laura.cebaldos@urjc.es)

Universidad Rey Juan Carlos

## Research Article

**Keywords:** White spot lesions, Enamel microhardness, Shear bond strength, Universal adhesive, Mesoporous silica nanoparticle, L-arginine

**Posted Date:** July 9th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-10259034/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.  
[Read Full License](#)

**Additional Declarations:** No competing interests reported.

---

# Abstract

## Objectives

To evaluate the effect of incorporating L-arginine-containing mesoporous silica nanoparticles (ArgC18@MSNs) into a commercial universal adhesive on enamel microhardness preservation under a biofilm-induced white spot lesion (WSL) model and on shear bond strength (SBS) following bracket bonding.

## Materials and Methods

Ambar Universal APS (FGM) was modified by incorporating 5 wt% ArgC18@MSNs; the unmodified adhesive served as the control. Forty-eight human premolars underwent bracket bonding after enamel etching using the modified or control adhesive and an orthodontic resin cement (Orthocem, FGM). Twelve teeth per adhesive were subjected to a 14-day *S. mutans* biofilm challenge to induce WSLs. Cross-sectional enamel microhardness was measured at 100, 200, and 300  $\mu\text{m}$  occlusal to the adhesive margin and at enamel depths of 25–200  $\mu\text{m}$ . The remaining twelve teeth per adhesive were tested for SBS, and the adhesive remnant index (ARI) was recorded. Data were analyzed using independent-samples *t*-tests or Mann–Whitney *U* tests ( $\alpha = 0.05$ ).

## Results

WSLs developed adjacent to brackets in both groups, although they appeared less pronounced with the ArgC18@MSNs-containing adhesive. Cross-sectional Knoop hardness was significantly higher in superficial enamel for the ArgC18@MSNs-containing adhesive (25–100  $\mu\text{m}$  depth at 100  $\mu\text{m}$ , 25–75  $\mu\text{m}$  at 200  $\mu\text{m}$ , and 25–50  $\mu\text{m}$  at 300  $\mu\text{m}$  from the bracket margin;  $p < 0.05$ ). SBS and ARI did not differ significantly between groups.

## Conclusions

Incorporating ArgC18@MSNs into a commercial universal adhesive preserved enamel microhardness adjacent to brackets under cariogenic challenge without compromising SBS.

## Clinical Relevance:

An ArgC18@MSNs-containing universal adhesive may help prevent bracket-associated white spot lesions without compromising orthodontic bond strength.

## 1. INTRODUCTION

The development of white spot lesions (WSLs) adjacent to orthodontic brackets represents one of the most frequent complications associated with fixed orthodontic treatment [1]. These lesions correspond to subsurface enamel demineralization that clinically manifests as opaque, matte, chalky-white areas on the tooth surface, resulting from differences in refractive index between sound and porous enamel [2]. Beyond their biological relevance as early caries lesions, WSLs constitute a significant aesthetic concern, may compromise the perceived success of orthodontic therapy, and negatively affect patient satisfaction [3]. Moreover, once established, these lesions exhibit a limited potential for spontaneous regression [4] and may require clinical interventions of varying invasiveness [5, 6], underscoring the need for effective preventive strategies throughout orthodontic treatment [7].

As any caries-related process, WSLs development is biofilm-associated [8]. Fixed orthodontic appliances favor plaque retention by creating niches that hinder effective oral hygiene, thereby promoting biofilm accumulation [9, 10]. A number of factors, including, but not limited to, changes in dietary habits during orthodontic treatment and the presence of biofilm-colonized, non-buffering surfaces of the brackets and their resin bonding agent, promote the shift in biofilm composition toward dysbiosis, fostering predominantly acidogenic and acid-tolerant species. This condition results in an imbalance between demineralization and remineralization, leading to a sustained acidic challenge [11]. Consequently, net mineral loss occurs in the regions immediately adjacent the bracket–enamel interface [12].

Unsurprisingly, conventional strategies aimed at preventing WSLs during orthodontic treatment mainly include reinforcement of oral hygiene measures, dietary counseling, and the use of fluoride-containing products [13]. However, such preventive approaches are often difficult to implement and sustain over time [14], as they largely depend on patients' compliance and motivation, which may be intermittent and inconsistent throughout the prolonged duration of orthodontic therapy [15]. Moreover, biofilms affect the delivery of ions and molecules to the underlying surfaces, making the use of external antimicrobial agents an impractical approach in many instances. Accordingly, increasing attention has been directed toward incorporating functional agents into orthodontic bonding materials that can locally modulate the biological environment at the susceptible sites, independently of patient behavior [16].

Within this framework, L-arginine has emerged as a particularly attractive agent for caries prevention [17, 18]. This semi-essential amino acid promotes ecological shifts within the dental biofilms towards eubiosis by serving as a substrate for arginolytic bacteria. Through the arginine deiminase system, its metabolism leads to ammonia production, contributing to local alkalization and buffering against acidic challenges. This mechanism helps counteract the predominance of acidogenic and aciduric species, favoring a microbial community more compatible with oral health [19–23]. In addition, L-arginine has also been associated with alterations in extracellular polysaccharide synthesis and biofilm matrix organization, potentially weakening bacterial adhesion and limiting the development of dense, structurally stable biofilms [24–26].

L-arginine has been incorporated into several dental materials, both in its free form [27–31] and through mesoporous silica nanoparticles (MSNs) [32–34], including conventional and resin-modified glass

ionomer cements, orthodontic resin cements, and dental adhesives [27–34]. Most studies have demonstrated that arginine incorporation enhances the antibacterial activity of these materials against different biofilm models, including mono-, dual-, and multispecies cultures [27–34]. However, evidence linking this antibacterial improvement to reduced enamel demineralization in the orthodontic context remains limited. To date, only one study has evaluated the preventive effect of a resin-modified glass ionomer cement containing 3 wt% free L-arginine on bracket-associated demineralization, demonstrating reduced enamel hardness loss under a dual-species challenge [29].

In contrast to the direct incorporation of the agent in its free form, MSNs exhibit high structural similarity to the inorganic silica fillers commonly present in resin-based materials [35], reduce dependence on material composition and drug concentration [36], and help prevent premature release or degradation of the agent [36], potentially favoring a more sustained local anticariogenic effect around orthodontic brackets [37].

In a previous investigation, L-arginine-containing mesoporous silica nanoparticles (ArgC18@MSNs) were developed and incorporated into a commercial universal adhesive. This modification significantly reduced the viability of planktonic and surface-adhered microorganisms derived from a polymicrobial dental plaque suspension, while preserving the adhesive's physico-mechanical properties [34]. However, it is still unclear whether the previously demonstrated antimicrobial activity of ArgC18@MSNs could effectively translate into reduced enamel demineralization around orthodontic brackets under cariogenic challenge. Therefore, this study investigated the effects of incorporating ArgC18@MSNs into a universal adhesive used for bracket bonding on: (I) enamel microhardness loss following a microbiologically induced WSL model and (II) shear bond strength. Therefore, the null hypotheses tested were that: (I) the incorporation of ArgC18@MSNs would have no effect on enamel microhardness under a biofilm-induced WSL model; and (II) it would not affect shear bond strength.

## 2. MATERIALS AND METHODS

The overall experimental workflow and specimen allocation are summarized in Fig. 1.

### 2.1. Adhesive groups

L-arginine-containing mesoporous silica nanoparticles (ArgC18@MSNs), previously synthesized and characterized, were employed in this study [34]. The commercially available universal adhesive Ambar Universal APS (AU, FGM Dental Group, Joinville, SC, Brazil; Table 1) was modified by incorporating ArgC18@MSNs at a 5 wt% concentration [AU\_5%ArgC18@MSNs]. To ensure homogeneous dispersion, the mixture was magnetically stirred at room temperature for 15 min. The original unmodified adhesive formulation served as the control [AU\_control].

### 2.2. Tooth selection

Forty-eight recently extracted sound human premolars were collected and cleaned to remove residual biological debris using an ultrasonic bath supplemented with periodontal curette instrumentation. Each tooth was carefully examined under a stereomicroscope (Lambda LEB-3, ATTO Instruments, Hong Kong, China) at 10× magnification to verify the absence of structural defects or caries lesions on the buccal enamel surface. The selected teeth were subsequently stored in distilled water at 5°C for a maximum of 3 mo until use.

\* FGM Dental Group, Joinville, SC, Brazil.

ArgC18@MSNs: L-arginine-containing mesoporous silica nanoparticles.

## 2.3. Bracket bonding to enamel

The buccal enamel surfaces were cleaned with distilled water using a prophylaxis brush mounted on a low-speed contra-angle handpiece for 15 s. The teeth were then extensively rinsed with distilled water, then air-dried. An area of the mid-buccal enamel surface approximately corresponding to the bracket base area was etched with 37% phosphoric acid (Condac 37, FGM Dental Group; Table 1) for 20 s, followed by a rinsing for 15 s and gentle air drying for 5 s until the characteristic frosty appearance of etched enamel was observed.

Table 1  
Composition of the materials used.

| Material (manufacturer)                                                                                                                                                                                           | Composition                                                                                                                                         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| 37% phosphoric acid etchant<br><br>Condac 37<br><br>(FGM Dental Group*)                                                                                                                                           | 37% phosphoric acid, thickener, pigment and deionized water                                                                                         |
| Universal adhesive system<br><br>Ambar Universal APS<br><br>(FGM Dental Group)                                                                                                                                    | 10-MDP, methacrylate monomers, photoinitiator complex (APS), co-initiators, stabilizers, inert filler (silica particles) and solvent (ethanol).     |
| Orthodontic adhesive resin cement<br><br>Orthocem<br><br>(FGM Dental Group)                                                                                                                                       | Methacrylate monomers (BisGMA, TEGDMA), methacrylate phosphatized monomers, stabilizers, camforoquinone, co-initiators, silicon filler and pigment. |
| * Joinville, SC, Brazil.<br><br>10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; APS: Advanced Polymerization System; Bis-GMA: bisphenol A glycidyl methacrylate; TEGDMA: triethylene glycol dimethacrylate. |                                                                                                                                                     |

The teeth were randomly assigned to one of the two adhesive groups, AU\_Control or AU\_5%ArgC18@MSNs (n = 24). Two adhesive layers (one drop per layer) were applied to the conditioned enamel surface following manufacturer's instructions. The first layer was vigorously applied by scrubbing a saturated microbrush for 10 s. A second layer was then applied using a new adhesive drop, followed by gentle air blowing for 10 s to evaporate the solvent. Finally, light curing was performed for 10 s using a VALO LED curing unit (Ultradent, South Jordan, UT, USA) operating at an irradiance of 1000 mW/cm<sup>2</sup>.

Premolar metal Roth brackets (Morelli Ortodontia, Sorocaba, SP, Brazil) with a base area of 12.25 mm<sup>2</sup> were bonded using the orthodontic resin cement Orthocem (FGM Dental Group; Table 1) according to the manufacturer's instructions. A small amount of cement was dispensed directly onto the bracket base, which was then positioned onto the tooth surface. Excess cement was removed without displacing the bracket, followed by light curing for a total of 40 s, with 10 s applied to each bracket margin (occlusal, gingival, mesial, and distal).

To ensure complete exposure of the enamel surface surrounding the bracket margins, any residual excess cement or adhesive was finished using aluminum oxide polishing discs (Sof-Lex, Solventum, St. Paul, MN, USA). The specimens were stored in distilled water for one week to remove leachable compounds (unpolymerized monomers) and minimize an initial burst release of L-arginine following bracket bonding.

## 2.4. Simulated microbiological white spot lesions

Twelve bracket-bonded teeth per adhesive group were subjected to a monospecies *S. mutans* biofilm model to simulate microbiological WSLs around brackets, following a previously described protocol [38], with minor modifications. These specimens were subsequently used for cross-sectional enamel microhardness analysis.

Each tooth was individually sterilized in a steam autoclave (Phoenix Ind. Brasileira, Araraquara, SP, Brazil) at 121°C for 15 min. Subsequently, specimens were immersed in an 8 mL Falcon tube containing an artificial caries solution composed of 9.25 g of brain heart infusion supplemented with 5.0 g of sucrose and 1.25 g of yeast extract dissolved in 250 mL of distilled water, along with 100 µL of a primary *S. mutans* culture (ATCC 25175), with an approximate pH of 4.0. Samples were incubated in an anaerobic jar (5% CO<sub>2</sub>) at 37°C for 48 h.

Every 48 h, the specimens were transferred to a new 8 mL Falcon tube containing fresh artificial cariogenic solution without additional *S. mutans* inoculation. This procedure was repeated over a total period of 14 days. After completion of the biofilm challenge, specimens were photographed under standardized conditions using a Canon EOS R mirrorless digital camera (Canon, Tokyo, Japan) equipped with a Sigma 105-mm F2.8 macro lens (Sigma, Kanagawa, Japan). Camera settings were fixed in manual mode (f/11, 1/125 s, ISO 200), and images were recorded in JPEG format.

## 2.5. Cross-sectional enamel microhardness

Each specimen was sectioned in the buccolingual direction through the bracket using a low-speed diamond saw under water irrigation (OCP 100 LC, Odeme Dental Research, Luzerna, SC, Brazil). One central slice, approximately 2 mm thick, was obtained and subsequently polished with silicon carbide abrasive paper up to 4000 grit. The slices were glued by self-cured acrylic resin to glass slides to ensure stability during the microhardness analysis, leaving the surface of interest exposed.

For the microhardness analysis, a microhardness tester equipped with a Knoop indenter (Shimadzu, Kyoto, Japan) was used with a 50 g load applied for 5 s. In each slice, 24 indentations were performed in the occlusal region adjacent to the adhesive margin: at distances of 100, 200, and 300  $\mu\text{m}$  from the adhesive margin and at eight enamel depths from the external enamel surface (25, 50, 75, 100, 125, 150, 175, and 200  $\mu\text{m}$ ). The Knoop Hardness Number (KHN) values measured at each distance from the adhesive margin and at each enamel depth were averaged for each adhesive group and used for the statistical analysis.

## **2.6. Shear bond strength (SBS)**

The remaining twelve bracket-bonded teeth per adhesive group, which were not subjected to the microbiological model, were subjected to the SBS test using a universal testing machine (3343, Instron, Norwood, MA, USA). Specimens were embedded in self-cured acrylic resin using a cylindrical polyvinyl siloxane mold, ensuring that the buccal surface was positioned perpendicular to the horizontal axis of the mold. The specimens were then secured in a testing device attached to the machine, and an occlusogingival load was applied at the enamel-bracket interface using a chisel blade at a crosshead speed of 1 mm/min until bracket debonding occurred. SBS was calculated in MPa by dividing the maximum applied load (N) by the bracket base area ( $\text{mm}^2$ ).

## **2.7. Adhesive remnant index (ARI)**

The amount of adhesive remaining on the tooth surface after bracket debonding was evaluated under a stereomicroscope (Lambda LEB-3, ATTO Instruments) at 10 $\times$  magnification following to the ARI scoring system: 0 (no adhesive remaining on the tooth surface), 1 (less than half of the adhesive remaining on the tooth surface), 2 (more than half of the adhesive remaining on the tooth surface), and 3 (all adhesive remaining on the tooth surface). After the ARI assessment, representative images of the observed score were captured as described previously (par. 2.4).

## **2.8. Statistical analysis**

The sample size of 12 teeth per adhesive group in each experiment (cross-sectional enamel microhardness and SBS) was established based on a previously published study evaluating the same outcome variables under comparable experimental conditions [39]. Shapiro-Wilk tests were used to assess the data normality (KHN values for each adhesive group at each distance from the adhesive margin and enamel depth, and SBS values), while Levene's test was applied to evaluate homogeneity of variances between adhesive groups (data not shown).

For the KHN analysis, normal distribution was confirmed in 93.75% of cases and homogeneity of variances in 91.67% of comparisons. Accordingly, independent-samples *t*-tests were predominantly used to compare adhesive groups at each distance from the adhesive margin and enamel depth, whereas the Mann–Whitney *U* test was applied when parametric assumptions were not met (25 µm enamel depth for the 100, 200, and 300 µm distances, and 50 µm enamel depth at the 200 µm distance). SBS values (MPa) were compared between adhesive groups using the Mann–Whitney *U* test. Differences in ARI score distribution between adhesive groups were evaluated using the Fisher–Freeman–Halton exact test. Statistical analyses were performed using SPSS v30 (IBM, Armonk, NY, USA) and the significance level was set at  $\alpha = 0.05$ .

## **3. RESULTS**

### **3.1. Simulated microbiological white spot lesions**

After the 14-day *S. mutans* biofilm challenge, specimens from both adhesive groups developed visible WSL on the buccal enamel surface adjacent to the bracket (Fig. 2). However, qualitative differences in WSL severity were observed between groups. Specimens bonded using AU\_Control exhibited a greater enamel opacity, characterized by a diffuse whitish appearance with poorly defined margins near the adhesive interface, consistent with enamel demineralization associated with WSL formation. Similar features were also observed in specimens bonded using AU\_5%ArgC18@MSNs; although the lesions appeared visibly less pronounced.

### **3.2. Cross-sectional enamel microhardness**

Cross-sectional enamel microhardness outcomes are presented in Table 2 and Fig. 3. Table 2 reports the mean and standard deviation Knoop Hardness Number (KHN) values obtained for each adhesive group at the evaluated enamel depths and distances from the bracket margin toward the occlusal direction, while Fig. 3 illustrates the distribution of KHN values across enamel depth at each evaluated distance.

Table 2

Mean (standard deviation) Knoop Hardness Number (KHN) values for each adhesive group at different distances from the bracket margin toward the occlusal direction (100, 200, and 300  $\mu\text{m}$ ) and enamel depths (25, 50, 75, 100, 125, 150, 175, and 200  $\mu\text{m}$ ), along with the corresponding statistical analysis.

| Distances ( $\mu\text{m}$ ) | Enamel depths ( $\mu\text{m}$ ) | KHN values (kgf/mm <sup>2</sup> ) for adhesive groups |                            | <i>p</i> -value               |
|-----------------------------|---------------------------------|-------------------------------------------------------|----------------------------|-------------------------------|
|                             |                                 | AU_Control                                            | AU_5%ArgC18@MSNs           |                               |
| 100                         | 25                              | 50.6 (32.2) <sup>B</sup>                              | 123.4 (74.1) <sup>A</sup>  | <i>p</i> = 0.007 <sub>‡</sub> |
|                             | 50                              | 94.6 (50.5) <sup>B</sup>                              | 232.4 (89.7) <sup>A</sup>  | <i>p</i> < 0.001 <sub>†</sub> |
|                             | 75                              | 154.1 (65.3) <sup>B</sup>                             | 290.8 (94.6) <sup>A</sup>  | <i>p</i> < 0.001 <sub>†</sub> |
|                             | 100                             | 279.0 (60.9) <sup>B</sup>                             | 362.3 (64.2) <sup>A</sup>  | <i>p</i> = 0.004 <sub>†</sub> |
|                             | 125                             | 311.9 (62.4) <sup>A</sup>                             | 364.7 (73.4) <sup>A</sup>  | <i>p</i> = 0.071 <sub>†</sub> |
|                             | 150                             | 315.8 (78.5) <sup>A</sup>                             | 373.3 (83.6) <sup>A</sup>  | <i>p</i> = 0.097 <sub>†</sub> |
|                             | 175                             | 335.5 (62.5) <sup>A</sup>                             | 350.6 (78.7) <sup>A</sup>  | <i>p</i> = 0.607 <sub>†</sub> |
|                             | 200                             | 366.2 (88.9) <sup>A</sup>                             | 377.3 (67.0) <sup>A</sup>  | <i>p</i> = 0.732 <sub>†</sub> |
| 200                         | 25                              | 52.5 (31.2) <sup>B</sup>                              | 124.8 (75.3) <sup>A</sup>  | <i>p</i> = 0.001 <sub>‡</sub> |
|                             | 50                              | 97.3 (37.5) <sup>B</sup>                              | 221.1 (108.8) <sup>A</sup> | <i>p</i> = 0.001 <sub>‡</sub> |
|                             | 75                              | 190.2 (72.5) <sup>B</sup>                             | 290.4 (102.9) <sup>A</sup> | <i>p</i> = 0.011 <sub>†</sub> |
|                             | 100                             | 255.1 (90.5) <sup>A</sup>                             | 319.1 (82.4) <sup>A</sup>  | <i>p</i> = 0.084 <sub>†</sub> |
|                             | 125                             | 325.5 (83.7) <sup>A</sup>                             | 364.8 (71.6) <sup>A</sup>  | <i>p</i> = 0.231 <sub>†</sub> |
|                             | 150                             | 345.4 (76.7) <sup>A</sup>                             | 357.7 (68.4) <sup>A</sup>  | <i>p</i> = 0.682 <sub>†</sub> |
|                             | 175                             | 318.0 (74.7) <sup>A</sup>                             | 336.7 (67.8) <sup>A</sup>  | <i>p</i> = 0.528 <sub>†</sub> |

| Distances (μm)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Enamel depths (μm) | KHN values (kgf/mm <sup>2</sup> ) for adhesive groups |                            | <i>p</i> -value               |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|-------------------------------------------------------|----------------------------|-------------------------------|
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                    | AU_Control                                            | AU_5%ArgC18@MSNs           |                               |
| 300                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 200                | 343.1 (83.4) <sup>A</sup>                             | 359.8 (73.1) <sup>A</sup>  | <i>p</i> = 0.606 <sub>†</sub> |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 25                 | 54.3 (38.8) <sup>B</sup>                              | 127.1 (53.0) <sup>A</sup>  | <i>p</i> = 0.001 <sub>‡</sub> |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 50                 | 93.1 (42.4) <sup>B</sup>                              | 198.6 (65.3) <sup>A</sup>  | <i>p</i> = 0.003 <sub>†</sub> |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 75                 | 233.8 (102.6) <sup>A</sup>                            | 297.3 (106.4) <sup>A</sup> | <i>p</i> = 0.151 <sub>†</sub> |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 100                | 271.7 (92.7) <sup>A</sup>                             | 320.4 (97.0) <sup>A</sup>  | <i>p</i> = 0.222 <sub>†</sub> |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 125                | 300.8 (88.6) <sup>A</sup>                             | 340.2 (81.4) <sup>A</sup>  | <i>p</i> = 0.269 <sub>†</sub> |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 150                | 311.6 (85.9) <sup>A</sup>                             | 340.9 (65.5) <sup>A</sup>  | <i>p</i> = 0.359 <sub>†</sub> |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 175                | 326.9 (78.4) <sup>A</sup>                             | 349.2 (60.4) <sup>A</sup>  | <i>p</i> = 0.444 <sub>†</sub> |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 200                | 306.8 (83.1) <sup>A</sup>                             | 363.2 (64.8) <sup>A</sup>  | <i>p</i> = 0.077 <sub>†</sub> |
| <p>Different uppercase superscript letters within the same enamel depth and distance from the bracket margin toward the occlusal direction indicate statistically significant differences between adhesive groups (independent-samples <i>t</i>-test [<sup>†</sup>] or Mann–Whitney <i>U</i> test [<sup>‡</sup>]; <i>p</i> &lt; 0.05). All <i>p</i>-values were two-sided, and Mann–Whitney <i>U</i> tests were computed using exact significance.</p> <p>AU_Control: unmodified Ambar Universal APS adhesive (AU; FGM Dental Group)</p> <p>AU_5%ArgC18@MSNs: AU loaded with 5 wt% L-arginine-containing mesoporous silica nanoparticles</p> |                    |                                                       |                            |                               |

At 100 μm from the bracket margin, AU\_5%ArgC18@MSNs yielded higher KHN values than AU\_Control at enamel depths of 25, 50, 75, and 100 μm (*p* < 0.05). No significant differences were detected at greater depths (125–200 μm; *p* > 0.05). At 200 μm from the bracket margin, higher KHN values were observed for AU\_5%ArgC18@MSNs at enamel depths of 25, 50, and 75 μm (*p* < 0.05), whereas no differences were found at depths ranging from 100–200 μm (*p* > 0.05). At 300 μm from the bracket margin, differences were limited to the most superficial enamel (25 and 50 μm; *p* < 0.05), with no significant differences observed at depths between 75 to 200 μm (*p* > 0.05).

### 3.3. Shear bond strength (SBS)

The SBS values for each adhesive group are shown in Fig. 4. The Mann–Whitney  $U$  test revealed no statistically significant differences ( $p = 0.50$ ) between AU\_Control (median: 31.50 MPa; Interquartile range [IQR]: 5.85) and AU\_5%ArgC18@MSNs (median: 30.06 MPa; IQR: 5.16).

### 3.4. Adhesive Remnant Index (ARI)

The frequency distribution of ARI scores for each adhesive is presented in Fig. 5, and representative images are shown in Fig. 6. The Fisher–Freeman–Halton exact test revealed no significant differences in the ARI scores distribution between groups ( $p = 0.57$ ). In the AU\_Control group, only ARI scores of 0 and 1 were observed, with most specimens presenting score 1 (66.7%). In the AU\_5%ArgC18@MSNs group, scores of 0, 1, and 2 were identified, with scored 1 (50.0%) also predominating. Overall, both groups exhibited failure modes predominantly at or near the adhesive–enamel interface, and no cohesive material failures (ARI score 3) were observed.

ARI scores: 0 (no adhesive remaining on the tooth surface), 1 (less than half of the adhesive remaining on the tooth surface), 2 (more than half of the adhesive remaining on the tooth surface), and 3 (all adhesive remaining on the tooth surface).

ARI scores: 0 (no adhesive remaining on the tooth surface), 1 (less than half of the adhesive remaining on the tooth surface), 2 (more than half of the adhesive remaining on the tooth surface), and 3 (all adhesive remaining on the tooth surface).

## 4. DISCUSSION

In the present *in vitro* study, a commercial universal adhesive was modified by incorporating 5 wt% ArgC18@MSNs and evaluated for use in bracket bonding, where it was applied to etched enamel prior to orthodontic resin cement placement as a potential anticariogenic strategy to reduce the development of WSLs during fixed orthodontic treatment.

To indirectly assess enamel demineralization adjacent to orthodontic brackets, a cross-sectional enamel microhardness test was performed after a 14-day *S. mutans*-based biofilm challenge, comparing the ArgC18@MSNs-modified adhesive with its unmodified counterpart. The microbiological model proved effective in inducing WSL formation in all specimens, as confirmed by photographic analysis, although lesions appeared qualitatively less pronounced in specimens bonded with the modified adhesive. Although cross-sectional microhardness testing does not directly quantify enamel mineral content, it may provide relevant information regarding lesion mechanical properties [40] and has been widely used in demineralization/remineralization studies [41–43], including investigations specifically involving orthodontic brackets [44–46].

The main results showed that the ArgC18@MSNs-modified adhesive significantly reduced enamel microhardness loss adjacent to the bracket margin compared with the unmodified counterpart, leading to the rejection of the first null hypothesis. Specifically, the protective effect was predominantly localized

to regions close to the bracket margin, with significantly higher hardness values observed in the modified adhesive group at superficial enamel depths up to 100 µm from the surface and 100 µm distance from the bracket margin, up to 75 µm at 200 µm, and up to 50 µm at 300 µm. These findings are consistent with a previous study in which the incorporation of 3 wt% free L-arginine into a commercial orthodontic resin-modified glass ionomer cement reduced enamel microhardness loss following a 24-h dual-species cariogenic challenge (*S. mutans* and *C. albicans*), with the protective effect likewise being more pronounced in enamel regions adjacent to the bracket margin [29]. Despite differences in material type and biofilm model, both studies collectively support the role of L-arginine as a promising additive for orthodontic bonding materials aimed at reducing enamel demineralization around brackets. Moreover, the localized nature of the protective effect suggests that the biological activity of L-arginine released from ArgC18@MSNs within the biofilm was concentrated near the tooth–material interface. Similar spatial patterns of protection have also been reported for Clearfil Protect Bond, an antibacterial adhesive containing MDPB and fluoride [47].

Considering that both the present and the aforementioned study employed simplified mono- and dual-species biofilm models [29], it is unlikely that the protective effect observed can be primarily explained by ecological shifts in microbial composition [19–23]. Simplified *in vitro* biofilm models are not meant to fully reproduce the ecological complexity of the oral environment [48]. Instead, the observed effect may be more plausibly attributed to direct alterations in the structural and adhesive properties of cariogenic biofilms induced by L-arginine. Previous studies have demonstrated that L-arginine interferes with extracellular polysaccharides (EPS) synthesis and organization in *S. mutans*, reducing water-insoluble glucans production and the EPS-to-bacteria ratio, thereby resulting in thinner, less cohesive biofilms with reduced adherence and a less dense extracellular matrix [24–26]. Additionally, L-arginine has been shown to inhibit both planktonic growth and biofilm formation of *S. mutans* and *C. albicans*, including mixed-species biofilms, potentially limiting cross-kingdom interactions and their synergistic cariogenic effects [49]. This enhanced antibacterial activity has also been reported when L-arginine was incorporated, either in free form or via mesoporous silica nanoparticles, into different orthodontic materials [28–30, 33], further supporting the protective effect observed in the present study.

Beyond L-arginine-based materials, the effectiveness of currently available orthodontic bonding materials in preventing enamel demineralization remains inconclusive. A recent systematic review of *in vivo* studies reported that, although certain modified systems, such as calcium-based nanoparticles or amorphous calcium phosphate-containing materials, may reduce demineralization, most conventional strategies, including fluoride-releasing (Opal Seal, Ultradent) and antibacterial monomer-containing adhesives (Clearfil Protect Bond, Kuraray), have not demonstrated clear clinical advantages over standard materials [16]. Similarly, nanoparticle-based systems such as titanium dioxide have shown conflicting results [16]. These findings highlight the limitations of currently available preventive approaches and underscore the difficulty of achieving clinically meaningful protection against WSL development. Therefore, although the present *in vitro* findings are promising, *in vivo* studies are warranted to confirm their clinical relevance.

From a clinical perspective, the applicability of a modified universal adhesive for bracket bonding depends not only on its anticariogenic potential but also on maintaining adequate bonding performance, since compositional changes may adversely affect adhesion [50, 51]. This consideration is particularly relevant because universal adhesives have been proposed as an alternative to conventional orthodontic primers with comparable bonding effectiveness [52].

Previous studies evaluating arginine-containing orthodontic bonding materials have reported variable SBS outcomes, depending on the arginine form, concentration, and material type. The addition of free L-arginine to orthodontic resin cements generally did not compromise SBS, even at relatively high concentrations (up to 7 wt%), and in some cases even enhanced bonding performance [28, 30]. In contrast, incorporation into resin-modified glass ionomer cements has shown concentration-dependent effects, with higher concentrations (5 wt%) associated with reduced SBS [29]. Similarly, ArgC18@MSNs incorporated into an orthodontic resin cement reduced immediate SBS at low concentrations (1 and 2 wt%), although this effect appeared to diminish after aging [33].

It must be noted that orthodontic resin cements and resin-modified glass ionomer cements differ substantially from dental adhesives or orthodontic primers in composition and chemical characteristics, which limits the direct extrapolation of previous findings. Furthermore, literature data have suggested that the incorporation of antimicrobial nanoparticles into orthodontic adhesives generally does not compromise SBS at concentrations up to 5 wt%, which is consistent with the present results [53].

The incorporation of ArgC18@MSNs did not significantly affect SBS, with comparable bond strength values observed between the modified and unmodified adhesives. Accordingly, the second null hypothesis was accepted. Notably, all SBS values exceeded the minimum threshold considered clinically acceptable for orthodontic bonding (6–8 MPa), further supporting the suitability of both adhesives for clinical use [54].

The preservation of bonding performance observed in the present study aligns with previous evidence demonstrating the chemical compatibility of ArgC18@MSNs within the AU adhesive matrix. Their incorporation did not adversely affect key physico-mechanical properties, including degree of conversion, surface microhardness, water sorption, mass change, ultimate tensile strength, and flexural strength, and has even been associated with improvements in resistance to ethanol-induced softening, water solubility, and elastic modulus [34].

Regarding the adhesive remnant index (ARI), incorporation of ArgC18@MSNs did not appear to influence the debonding pattern, as both adhesives exhibited similar distributions of failure modes. In both groups, lower ARI scores predominated, indicating failures mainly occurred at or near the adhesive–enamel interface. From a clinical standpoint, the predominance of scores 0 and 1 may be advantageous, as these scores have been associated with reduced risk of enamel damage during bracket debonding and subsequent resin removal procedures [55]. Overall, these findings suggest that the modified adhesive preserves a debonding behavior comparable to that of the unmodified material without introducing unfavorable failure patterns.

Despite the controlled experimental design, the present findings should be interpreted with caution. The *in vitro* model and simplified *S. mutans* biofilm do not fully reproduce the biological complexity of the oral environment and do not allow full elucidation of the biofilm-modulating mechanism of L-arginine. Additionally, enamel demineralization was assessed indirectly through cross-sectional microhardness testing, which, although widely accepted, does not directly quantify mineral loss. It should also be considered that only one universal adhesive was evaluated. Therefore, given the compositional variability among commercially available adhesives [50], the present results cannot be directly extrapolated to other formulations. More comprehensive experimental models and further *in vivo* studies are required to confirm the clinical relevance of these findings.

## 5. CONCLUSIONS

Within the limitations of this *in vitro* study, the incorporation of 5 wt% L-arginine-containing mesoporous silica nanoparticles (ArgC18@MSNs) into a commercial universal adhesive used for bracket bonding reduced enamel microhardness loss adjacent to brackets under a *S. mutans* microbiological white spot lesion induction model, particularly in the superficial enamel near the bracket margin. At the same time, this modification did not significantly affect shear bond strength or the distribution of adhesive remnant index scores. These findings suggest that ArgC18@MSNs may represent a promising strategy for development of universal adhesives with anticariogenic potential for bracket bonding without compromising bonding performance.

## Declarations

### Acknowledgments

This paper was conducted by Daniel Jiménez-Díez as part of the requirements for the international doctoral degree jointly undertaken at Rey Juan Carlos University (URJC; Mostoles, Madrid, Spain) and State University of Ponta Grossa (UEPG, Ponta Grossa, PR, Brazil). The research was presented at the 2026 CRADO (Conferencia de Decanos/as y Directores Académicos de las Facultades de Odontología de España) & CED-IADR (Continental European Division of the International Association for Dental, Oral, and Craniofacial Research) Award Session (ExpoDental, Madrid, Spain). The authors gratefully acknowledge FGM Dental Group (Joinville, SC, Brazil) for generously providing the universal adhesive system used. Special thanks are also extended to Jana Baptista (Laboratory Assistant in the Department of Microbiology at UEPG) for her valuable assistance.

### Funding

This work was supported by Rey Juan Carlos University (URJC, Móstoles, Madrid, Spain) [DJD, Grant No. PREDOC22-015], Ilustre Colegio Oficial de Odontólogos y Estomatólogos de la 1ª Región (COEM, Madrid, Spain) [DJD, 2023 COEM Research Grant], Brazilian Government by National Council for Scientific and Technological Development (CNPq) [ADL, Grant No. 304444/2025-1] and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 [CF-P, GC, and BC-S],

and by the Agencia Estatal de Investigación/Ministerio de Ciencia e Innovación de España MCIN/AEI/10.13039/501100011033 (Grants No. PID2021-125216OB-I00 and PID2024-160428OB-I00).

### **Ethical approval**

This *in vitro* study was approved by the Research Ethics Committee of Rey Juan Carlos University (URJC, Móstoles, Madrid, Spain): internal registration number 120120260822026.

### **Competing interests**

The authors declare that they have no conflict of interest.

### **Author contribution**

D.J.-D., A.D.L., V.F. and L.C. conceived and designed the study. D.J.-D., A.D.L., V.F. and L.C. developed the methodology. D.J.-D., C.M.-F., G.C., B.C.-S. and F.N. conducted the investigation. D.J.-D. and V.F. performed the data curation and formal analysis. D.J.-D. wrote the original draft of the manuscript. V.M., R.S., R.A.G.-M., A.C.I., A.D.L., V.F. and L.C. reviewed and edited the manuscript. D.J.-D., C.M.-F., G.C., B.C.-S., R.S., R.A.G.-M., A.D.L. and L.C. acquired the funding. R.S., R.A.G.-M., A.D.L. and L.C. provided resources. A.C.I., A.D.L., V.F. and L.C. supervised the study. All authors read and approved the final manuscript.

## **References**

1. Hussain U, Wahab A, Kamran MA, Alnazeh AA, Almoammar S, Alshahrani SSM, Niazi FH, Alam S, Arif N, Campobasso A, Pandis N (2025) Prevalence, incidence and risk factors of White Spot lesions associated with orthodontic treatment - A systematic review and meta-analysis. *Orthod Craniofac Res* 28:379–399. <https://doi.org/10.1111/ocr.12888>
2. Denis M, Atlan A, Vennat E, Tirlet G, Attal J-P (2013) White defects on enamel: diagnosis and anatomopathology: two essential factors for proper treatment (part 1). *Int Orthod* 11:139–165. <https://doi.org/10.1016/j.ortho.2013.02.014>
3. Huang X, Qin D, Ngan P, Cao L, Hua F, He H (2024) White spot lesion-related perception of aesthetics and treatment satisfaction among orthodontic patients, orthodontists and other dental specialists. *Orthod Craniofac Res* 27:626–634. <https://doi.org/10.1111/ocr.12776>
4. Mattousch TJH, van der Veen MH, Zentner A (2007) Caries lesions after orthodontic treatment followed by quantitative light-induced fluorescence: a 2-year follow-up. *Eur J Orthod* 29:294–298. <https://doi.org/10.1093/ejo/cjm008>
5. Höchli D, Hersberger-Zurfluh M, Papageorgiou SN, Eliades T (2017) Interventions for orthodontically induced white spot lesions: a systematic review and meta-analysis. *Eur J Orthod* 39:122–133. <https://doi.org/10.1093/ejo/cjw065>

6. Puleio F, Fiorillo L, Gorassini F, Iandolo A, Meto A, D'Amico C, Cervino G, Pinizzotto M, Bruno G, Portelli M, Amato A, Lo Giudice R (2022) Systematic review on White Spot lesions treatments. *Eur J Dent* 16:41–48. <https://doi.org/10.1055/s-0041-1731931>
7. Sardana D, Schwendicke F, Kosan E, Tüfekçi E (2023) White spot lesions in orthodontics: consensus statements for prevention and management. *Angle Orthod* 93:621–628. <https://doi.org/10.2319/062523-440.1>
8. Machiulskiene V, Campus G, Carvalho JC, Dige I, Ekstrand KR, Jablonski-Momeni A, Maltz M, Manton DJ, Martignon S, Martinez-Mier EA, Pitts NB, Schulte AG, Splieth CH, Tenuta LMA, Ferreira Zandona A, Nyvad B (2020) Terminology of dental caries and dental caries management: Consensus report of a workshop organized by ORCA and Cariology Research Group of IADR. *Caries Res* 54:7–14. <https://doi.org/10.1159/000503309>
9. Santonocito S, Polizzi A (2022) Oral Microbiota Changes during Orthodontic Treatment. *Front Biosci (Elite Ed)* 14:19. <https://doi.org/10.31083/j.fbe1403019>
10. Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, Bei K (2024) Oral microbiome: A review of its impact on oral and systemic health. *Microorganisms* 12:1797. <https://doi.org/10.3390/microorganisms12091797>
11. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G, Ismail A (2017) Dental caries. *Nat Rev Dis Primers* 3:17030. <https://doi.org/10.1038/nrdp.2017.30>
12. Raghavan S, Abu Alhaija ES, Duggal MS, Narasimhan S, Al-Maweri SA (2023) White spot lesions, plaque accumulation and salivary caries-associated bacteria in clear aligners compared to fixed orthodontic treatment. A systematic review and meta- analysis. *BMC Oral Health* 23:599. <https://doi.org/10.1186/s12903-023-03257-8>
13. Xia L, Zhou C, Mei P, Jin Z, He H, Wang L, Bai Y, Chen L, Li W, Wang J, Hu M, Song J, Cao Y, Liu Y, Hou B, Wei X, Niu L, Lu H, Ma W, Wang P, Zhang G, Guo J, Li Z, Lu H, Ren L, Xu L, Wu X, Lu Y, Hu J, Yue L, Zhang X, Fang B (2025) Expert consensus on the prevention and treatment of enamel demineralization in orthodontic treatment. *Int J Oral Sci* 17:13. <https://doi.org/10.1038/s41368-024-00335-7>
14. Werner H, Hakeberg M, Dahlström L, Eriksson M, Sjögren P, Strandell A, Svanberg T, Svensson L, Wide Boman U (2016) Psychological interventions for poor oral health: A systematic review. *J Dent Res* 95:506–514. <https://doi.org/10.1177/0022034516628506>
15. Mansoor M, Monis D, Anjum R, Siddiqui TA, Mir HA, Nazir R (2024) A cross-sectional study to correlate oral hygiene habit among orthodontic patients with their clinical findings and periodontal treatment need. *BMC Oral Health* 24:903. <https://doi.org/10.1186/s12903-024-04678-9>
16. El Helou M, Chakar S, Nicolas E, Estephan E, Cuisinier F, Barthélemy S (2024) Can orthodontic adhesive systems inhibit the formation and development of White Spot lesions during fixed orthodontic treatment? A systematic review. *J Adhes Dent* 26:241–252. <https://doi.org/10.3290/j.jad.b5781299>

17. Nascimento MM (2018) Potential uses of arginine in dentistry. *Adv Dent Res* 29:98–103.  
<https://doi.org/10.1177/0022034517735294>
18. Zaura E, Twetman S (2019) Critical appraisal of oral pre- and probiotics for caries prevention and care. *Caries Res* 53:514–526. <https://doi.org/10.1159/000499037>
19. Agnello M, Cen L, Tran NC, Shi W, McLean JS, He X (2017) Arginine improves pH homeostasis via metabolism and microbiome modulation. *J Dent Res* 96:924–930.  
<https://doi.org/10.1177/0022034517707512>
20. Koopman JE, Röling WFM, Buijs MJ, Sissons CH, ten Cate JM, Keijser BJJ, Crielaard W, Zaura E (2015) Stability and resilience of oral microcosms toward acidification and *Candida* outgrowth by arginine supplementation. *Microb Ecol* 69:422–433. <https://doi.org/10.1007/s00248-014-0535-x>
21. Ledder RG, Mistry H, Sreenivasan PK, Humphreys G, McBain AJ (2017) Arginine exposure decreases acidogenesis in long-term oral biofilm microcosms. *mSphere* 2:e00295–e00217.  
<https://doi.org/10.1128/mSphere.00295-17>
22. Zheng X, He J, Wang L, Zhou S, Peng X, Huang S, Zheng L, Cheng L, Hao Y, Li J, Xu J, Xu X, Zhou X (2017) Ecological effect of arginine on oral Microbiota. *Sci Rep* 7:7206.  
<https://doi.org/10.1038/s41598-017-07042-w>
23. Assar S, Kristensen MF, Del Rey YC, Lund MB, Raittio EJ, Schramm A, Meyer RL, Schlafer S (2025) Microscale analyses of the effect of arginine on oral biofilms. *J Dent* 160:105863.  
<https://doi.org/10.1016/j.jdent.2025.105863>
24. Huang X, Zhang K, Deng M, Exterkate RAM, Liu C, Zhou X, Cheng L, Ten Cate JM (2017) Effect of arginine on the growth and biofilm formation of oral bacteria. *Arch Oral Biol* 82:256–262.  
<https://doi.org/10.1016/j.archoralbio.2017.06.026>
25. Sharma S, Lavender S, Woo J, Guo L, Shi W, Kilpatrick-Liverman L, Gimzewski JK (2014) Nanoscale characterization of effect of L-arginine on *Streptococcus mutans* biofilm adhesion by atomic force microscopy. *Microbiology* 160:1466–1473. <https://doi.org/10.1099/mic.0.075267-0>
26. He J, Hwang G, Liu Y, Gao L, Kilpatrick-Liverman L, Santarpia P, Zhou X, Koo H (2016) L-arginine modifies the exopolysaccharide matrix and thwarts *Streptococcus mutans* outgrowth within mixed-species oral biofilms. *J Bacteriol* 198:2651–2661. <https://doi.org/10.1128/JB.00021-16>
27. Bijle MN, Ekambaram M, Lo ECM, Yiu CKY (2020) Antibacterial and mechanical properties of arginine-containing glass ionomer cements. *Dent Mater* 36:1226–1240.  
<https://doi.org/10.1016/j.dental.2020.05.012>
28. Geraldeli S, Maia Carvalho L, de Souza Araújo A, Guarda IJ, Nascimento MB, Bertolo MM, Di Nizo MVL, Sinhoreti PT, McCarlie MAC Jr (2021) Incorporation of arginine to commercial orthodontic light-cured resin cements-physical, adhesive, and antibacterial properties. *Mater (Basel)* 14:4391.  
<https://doi.org/10.3390/ma14164391>
29. Ma Y, Wu X, Gao H, Fang D, Bai Y (2025) Arginine-modified orthodontic resin cement inhibits cariogenic bacterial-fungal co-adherence and enamel hardness loss. *J Dent* 159:105822.  
<https://doi.org/10.1016/j.jdent.2025.105822>

30. Santos ALC, Correr-Sobrinho L, Tsuzuki FM, Facury Ferraz AG, Neves JG, Sinhoreti MAC, Franco EM, Costa AR (2024) Effect of adding arginine at different concentrations to experimental orthodontic resins: an in vitro study. *Braz Oral Res* 38:e078. <https://doi.org/10.1590/1807-3107bor-2024.vol38.0078>
31. Geraldeli S, Soares EF, Alvarez AJ, Farivar T, Shields RC, Sinhoreti MAC, Nascimento MM (2017) A new arginine-based dental adhesive system: formulation, mechanical and anti-caries properties. *J Dent* 63:72–80. <https://doi.org/10.1016/j.jdent.2017.05.024>
32. López-Ruiz M, Navas F, Fernández-García P, Martínez-Erro S, Fuentes MV, Giráldez I, Ceballos L, Ferrer-Luque CM, Ruiz-Linares M, Morales V, Sanz R, García-Muñoz RA (2022) L-arginine-containing mesoporous silica nanoparticles embedded in dental adhesive (Arg@MSN@DAdh) for targeting cariogenic bacteria. *J Nanobiotechnol* 20:502. <https://doi.org/10.1186/s12951-022-01714-0>
33. An J, Shen X, Peng T, Qiao M, Xu B (2024) Formulation of arginine-loaded mesoporous silica nanoparticles (Arg@MSNs) modified orthodontic adhesive. *J Dent* 145:104992. <https://doi.org/10.1016/j.jdent.2024.104992>
34. Jiménez-Díez D, Fernández-García P, Navas F, Morales V, Sanz R, García-Muñoz RA, Ruiz-Linares M, Ferrer-Luque CM, Loguercio AD, Fuentes V, Ceballos L (2025) Antimicrobial and physico-mechanical properties of a universal adhesive loaded with L-arginine-containing mesoporous silica nanoparticles (ArgC18@MSNs). *Dent Mater* 41:1465–1477. <https://doi.org/10.1016/j.dental.2025.08.004>
35. Ferracane JL (2024) A historical perspective on dental composite restorative materials. *J Funct Biomater* 15:173. <https://doi.org/10.3390/jfb15070173>
36. Cadenaro M, Maravic T, Comba A, Mazzoni A, Fanfoni L, Hilton T, Ferracane J, Breschi L (2019) The role of polymerization in adhesive dentistry. *Dent Mater* 35:e1–e22. <https://doi.org/10.1016/j.dental.2018.11.012>
37. Djayanti K, Maharjan P, Cho KH, Jeong S, Kim MS, Shin MC, Min KA (2023) Mesoporous silica nanoparticles as a potential nanoplatform: Therapeutic applications and considerations. *Int J Mol Sci* 24:6349. <https://doi.org/10.3390/ijms24076349>
38. Falconi-Páez C, Aldaz MAN, Navarro TG, González-Vaca CC, Campos LA, Davila-Sanchez A, Arrais CAG (2025) How universal adhesive systems with nanoencapsulated flavonoids improve long-term bonding to caries-affected dentin. *J Esthet Restor Dent* 37:1546–1556. <https://doi.org/10.1111/jerd.13426>
39. Ma Y, Zhang N, Weir MD, Bai Y, Xu HHK (2017) Novel multifunctional dental cement to prevent enamel demineralization near orthodontic brackets. *J Dent* 64:58–67. <https://doi.org/10.1016/j.jdent.2017.06.004>
40. Magalhães AC, Moron BM, Comar LP, Wiegand A, Buchalla W, Buzalaf MAR (2009) Comparison of cross-sectional hardness and transverse microradiography of artificial carious enamel lesions induced by different demineralising solutions and gels. *Caries Res* 43:474–483. <https://doi.org/10.1159/000264685>

41. Shaheen SD, Aboelwafa MR (2025) The impact of fluorinated graphene versus nanohydroxyapatite crystals on artificial white spot lesion microhardness and color change. *BMC Oral Health* 25:1041. <https://doi.org/10.1186/s12903-025-06410-7>
42. Martins TP, Báez-Quintero LC, Gomes IMP, Wiegand A, Delbem ACB, Pessan JP (2025) Effect of fluoride gels supplemented with nano-sized sodium trimetaphosphate on enamel remineralization in situ. *J Dent* 162:105964. <https://doi.org/10.1016/j.jdent.2025.105964>
43. Cuéllar-Mansilla JOF, Pessan JP, Gonçalves DFM, Danelon M, Ferraresso LF, de OT, Hosida TY, Sampaio C, Delbem ACB (2025) Development of a cycling protocol for the in vitro assessment of enamel erosion and the effect of a fluoridated mouthrinse containing micrometric- and nano-sized trimetaphosphate on erosion prevention. *Clin Oral Investig* 29:607. <https://doi.org/10.1007/s00784-025-06644-3>
44. Babanouri N, Heidari F, Hajipour F, Pakshir H (2025) The effect of nanohydroxyapatite serum and toothpaste on prevention of enamel demineralization around orthodontic brackets: an in vitro study. *BMC Oral Health* 25:1619. <https://doi.org/10.1186/s12903-025-07014-x>
45. Silva-Fialho P, Ferreira R, Leal J, Tabchoury C, Vale G (2021) Effect of high-fluoride dentifrice and bracket bonding composite material on enamel demineralization adjacent to orthodontic brackets in vitro. *J Clin Exp Dent* 13:e493–e498. <https://doi.org/10.4317/jced.57673>
46. Yi J, Dai Q, Weir MD, Melo MAS, Lynch CD, Oates TW, Zhang K, Zhao Z, Xu HHK (2019) A nano-CaF<sub>2</sub>-containing orthodontic cement with antibacterial and remineralization capabilities to combat enamel white spot lesions. *J Dent* 89:103172. <https://doi.org/10.1016/j.jdent.2019.07.010>
47. Uysal T, Amasyali M, Ozcan S, Koyuturk AE, Sagdic D (2011) Effect of antibacterial monomer-containing adhesive on enamel demineralization around orthodontic brackets: an in-vivo study. *Am J Orthod Dentofac Orthop* 139:650–656. <https://doi.org/10.1016/j.ajodo.2009.06.038>
48. Ibrahim MS, Garcia IM, Kensara A, Balhaddad AA, Collares FM, Williams MA, Ibrahim AS, Lin NJ, Weir MD, Xu HHK, Melo MAS (2020) How we are assessing the developing antibacterial resin-based dental materials? A scoping review. *J Dent* 99:103369. <https://doi.org/10.1016/j.jdent.2020.103369>
49. Gao H-Y, Yang H, Wang H-M, Li H-M, Ma Y-S, Bai Y-X (2025) Arginine inhibits cross-kingdom interactions and synergistic cariogenicity between *Streptococcus mutans* and *Candida albicans*. *Front Cell Infect Microbiol* 15:1633342. <https://doi.org/10.3389/fcimb.2025.1633342>
50. Dressano D, Salvador MV, Oliveira MT, Marchi GM, Fronza BM, Hadis M, Palin WM, Lima AF (2020) Chemistry of novel and contemporary resin-based dental adhesives. *J Mech Behav Biomed Mater* 110:103875. <https://doi.org/10.1016/j.jmbbm.2020.103875>
51. Mokeem LS, Balhaddad AA, Garcia IM, Collares FM, Melo MAS (2022) Benzyltrimethylammonium chloride doped dental adhesive: Impact on core's properties, biosafety, and antibacterial/bonding performance after aging. *J Funct Biomater* 13:190. <https://doi.org/10.3390/jfb13040190>
52. de Resende I-S-L, Cunha D-A, Silva P-GB, Damasceno J-X, Aragão L-R, Vieira-Meyer A-P-G-F, Neri J-R (2024) Does the use of universal adhesive systems improve the durability of the bond strength of

- orthodontic brackets to enamel? J Clin Exp Dent 16:e178–e185. <https://doi.org/10.4317/jced.61247>
53. Pourhajibagher M, Sodagar A, Bahador A (2020) An in vitro evaluation of the effects of nanoparticles on shear bond strength and antimicrobial properties of orthodontic adhesives: A systematic review and meta-analysis study. Int Orthod 18:203–213. <https://doi.org/10.1016/j.ortho.2020.01.011>
54. González-Serrano C, Phark J-H, Fuentes MV, Albaladejo A, Sánchez-Monescillo A, Duarte S Jr, Ceballos L (2021) Effect of a single-component ceramic conditioner on shear bond strength of precoated brackets to different CAD/CAM materials. Clin Oral Investig 25:1953–1965. <https://doi.org/10.1007/s00784-020-03504-0>
55. Malavasi CV, da Silva EM, Rodrigues FP, Guimarães JGA, Portela MB, Cabral-Oliveira GG, Poskus LT (2026) Effectiveness of experimental composites containing antimicrobial agents for orthodontic applications. Odontology <https://doi.org/10.1007/s10266-026-01319-1>

## Figures

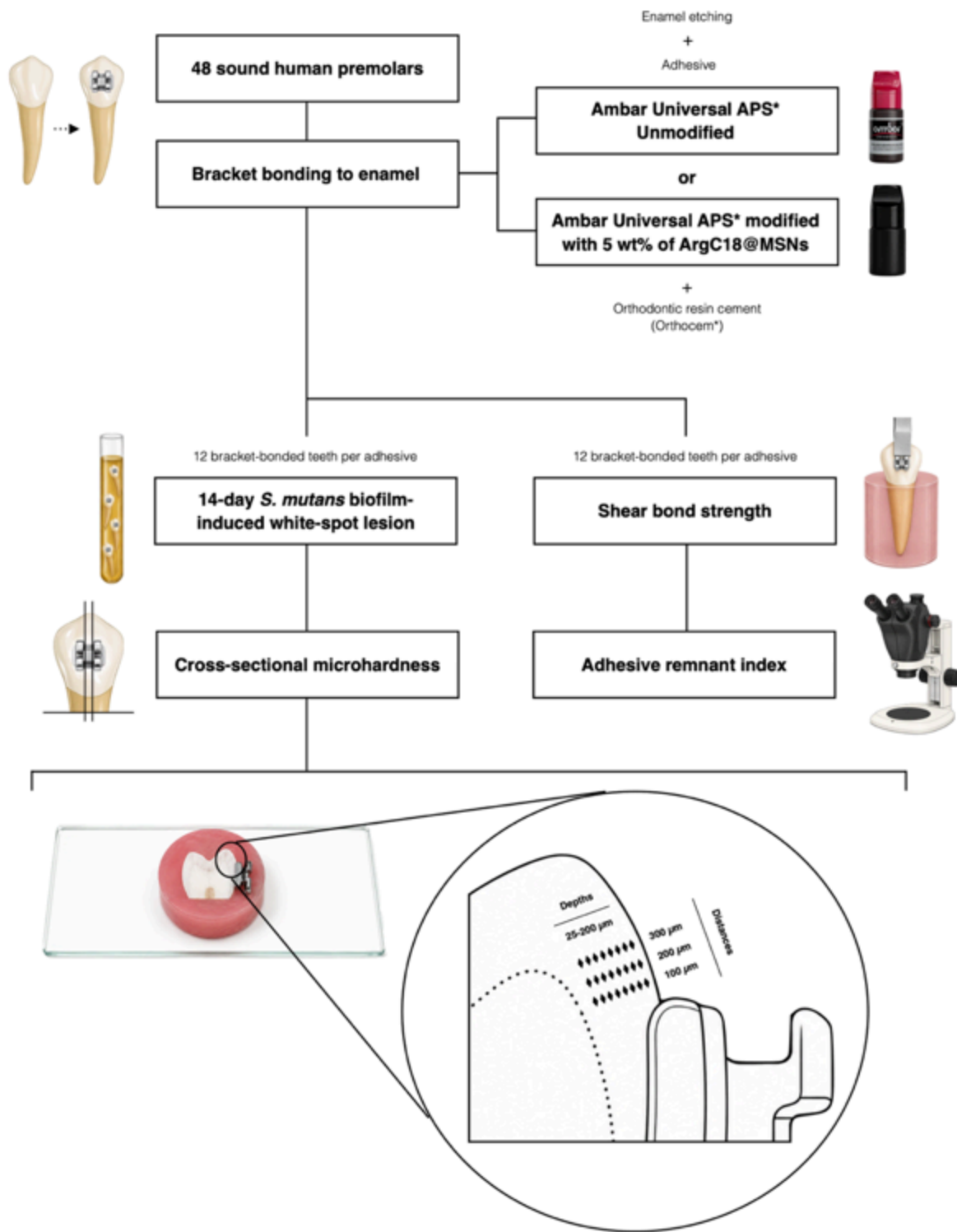


**Figure 1**

Schematic representation of the experimental workflow and specimen allocation.

\* FGM Dental Group, Joinville, SC, Brazil.

ArgC18@MSNs: L-arginine-containing mesoporous silica nanoparticles.

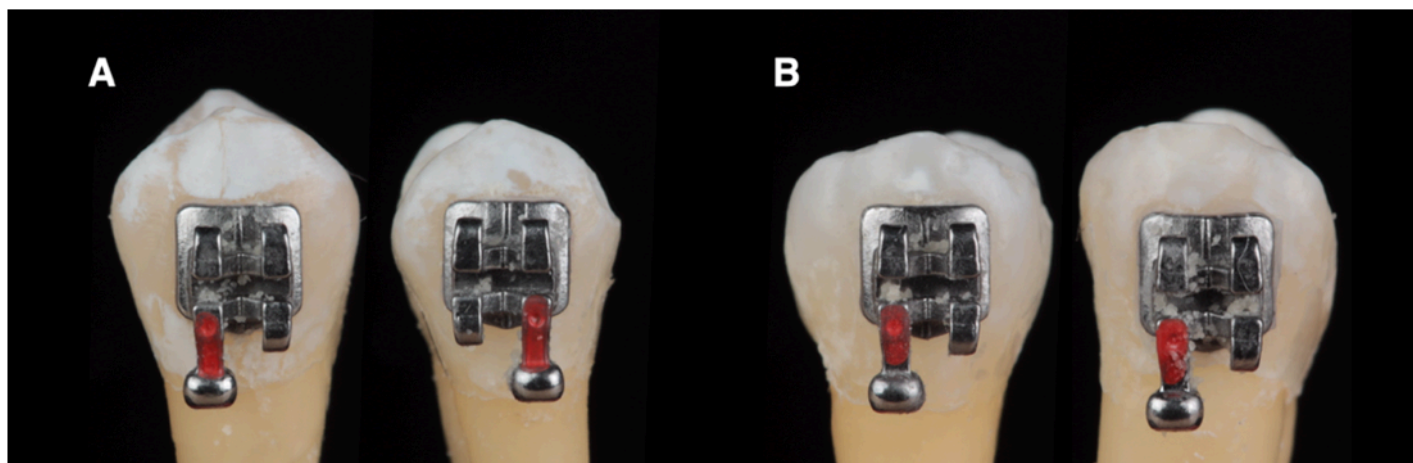


**Figure 2**

Representative images of WSLs formed after a 14-day *S. mutans* biofilm challenge for each adhesive group (A: unmodified Ambar Universal APS adhesive [AU; FGM Dental Group]; B: AU loaded with 5 wt% L-arginine-containing mesoporous silica nanoparticles [ArgC18@MSNs]).

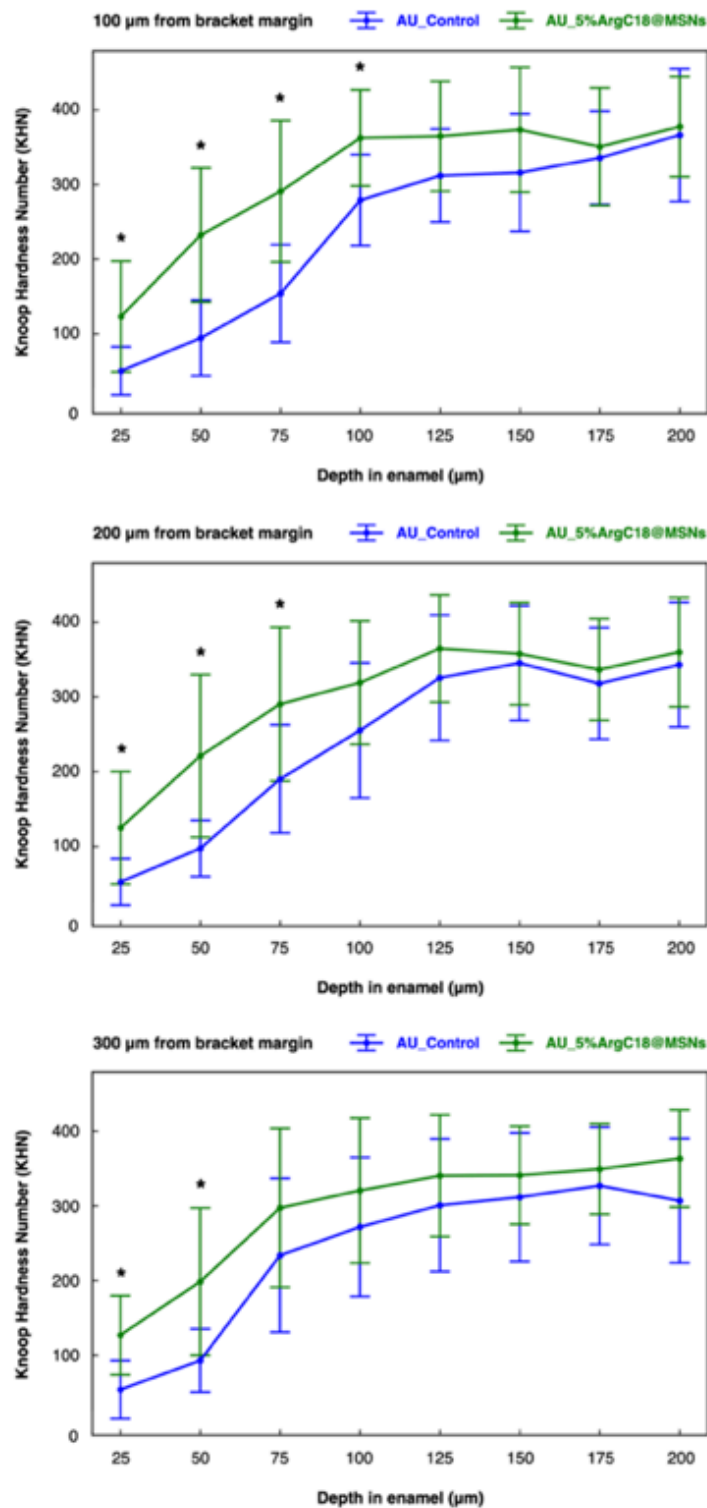


**Figure 3**

Knoop Hardness Number (KHN) values ( $\text{kgf/mm}^2$ ) as a function of enamel depth (25–200  $\mu\text{m}$ ) at three distances from the bracket margin toward the occlusal direction (100, 200, and 300  $\mu\text{m}$ ) for each adhesive group. Mean values are shown with error bars representing standard deviations, and exact numerical values are reported in Table 2. Asterisks indicate statistically significant differences between adhesive groups at each enamel depth (independent samples *t*-test,  $p < 0.05$ ).

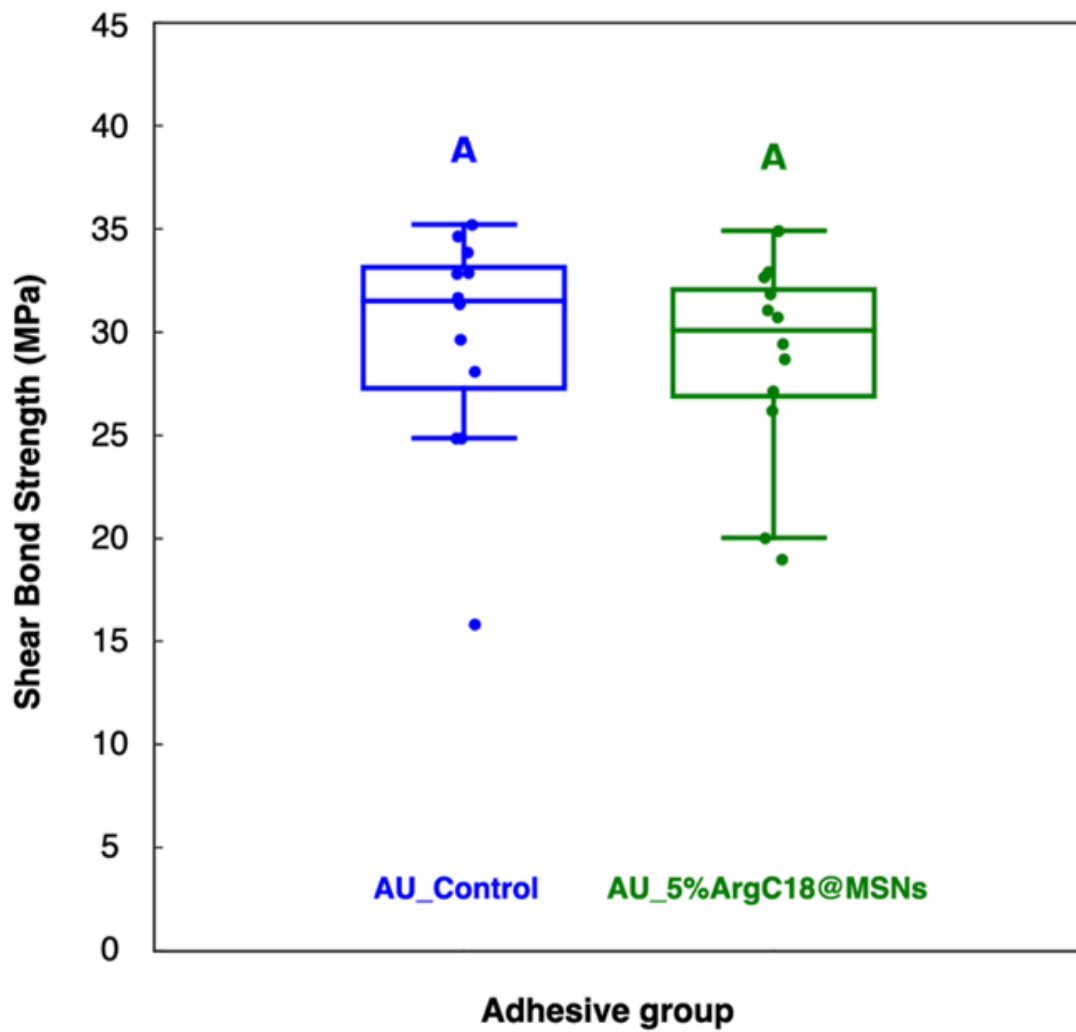


Figure 4

Box-and-whisker plot of shear bond strength values in MPa for each adhesive group. Identical uppercase letters indicate no statistically significant differences between adhesive groups (Mann–Whitney  $U$  test;  $p=0.50$ ).

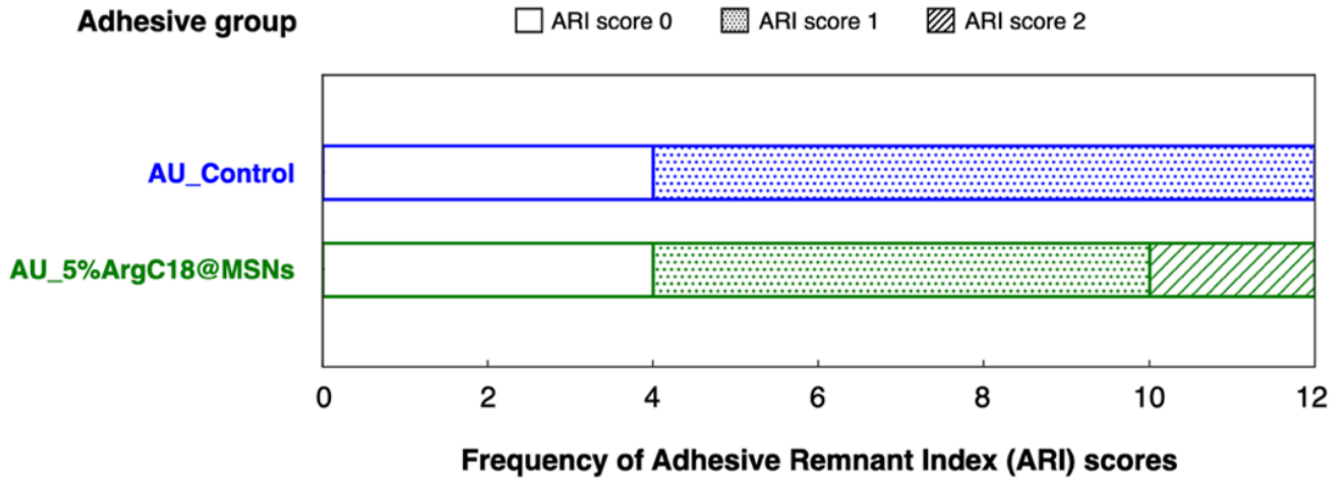


**Figure 5**

Absolute frequency distribution of Adhesive Remnant Index (ARI) scores for each adhesive group after bracket debonding.

ARI scores: 0 (no adhesive remaining on the tooth surface), 1 (less than half of the adhesive remaining on the tooth surface), 2 (more than half of the adhesive remaining on the tooth surface), and 3 (all adhesive remaining on the tooth surface).

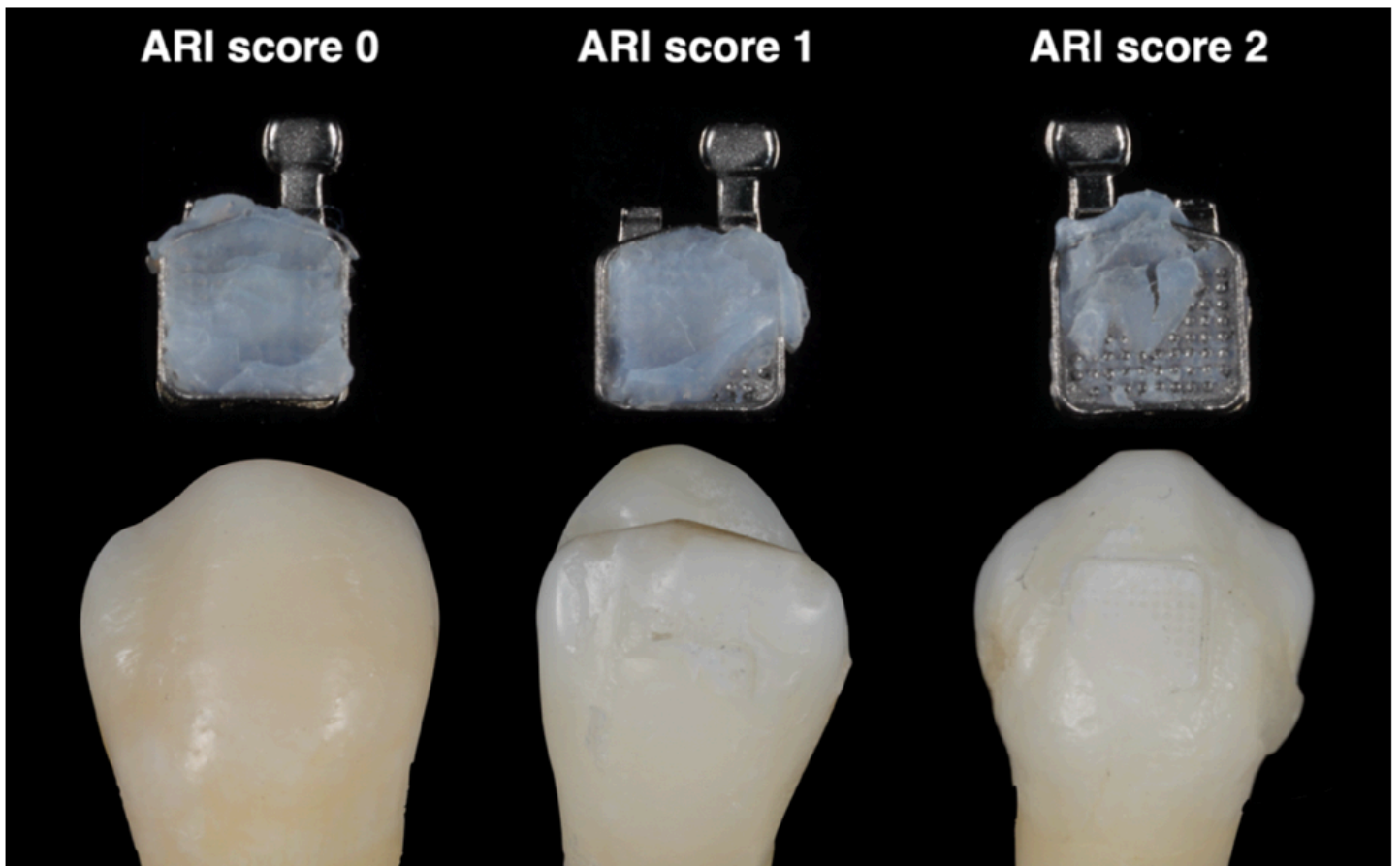


**Figure 6**

Representative images of Adhesive Remnant Index (ARI) scores observed in the study (scores 0, 1, and 2). ARI score 3 was not observed in any specimen.

ARI scores: 0 (no adhesive remaining on the tooth surface), 1 (less than half of the adhesive remaining on the tooth surface), 2 (more than half of the adhesive remaining on the tooth surface), and 3 (all adhesive remaining on the tooth surface).