Comparative evaluation of eighth-generation bonding agent modified with 7% arginine and 0.12% chitosan for antibacterial property and microtensile bond strength

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Abstract

Aim: The aim of this study was to evaluate and compare eighth-generation bonding agent (G-Premio BOND) modified with 7% arginine and 0.12% chitosan for antibacterial property and microtensile bond strength.

Methods: To evaluate antibacterial property, the sterile 96-microtiter plate was taken. The sidewalls of nine wells were coated with 15 µl of adhesive from each group. Suspension of Streptococcus mutans bacteria was placed on each group of adhesive and antibacterial property was checked using Spectrophotometer. Thirty-three healthy extracted premolars were taken and adhesive from each group was applied on the exposed surface of dentin, and the specimens were tested using a universal testing machine at crosshead speed of 1 mm/min.

Results: The least antibacterial efficacy was seen with unmodified eighth-generation bonding agent. This result was statistically significant when all study groups were compared. Microtensile bond strength was evaluated and the highest mean value (5.07) was seen with adhesive modified with 7% arginine, followed by adhesive modified with 0.12% chitosan (mean value: 4.14), and unmodified adhesive had the lowest mean bond strength value (4.07).

Conclusions: The eighth-generation bonding agent modified with 7% arginine and 0.12% chitosan showed antibacterial efficacy against S. mutans. In addition to this, they also had higher tensile bond strength values as compared to unmodified adhesive.

Keywords: Adhesive; antibacterial property; arginine; chitosan; microtensile bond strength

INTRODUCTION

Dental caries is a global disease. There are various materials used for restoration of cavities such as dental amalgam, GIC, RMGIC, and composite resins. Secondary caries remains one of the main reasons for failure of dental composite restorations, and replacement of the failed restorations accounts for up to 75% of the operative work.1

Adhesive resins are designed to provide strong coupling between resin composites and enamel and dentin. In light of minimal-invasive dentistry, this new approach promotes a more conservative cavity design, which relies on the effectiveness of current enamel-dentine adhesives.2
In 2010, America introduced VOCO Futurabond DC as eighth-generation bonding agent, which contains nanosized fillers. In the new agents, the addition of nano-fillers with an average particle size of 12 nm increases the penetration of resin monomers and the hybrid layer thickness, which in turn improves the mechanical properties of the bonding systems.[3]

The inherent biodegradation of the interface between the tooth and the adhesive layer produces crevices that are readily colonized by caries pathogens such as Streptococcus mutans. Those crevices are also derived from polymerization shrinkage and improper resin-based composite layering. Although it is unlikely that biofilms can be eliminated from the crevices, the engineering of novel dentin adhesives that can shift the microbial ecology from a disease to a health state is greatly desirable. Addition of composite resin with antibacterial agents like chlorhexidine (CHX) has been documented in literature.[1]

This study attempts to modify the adhesive with arginine and chitosan and evaluate the antibacterial property against S. mutans. The addition of arginine and chitosan may alter the adhesive properties like bond strength of composite resin to tooth substrate. Hence, this research also attempts to evaluate the antibacterial properties of modified adhesive and microtensile bond strength of composite resin using eighth-generation bonding agent with or without modification with 7% arginine and 0.12% chitosan.

METHODS

Study groups were as follows:

1. Group I – Eighth-generation bonding agent
   - Group IA – Antibacterial property of adhesive (n = 9)
   - Group IB – Microtensile bond strength of adhesive (n = 11)
2. Group II – Eighth-generation bonding agent modified with 7% arginine
   - Group IIA – Antibacterial property of modified adhesive (n = 9)
   - Group IIB – Microtensile bond strength of modified adhesive (n = 11)
3. Group III – Eighth-generation bonding agent modified with 0.12% chitosan
   - Group IIIA – Antibacterial property of modified adhesive (n = 9)
   - Group IIIB – Microtensile bond strength of modified adhesive (n = 11).

Adhesive formulation

1. G-Premio BOND (eighth-generation adhesive) was taken; this was considered the control group (Group I)
2. Arginine-modified adhesive (Group II)
   - 7% of L-arginine was added to the adhesive
   - Experimental adhesive was prepared in a dark room under controlled temperature and humidity
3. Chitosan-modified adhesive (Group III)
   - 0.12% (w/w) of chitosan was added to adhesive
   - Chitosan solution was prepared by dissolving chitosan powder in 1% (v/v) acetic acid as per the formula 1% = 1 g/10 ml.

To evaluate antibacterial property [Figure 1]

1. S. mutans was obtained from the Department of Microbiology, Dr. D Y Patil Medical College and Hospital, Pimpri
2. S. mutans was obtained aerobically from frozen stock cultures in brain–infusion broth containing 8 µg/ml of bacitracin for 48 h at 37°C before use
3. Antibacterial property was evaluated by using direct contact test (DCT)
4. The sterile 96-microtiter plate was held vertically, and the sidewalls of nine wells were coated with 15 µl of each adhesive (Group IA, Group IIA, and Group IIIA) without it flowing and wetting the bottom of the well
5. The experimental adhesive was light cured for 10 s using LED-curing device
6. The experimental adhesive in each well was light cured at the same distance from the light-curing device and without exposing the adjacent wells
7. A 10-µl suspension containing S. mutans bacteria and 235 µl of bacitracin was placed on the material, and the plates were incubated in vertical position for 1 h 100 at 37°C
8. The control consisting of one set of nine wells coated with unmodified adhesive (Group IA) containing equal volume of uninoculated fresh medium was taken
9. Absorbance at 650 nm (A650) was determined in each well by spectrophotometer set at 37°C to determine the maximum change in A650.

To evaluate microtensile bond strength

Inclusion criteria

1. Healthy premolars (maxillary and mandibular), which are indicated for extraction due to orthodontic treatment or periodontal condition
2. Single-rooted premolar.

Exclusion criteria

1. Carious teeth
2. Teeth with enamel or dentinal cracks
3. Hypoplastic teeth
4. Teeth with occlusal facets
5. Restored teeth
6. Teeth with abrasion, attrition, erosion, and abfraction
7. Fractured teeth.
Thirty-three healthy extracted premolars (as per inclusion criteria) were taken; each tooth was sectioned horizontally to exposed dentin.

Two consecutive coats of the adhesive (control/experimental) were applied on the dentin surface of the premolars by using a disposable brush, which was air-dried for 5 s and light cured for 10 s.

Following adhesive application, 4-mm thickness of composite resin was built up and light cured for 40 s.

After that, the specimens were stored in distilled water at 37°C, 100% humidity for 24 h before sectioning.

After 24 h storage in distilled water at 37°C, the bonded teeth were vertically sectioned into serial slabs and further into beams with cross-sectional area of 1 mm² using a low-speed diamond saw under water cooling.

Table 1: Post hoc test to assess the difference between the study groups

| Groups | Mean | Difference | Significance (P) |
|--------|------|------------|-----------------|
| IA     | 0.08 | 0.07       | 0.01*           |
| IIA    | 0.15 |            |                 |
| IA     | 0.08 | 0.08       | 0.007*          |
| IIIA   | 0.16 |            |                 |
| IIA    | 0.15 | 0.004      | 0.97            |
| IIIA   | 0.16 |            |                 |

*Significance at P<0.05. A significant difference was found between IA and IIA and IA and IIIA, but no significant difference was found between IIA and IIIA.

Table 2: Difference between study groups for microtensile bond strength

| Groups | n  | Mean | F    | Significance (P) |
|--------|----|------|------|-----------------|
| IB     | 11 | 4.07 | 0.979| 0.387           |
| IIB    | 11 | 5.07 |      |                 |
| IIB    | 11 | 4.14 |      |                 |

No significant difference was found between the study groups, concluding that IB, IIB, and IIB have similar microtensile bond strength.

RESULTS

According to the results of the present study, the mean value for antibacterial property of Group IA was 0.8, Group IIA was 0.15, and Group IIIA was 0.16. Maximum antibacterial efficacy against S. mutans was seen in Group IIIA (adhesive modified with 0.12% chitosan). This was followed by Group IIA (adhesive modified with 7% arginine). The least antibacterial efficacy was seen in Group IA which is an unmodified eighth-generation bonding agent. This result was statistically significant when all study groups were compared. However, there was no statistical difference between Groups IIA and IIIA. This result suggests that both 7% arginine and 0.2% chitosan had similar antibacterial action on S. mutans [Tables 1 and 2].

Microtensile bond strength was evaluated and the highest mean value (5.07) was seen in Group IIB, i.e., adhesive modified with 0.12% chitosan. This was followed by Group IIB which is adhesive modified with 0.1% arginine. The least antibacterial efficacy was seen in Group IA which is an unmodified eighth-generation bonding agent. This result was statistically significant when all study groups were compared. However, there was no statistical difference between Groups IIA and IIIA. This result suggests that both 7% arginine and 0.2% chitosan had similar antibacterial action on S. mutans [Tables 1 and 2].

DISCUSSION

Secondary caries is a significant challenge in restorative dentistry. Bacteria may remain trapped in the dental tissue during removal of carious substrate, since neither the clinical parameters of dentin hardness and color nor the caries-detector dyes are capable to ensure the complete removal of microorganisms. The inherent biodegradation of the interface between the tooth and the adhesive layer produces crevices that are readily colonized by caries.
pathogens such as S. mutans. Those crevices are also derived from polymerization shrinkage and improper resin-based composite layering. Although it is unlikely that biofilms can be eliminated from the crevices, the engineering of novel dentin adhesives that can shift the microbial ecology from a disease to a health state is greatly desirable.

According to the previous literature, 2% solution of CHX is bactericidal by precipitating cytoplasmic contents and leading to cell death. CHX is a popular antimicrobial agent and matrix metalloproteinase inhibitor.[4,5]

In the present study, the eighth-generation bonding agent (G-Premio BOND, GC) was modified with 7% arginine and 0.12% chitosan. The eighth-generation bonding agent (GC BRAND) uses nanosized fillers which increases the penetration of the resin monomers and the hybrid layer thickness, thus improving the mechanical properties of the adhesive. Other advantages include bonding with all substrates and being a dual-cure mode of polymerization.

Arginine entering the mouth can be metabolized by certain oral bacteria via the Arginine Deiminase Pathway (ADI Pathway) to produce ammonia, which neutralizes glycolytic acids and contributes to the pH rise of oral biofilms. Ammonia production via the ADS results in cytoplasmic and environmental pH increases and benefits oral bacteria in the following ways: (i) Protecting them against acid killing, (ii) providing bioenergetic advantages that include increasing ΔpH and synthesizing ATP, and (iii) maintaining a relatively neutral environmental pH that is less favorable for the outgrowth of a cariogenic microflora.[1] One of the suggested mechanisms of chitosan’s antibacterial characteristics is that the interaction between positively charged chitosan and a negatively charged bacterial cell could change the bacterial cell permeability, leading to the leakage of intercellular components and cell death.[6]

In this study, DCT was used to evaluate the antibacterial property of modified adhesives. DCT was introduced to determine the effect of direct contact between the tested materials and the test microorganism. The DCT is a quantitative method and provides information on the viability and bacterial growth rate.[6] The microtensile bond strength was checked to evaluate whether the modified adhesive hampers the original properties of adhesive or no.

The results of this study reject the null hypothesis since the incorporation of increasing concentration of 7% arginine and 0.12% chitosan enhanced the antibacterial activity of the adhesive resin. The addition of 7% arginine and 0.12% chitosan into a generic dental adhesive formulation did not compromise the physical and mechanical properties tested; however, the higher concentrations of arginine and chitosan solution (0.5% and 1% [w/v]) incorporated into the adhesive resin affected the adhesive properties.

**CONCLUSIONS**

Under the limitations of this study, the following conclusions can be drawn – the eighth-generation bonding agent modified with 7% arginine and 0.12% chitosan showed antibacterial efficacy against S. mutans. This was statistically significant when compared to unmodified adhesive. Eighth-generation bonding agent modified with 7% arginine and 0.12% chitosan presented similar antibacterial efficacy. The eighth-generation bonding agent modified with 7% arginine and 0.12% chitosan had higher tensile bond strength values as compared to unmodified adhesive; however, this was not statistically significant.

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**Conflicts of interest**
There are no conflicts of interest.

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