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Dental Materials

Effect of adding arginine at different concentrations to experimental orthodontic resins: an *in vitro* study

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Abstract: The aim of this study was to assess the effect of adding arginine at different concentrations to commercial and experimental orthodontic resins on shear bond strength (SBS), as well as on the antimicrobial activity of arginine against . Metal brackets were bonded onto the surface of 120 bovine incisors using Transbond, OrthoCem, and an experimental resin (ER), adding 0, 2.5, 5, and 7 wt.% of arginine. The SBS test was performed in deionized water at 37 ºC for 24 h, at 0.5 mm/min. SBS test results were subjected to two-way ANOVA and Tukey's test ($\alpha = 0.05$). CFU/mL data (antimicrobial assessment) were assessed by Kruskal-Wallis and Dunn's tests ($\alpha = 0.05$). No statistical difference between the resins was observed in untreated groups ($p > 0.05$). The addition of arginine at 2.5% (27.7 MPa) and 5% (29.0 MPa) increased the SBS of Transbond when compared ($p < 0.05$) to OrthoCem (18.5 and 15.6 MPa, respectively) and ER (16.3 and 18.1 MPa, respectively). Arginine at 7% improved the SBS of Transbond (24.1 MPa) and ER (21.0 MPa), which was statistically higher ($p < 0.05$) than OrthoCem (12.6 MPa). OrthoCem did not show a statistically

addition of arginine to resins reduced the count of ($p < 0.05$).

reduced CFU/mL. The addition of arginine did not interfere with the bond strength and demonstrated antibacterial activity against S. .

Keywords: Orthodontics; Composite resins; Arginine; Mechanical tests; .

Introduction

Dental caries is one of the most common oral diseases[1-3] and it is caused

poor oral hygiene conditions.[1,4,5] Another very common problem in the oral cavity is malocclusion, whose recommended treatment consists in bonding orthodontic brackets to the enamel to correct malpositioned teeth.[6] Food remains get trapped around the wires and accessories of orthodontic

the tooth structure and changes in the properties of saliva and microbial count.[7-14] One of the side effects of orthodontic treatment is the development of white spot lesions, with a reported incidence of 32% to 72.9%,[10] found

more frequently at the cervical third and on the margins around the brackets.[10,15]

In addition to the orthodontic appliance and its accessories, the resin used for bonding the brackets may be a predisposing factor for caries, as adhesive failure and [11,15,16]

Note that the ideal orthodontic bonding material should have a minimum bond strength from 5.9 to 7.8 MPa so it can withstand masticatory forces and the forces applied during the treatment.[14,17,18]

The well-structured organization of the oral biofilm hinders the development of an efficient therapy for dental caries control.[5,18] The use of

demineralization.[6,13,14,18,19] Nevertheless, using dentifrice,

patient collaboration and constant reapplication. Another preventive alternative, which does not rely on patient collaboration, is using bonding agents with [13,14]

The addition of antibacterial properties to orthodontic bonding agents is another way to reduce the development of white spot lesions.[8,9,11,18] Arginine, increasingly used in dentistry nowadays, is an amino acid present in foods and naturally found in the saliva. It is hydrolyzed by arginine deiminase (ADI), generating ammonia.[2-4,6,20-23] Ammonia production inhibits dental demineralization, neutralizes acids, and positively affects the development of bacterial ecology and the pathogenicity of the oral microbiota.[4]

pH homeostasis.[1,2,4,21-24] Thus, arginine can be used for the prevention and treatment of caries.[4]

To the best of our knowledge, only one study has assessed the addition of arginine to orthodontic resins[6] and no study has evaluated the addition of arginine at different concentrations. The necessity for developing antibacterial orthodontic resins combined with the thriving outcomes of arginine addition requires further studies to verify whether an increase in arginine concentration will reduce microbial count and whether the mechanical properties of the bonding materials will be affected.

Accordingly, the aim of the present study was to assess the effect of adding arginine at concentrations of 2.5%, 5%, and 7% to commercial and experimental orthodontic resins on bond strength and antimicrobial activity. The null hypotheses stated that: a) The addition of arginine at different concentrations would not interfere with the mechanical properties of the materials; b) The addition of arginine at different concentrations would not interfere with antimicrobial activity.

Methods

The present study was approved by the local Research Ethics Committee, process no. 1038/2020.

Study design

The present study consisted of 12 groups: G1 – Transbond XT (TXT) commercial resin without arginine addition; G2 – OrthoCem (O) commercial resin without arginine addition; G3 – experimental resin (ER) without arginine addition; G4 – TXT with 2.5 wt.% of arginine; G5 – O with 2.5 wt.% of arginine; G6 – ER with 2.5 wt.% of arginine; G7 – TXT with 5 wt.% of arginine; G8 – O with 5 wt.% of arginine; G9 – ER with 5 wt.% of arginine; G10 – TXT with 7 wt.% of arginine; G11 – O with 7 wt.% of arginine, and G12 – ER with 7 wt.% of arginine, all of which were assessed as to their mechanical properties and antimicrobial activity (Figure 1).

Manipulation of experimental and commercial orthodontic resins

The experimental resin (ER) used in this study was composed of bisphenol-A-glycidyl methacrylate monomers (Bis-GMA; Sigma-Aldrich Inc., St. Louis, USA) mixed with triethylene glycol dimethacrylate (TEGDMA; Sigma-Aldrich Inc., St. Louis, USA) in a 70:30 ratio (wt.%). The photoactivation system was composed of ethyl-4-(dimethylamino) benzoate (EDAB; Sigma-Aldrich) and camphorquinone (CQ; Sigma-Aldrich Inc., St. Louis, USA) at a concentration of 1 wt.% each and 0.1 wt.% BHI.[25] Twenty-weight percent

0.04 μm) were added to the mixture (Nippon Aerosil Co. Ltd., Yokkaichi, Tokyo, Japan). The commercial orthodontic resins used were Transbond XT (3M ESPE, St Paul, USA) and OrthoCem (FGM, Joinville, Santa Catarina, Brazil) (Table 1).

The total volume of each syringe material was weighed (AG 200 - GEHAKA, Ind. e Com. Eletro-Eletronica Gehaka Ltda., Sao Paulo, Brazil) under orange light and stored in dark vials to avoid its exposure to light. Thereafter, different concentrations (2.5%, 5.0%, and 7.0%) of arginine (Sigma-Aldrich Inc., St. Louis, USA) were added to commercial and experimental orthodontic resins and mechanically mixed in a centrifuge (DAC 150 Speed Mixer; Flacktek, Landrum, USA) at 3,000 rpm for 2 min, maintaining the temperature below 37°C.

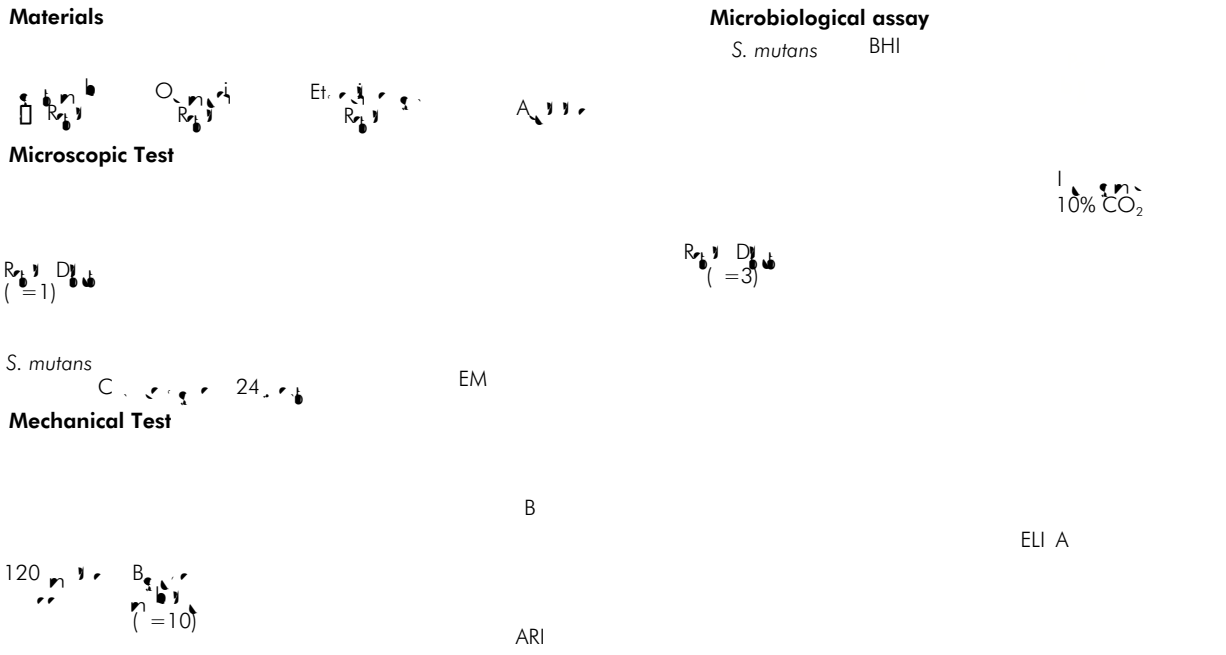

Figure 1. Experimental design.

Table 1. Materials used and application methods.

Bonding material	Composition (% weight)	Application method
Transbond TXT*	Primer: Bis-GMA and TEGDMA (45-55%), 4 – (Dimethylamino) -benzeneethanol (< 0.5%). Resin: Bis-GMA (10-20%), Bisphenol A-dimethacrylate (5-10%), silane-treated quartz (70-80%), silane-treated silica (< 2%), diphenyliodonium hexafluorophosphate (< 1%), triphenylantimony (< 1%).	Active primer application for 10 s, resin application and light curing for 10 s on each side of the support.
Orthocem**	Bis-GMA, TEGDMA, methacrylic phosphate monomers, camphorquinone, tertiary amine, silicon dioxide	Application of two layers of Single Bond 2 adhesive and light curing for 10 s. Application of the resin and light curing for 10 s on each side of the support.
Experimental Resin	Bis-GMA, TEGDMA, EDAB, camphorquinone, BHI, colloidal silica.	Application of two layers of Single Bond 2 adhesive and light curing for 10 s. Application of the experimental resin and light curing for 10 s on each side of the support.
Single Bond 2 Adhesive*	Bis-GMA (10-20%), HEMA (5-15%), UDMA (< 5%), Copolymer of acrylic and Itaconic acids (5-10%), ethyl alcohol (25-35%), water (< 5%), glycerol 1,3-dimethacrylate (5-10%), diphenyliodonium hexafluorophosphate (< 0.5%), silane-treated silica (10-20%), EDMAB.	Application of two layers of Single Bond 2 adhesive, air blasting, and light curing for 10 s.

*Information from Safety Data Sheets; **Manufacturer's information.

Mechanical test

Selection and preparation of teeth and orthodontic brackets bonding

For the adhesive analysis, 120 bovine central incisors were extracted, cleaned, and stored in 0.1% thymol aqueous solution to inhibit bacterial growth at room temperature. The teeth were placed in a PVC cylinder with chemically activated Jet® acrylic resin (Clássico, Sao Paulo, Brazil), with the buccal surface perpendicular to the horizontal axis, and then assigned randomly to 12 groups (n = 10), as described earlier, according to the resins used for bracket bonding. Before bonding, the buccal surfaces of bovine teeth

the same conditions for 18 h. After growth in 2-mL lidded tubes containing the resin discs (n = 6), 1 mL

($p < 0.05$). No statistical difference was observed between arginine concentrations of 7%, 5%, and 2.5% and between the concentrations of 5% and 2.5% and the untreated groups ($p > 0.05$). SBS for the 5%

TXT than for the untreated groups ($p < 0.05$). No statistical difference was observed between the 5%, 7%, and 2.5% concentrations of arginine and between the 7% and 2.5% concentrations and the untreated groups ($p > 0.05$). As for the Ortho resin, there was no statistical difference between the three concentrations of arginine and the untreated groups ($p > 0.05$).

predominant for TXT and ER and score 1 for Ortho without adding arginine. Among the groups with the addition of 2.5% and 7% arginine, score 0 was observed for TXT and ER and score 1 for Ortho. In the groups treated with 5% arginine, score 1 was predominant in Ortho and TXT, whereas scores 0 and 3 were observed for ER.

Chemical and microbiological analyses – *CFU/mL*

The addition of arginine reduced the CFU/mL of S. in all assessed resins (Figure 3). The

lower with the addition of 7% arginine compared to the Ortho resin without the addition of arginine ($p < 0.05$). No statistical difference was observed for arginine concentrations of 0%, 2.5%, and 5.0%. There was no significant difference between the Ortho resin and the Ortho resin after addition of arginine at different concentrations ($p > 0.05$). The addition of

CFU/mL when compared with the 0%, 2.5%, and 5% concentrations ($p < 0.05$). No statistical difference was observed between TXT without arginine and TXT with the addition of 2.5% and 5.0% of arginine ($p < 0.05$). All the concentrations of arginine added

CFU/mL ($p < 0.05$).

Surface morphology (SEM)

The initial morphology of the specimens (Figure 4)

are described in Figure 5. The initial morphological analysis, without adhesion, showed penetration of arginine at different concentrations, regardless of the type of bonding resin (Figure 4). In the morphological analysis after adhesion, there was a reduction in the count of bacteria adhered to the surface when compared to resins without the addition of arginine. Apparently, there was an increase in the count of

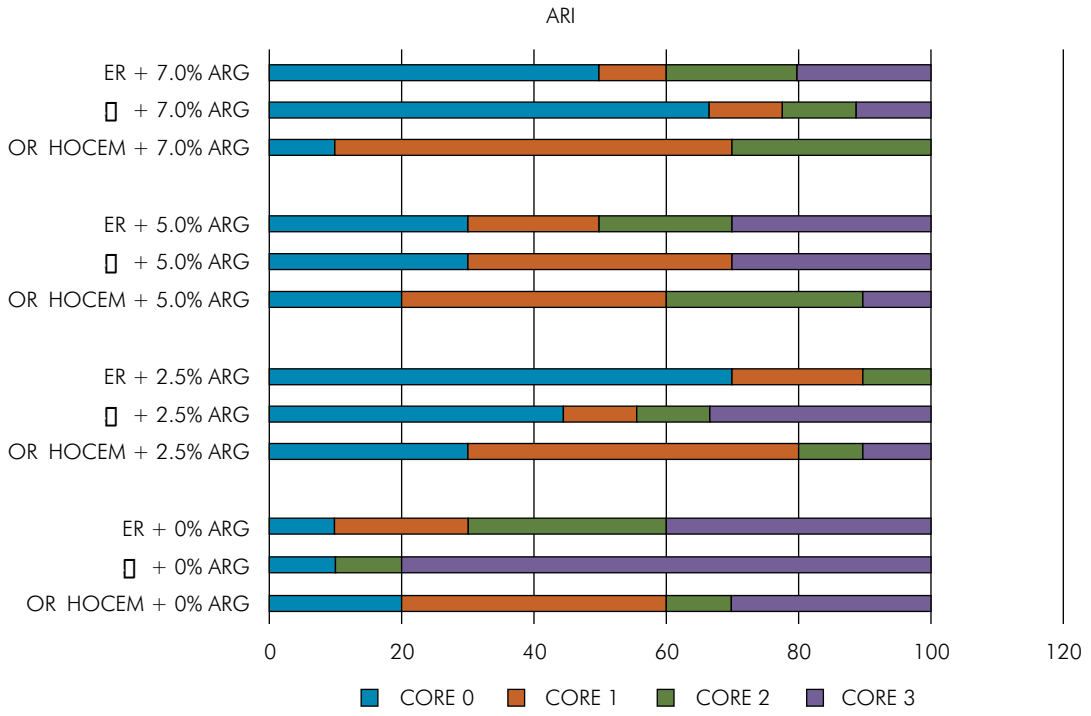

Figure 2. Adhesive remnant indices (ARIs).

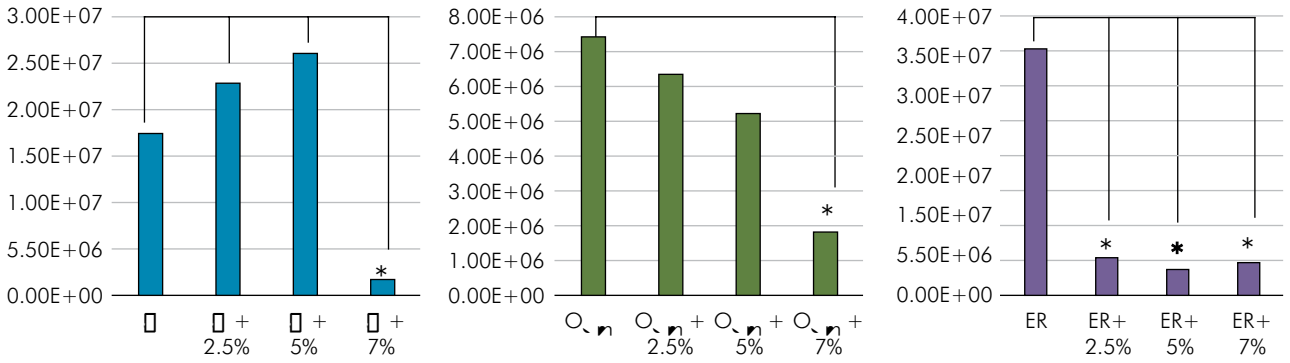

Figure 3. CFU/mL. The asterisk indicates statistical difference.

groups, the SBS found in the present study ranged from 12.6 to 29.0 MPa. It has been recommended in the literature that orthodontic adhesives have bond strengths between 5.9 and 7.8 MPa to allow retention of the bracket throughout the duration of the treatment while also allowing for of its easier removal.[14,17,18]

In general, the highest SBS in the present study was that of TXT with or without arginine, in line [6] This could be explained by the presence of a higher percentage of filler particles in TXT (70% of silanized inorganic silica) when compared to Ortho (48% load) and ER (20%

strengthen the orthodontic resin matrix. The lowest percentages of ARI with scores 1 and 2 for TXT, with adhesive failure, when compared to Ortho and ER,

In the untreated groups, there was no statistical

the present study are at odds with those of a previous

than that of Ortho.[6] This could probably be explained

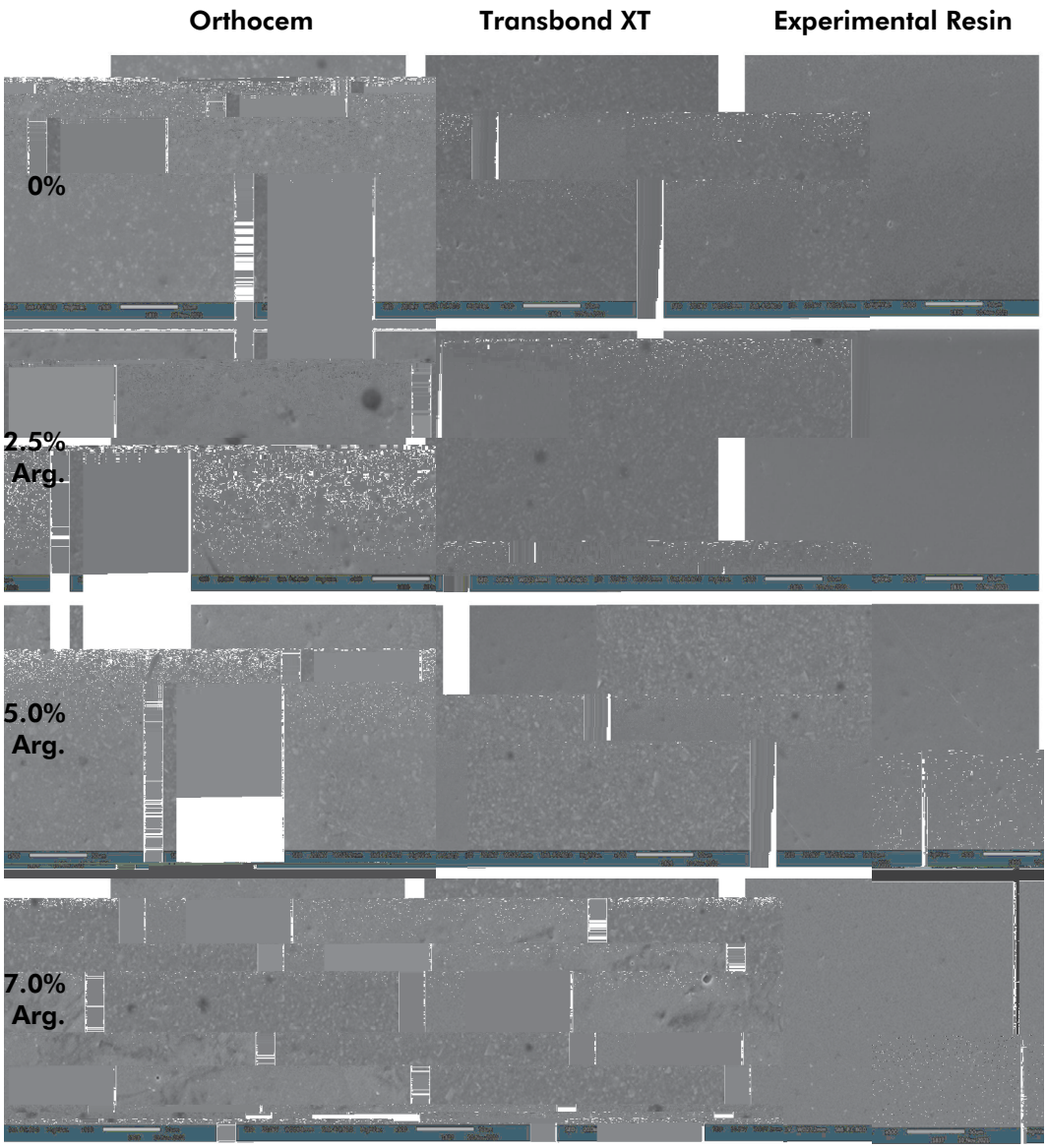

Figure 4. Initial surface morphology of the tested groups at 500x magnification.

by the application of Single Bond 2 adhesive prior to bracket bonding with Ortho and ER resins, which had a positive impact on the bond strength of these resins, as no statistical difference in SBS was found between these resins and TXT, although the literature shows that the use of adhesive prior to orthodontic [27]

Applying the adhesive before the orthodontic adhesive improved penetration into the enamel micropores produced by acid etching, favoring the retention of the bracket to the tooth enamel.[28] However, adding arginine to TXT at 2.5% and 5% showed statistically higher SBS than that of Ortho and ER resins at 2.5% and 5% of arginine. It can be inferred that the addition of arginine might have increased the viscosity of the assessed resins, reducing their penetration into the micropores created by the dissolution of the interrod enamel caused by acid etching.[29] On the other hand, as the primer of TXT was not light-cured before resin bonding, this might have led to a greater interaction between the primer and the resin, allowing the set to

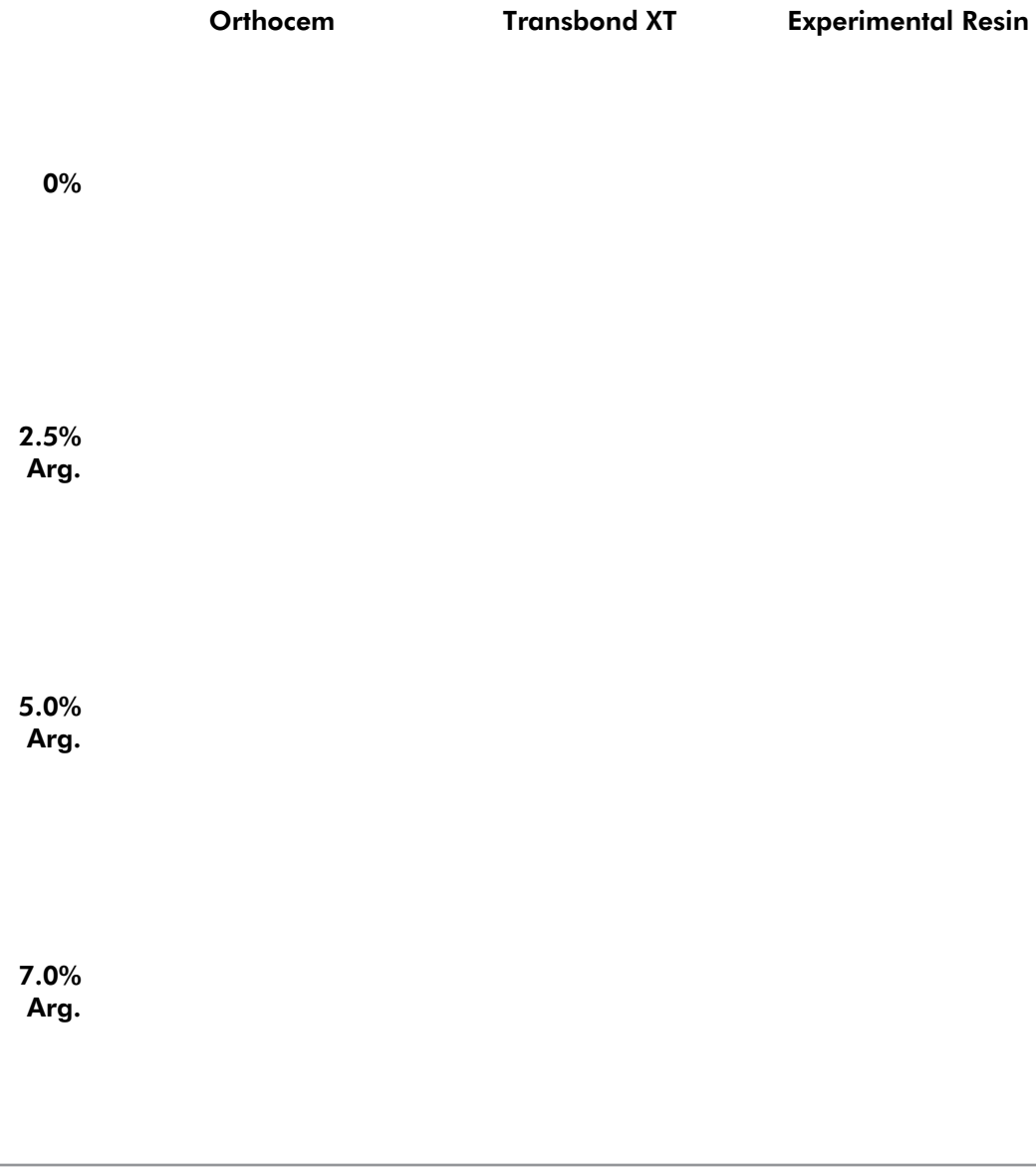

Figure 5. Surface morphology of the different tested groups after adhesion of S. *mutans* at 1.000x magnification.

In the case of Ortho and ER, the Single Bond 2 adhesive system was applied prior to bonding and light-cured before the placement of the resin/bracket set; therefore, the micropores might have not allow the resin to penetrate deeper, increasing its viscosity after the addition of arginine. Moreover, it is widely known that the viscosity of the adhesive is larger than that of the primer, allowing the primer to penetrate the micropores, which does not occur with the Single Bond 2 adhesive system. This is in line with [9] which demonstrated that when of resin used as a primer prior to the application of the orthodontic resin can improve the interlocking between the resin and the enamel. Furthermore, as mentioned earlier, Ortho and ER have a lower the present study (Figure 2).

ARI has been used to show where and in what quantity orthodontic adhesive was retained after debonding. In orthodontics, the site of the bonding failure after the removal of an orthodontic bracket

is important because it is necessary to treat the solid and intact enamel surface.[17] In the groups with the addition of 5% arginine, there was a predominance of score 3 for TXT and ER, with adhesive failure at the interface between the bracket and the bonding material. The score of Ortho resin, even after the addition of arginine at different concentrations, was 1, with failures at the interface between the bonding materials and the enamel. The other resins with the addition of arginine showed a larger variation in their scores, but most had scores lower than 3. A way to minimize the risk of enamel fracture during the removal of the orthodontic bracket would be a failure at the bracket/orthodontic adhesive interface or cohesively in the orthodontic adhesive than at the bonding material/enamel interface since the adhesive residues could be removed with suitable manual or rotary instruments, in a safer way. A smaller amount of bonding material on the tooth surface indicates a shorter length of treatment.[17]

The second hypothesis – that the addition of arginine would not interfere with antimicrobial

adding arginine to the orthodontic resins provided antimicrobial activity by reducing the growth of . For the Ortho and TXT resins, only the addition of 7% arginine demonstrated a statistically when compared to the resin without the addition of arginine. As for ER, adding 2.5% arginine was enough for a statistically when compared to ER without the addition of arginine.

those of a previous study,[6] given that adding 2.5% arginine to TXT did not statistically influence CFU/mL. On the other hand, the addition of 2.5%

of the present study. It can be assumed that this discrepancy might have occurred because of the difference in the method used for counting the CFU/mL. In this study, the bacterial count subtracted the supernatant from the adhered material. Importantly, the increase in CFU/mL in TXT with the addition of 5% arginine might have occurred because of the antimicrobial agent (arginine), which strengthened the virulence of the strain and activated resistance genes that boosted growth,[30] but the addition of 5% arginine to TXT did not demonstrate statistical difference from TXT with and without the addition of 2.5% arginine.

that the addition of arginine allowed reducing the growth of (Figure 5) However, the increase in the number of bacteria adhered to the specimens with the addition of 2.5% arginine to the Ortho resin and 5% and 7% arginine to TXT and ER can be evaluated by the live-dead assay, which indicates the number of live bacteria on surface morphology by green staining and the number of dead bacteria by reddish staining. This

of on the surfaces of the analyzed resins. Arginine, with proven antimicrobial activity, has been widely used in dentistry (dentifrices, chews, and mouthwashes) in recent years.[4] It neutralizes acids and modulates the pH homeostasis in the oral

down its structure.[1, 5,6,16,22-24]

Previous studies have suggested the presence of arginine in the oral cavity can influence the adhesion of to the tooth surface, as a denser

without arginine.[22,24] Another study has shown that treatment with 2.5% arginine can inhibit the growth of

growth, whereas 5% and 10% arginine have more remarkable inhibition of planktonic growth and biofilm accumulation of this species.[2] Moreover, Kolderman et al.[31] demonstrated that L-arginine monohydrochloride inhibits bacterial growth to some

on its concentration. Geraldeli et al.[16] assessed different adhesive systems by adding arginine at different concentrations (5%, 7%, and 10%) and noted that dentin hybridization occurred properly with the addition

accumulation in the presence of an adhesive with 7%

present study. They also observed that arginine was released at 30 days, but such release had decreased

Of note, over 50% of orthodontic treatment patients had white spot lesions, even after receiving complementary treatment with mouthwashes and fluoride varnishes.[6,11] Accordingly, some recent

as to allow an antimicrobial activity on orthodontic bonding resins without hindering their mechanical properties, adding different materials such as silver-doped hydroxyapatite nanoparticles;[8] titanium dioxide nanoparticles;[15] boron nitrate, and alkyl trimethyl ammonium bromide;[9] methacrylate or methacrylamide monomers containing quaternary [18] propolis powder;[11] and 2.5% arginine to commercial resins.[6]

Therefore, the findings of the present study seem to be very promising. The ER with addition of arginine at different concentrations used in this study and the commercial resins with 7% arginine showed antimicrobial activity without interfering with adhesive properties, indicating that arginine could be the new alternative for the prevention of white spot lesions and caries in orthodontic patients. Further studies are, however, needed to assess the effect of arginine on biofilm and its interference in the addition of experimental bonding resins, as well as its effects in the long term.

Conclusion

The addition of different concentrations of arginine to commercial and experimental resins had a positive impact on bond strength to enamel and reduced the growth of at the concentration of 7% in commercial resins and at all concentrations in the experimental resin; therefore, it may be an alternative for minimizing the development of white spot lesions without interfering with the mechanical properties of materials.

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