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Original Research Article

Comparative evaluation of various surface contaminants on the microleakage between 7^{th} and 8^{th} generation bonding agent: An in vitro study

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ABSTRACT

Aim: To evaluate, the effect of, various surface contaminants on the microleakage between 7^{th} Generation (G-Bond, GC) and 8^{th} Generation Bonding Agent (G-Premio, GC).
Materials and Methods: Ninety freshly extracted maxillary human premolars were collected for the study. They were randomly divided into two groups (n=45), Group 1-7^{th} Generation and Group 2- 8^{th} Generation. They were further subdivided into 3 sub-groups (n=15): a) Control b) Saliva c) Blood. A Class V cavity was prepared on the buccal surface. Samples of both the groups were applied with bonding agent and according to respective sub-group were contaminated with saliva and blood, before curing the bonding agent, then restored with Composite material. The samples were subjected to thermocycling and prepared for dye immersion. Samples were immersed in 2% Methylene Blue dye for 24 hours and later sectioned buccolingually. Each half of the buccolingually sectioned samples was observed under stereomicroscope under the power of 10x and 40x.
Statistical analysis used: Result was obtained using the Chi Square Test.
Results: In Group 1, minimum microleakage is observed in control group, and maximum in those contaminated with blood. In Group 2, maximum microleakage is observed in samples contaminated with blood and the microleakage in samples of control group and those contaminated with saliva is almost same and less than that observed in blood.
Conclusion: 8^{th} Generation shows better performance than 7^{th} Generation Bonding Agent.
Key Message: Contamination from blood and saliva, during bonding leads to a grave outcome of microleakage, during restoration with composite. But, with newer generation of bonding agents, it seems to be a ray of hope at the dawn of preventing microleakage to quite an extent.

1. Introduction

Since years, adhesion to the tooth structure has been a challenging concept.[1] The advent in inventions have developed techniques and modalities, which help in adhesion that reduces gap between tooth and restoration.[2] In 1955, Buonocore founded the modern adhesive dentistry by suggesting that acids altered the surface of enamel making it more receptive to adhesion.[1,3] However, acid etching was not that promising in dentin.[1] In past two decades, dentinal adhesives have shown a considerable improvement and development.[4]

Many classifications, for dentin bonding agents, have been proposed by various authorities.[5,6] Clinical success and longevity of the restoration is adversely affected if contaminants come in contact during any of the clinical procedural steps. Saliva, Blood and Gingival Crevicular fluid are the common contaminants which may affect the

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bonding to the tooth surface. Microleakage occurs due to contamination, which may cause unwanted sequelae like post-operative sensitivity and secondary caries.[7]

Improved physical properties and ease of operation are included in the evolved products[2,5] Nano dentistry has positively influenced restorative dentistry.[5] Nano sized fillers in nano composites and nano adhesives are a breakthrough due to nano dentistry.[5]

Recently the manufacturer of nanofilled dentin adhesives (G-premio, GC) has claimed it as 8^{th} generation bonding agent.

Thus, the purpose of this study is to compare the effect of contaminants (blood and saliva) on the microleakage between 7^{th} (G – Bond, GC) and 8^{th}(G-Premio, GC) generation bonding agent.

2. Materials and Methods

Ninety freshly extracted maxillary human premolars were collected for the study. The premolars with intact coronal tooth structure and without any structural defects like caries, attrition, abrasion, erosion or fracture were selected for the study. The samples were cleaned off debris and residual tissues with the help of an ultrasonic scaler and stored in the solution of 10% Formalin, until use.

All the samples were randomly divided into two groups, with 45 samples in each group.

Group 1: 7^{th} Generation Bonding Agent

Group 2- 8^{th} Generation Bonding Agent

The samples of each group were further subdivided as follows, with n=15 in each sub-group:-

Group 1 (7^{th} Generation Bonding Agent)	**Group 2 (8^{th} Generation Bonding Agent)**
a) Control	a) Control
b) Saliva	b) Saliva
c) Blood	c) Blood

For the ease of analysis, all the groups were renamed in continuation from 1 to 6 as follows:-

Group 1: 7th Generation Control

Group 2: 7th Generation Saliva

Group 3: 7th Generation Blood

Group 4: 8th Generation Control

Group 5: 8th Generation Saliva

Group 6: 8th Generation Blood

2.1. Tooth preparation

A Class V cavity was prepared on the buccal surface of each sample. The dimensions of the prepared cavity were as follows: 3mm width i.e. parallel to cementoenamel junction, 2mm height i.e. occluso-gingivally with 0.5 mm below CEJ and 1.5mm depth. The enamel margins were beveled (45°). After the tooth preparation was done, the cavity was thoroughly dried.

2.2. Collection of The Contaminants: Fresh contaminants were collected each time, before performing the procedure.

Saliva: Fresh, unstimulated human saliva was collected in a sterile beaker.

Blood: Fresh human blood was obtained by using a sterile lancet to prick the finger.

Self-Etch method was used for samples of all the groups.

2.3. Application of the Bonding Agent

After thoroughly shaking the bottle of the bonding agent, the bonding agent was dispensed in the dispensing dish. With the help of the disposable applicator tip, the bonding agent was applied to the Class V cavity prepared on the samples.

1. Group 1 and 4 (Control): According to the manufacturer's instructions, the cavity was left undisturbed for 5-10 seconds, and then dried for 5 seconds, under Maximum air pressure, in the presence of vacuum suction. Then it was light cured using LED curing light (700mW/cm^2) for 10 seconds, at a distance of less than 10mm.
2. Group 2 and 5 (Saliva): Before curing, the samples were contaminated with Fresh Collected Unstimulated Human Saliva, using a separate disposable applicator tip. According to the manufacturer's instructions, the cavity was left undisturbed for 5-10 seconds, and then dried for 5 seconds, under Maximum air pressure, in the presence of vacuum suction. Then it was light cured using LED curing light (700mW/cm^2) for 10 seconds, at a distance of less than 10mm.
3. Group 3 and 6 (Blood): Before curing, the samples were contaminated with Fresh Pricked Human Blood, using a separate disposable applicator tip. According to the manufacturer's instructions, the cavity was left undisturbed for 5-10 seconds, and then dried for 5 seconds, under Maximum air pressure, in the presence of vacuum suction. Then it was light cured using LED curing light (700mW/cm^2) for 10 seconds, at a distance of less than 10mm.

2.4. Restoration of the cavity

All the samples, after application of bonding agent, were then restored with Composite restorative material (Solare X, GC). The final restoration was finished and polished.

1. The samples were then immersed in distilled water for 24 hours, at room temperature.
2. After 24 hours, the samples were subjected to 500 cycles of thermocycling with the temperature range of 55°C ±5 to 5°C ±.

2.5. Preparation of the samples for dye immersion

The apical tip of all the samples were sealed with the help of the modelling wax. The entire tooth surface, except the restored cavity and margins of 1 mm surrounding it, was coated with nail varnish and allowed to dry.

1. The samples were then immersed in 2% Methylene Blue dye for 24 hours.
2. After 24 hours the samples were removed from the dye solution and then rinsed under running water.

2.6. Preparation of the samples for observation

The samples were sectioned buccolingually, using diamond sectioning disk, to observe the dye penetration at the occlusal and the gingival surface of the samples. Each half of the buccolingually sectioned samples was observed under stereomicroscope under the power of 10x and 40x.

2.7. Scoring criteria:[8]

0 = No dye penetration
1 = Dye penetration up to, but not beyond $\frac{1}{2}$ to occlusal or gingival wall.
2 = Dye penetration up to, but not contacting the axial wall.
3 = Dye penetration along the axial wall

3. Results

The observations obtained were subjected to the statistical analysis, using the Chi Square Test. The Pearson Chi Square ratio (P-value) was calculated and value ≤ 0.05 was considered to be statistically significant. The results obtained were as follows:

1. P-value for occlusal surface= 0.044
2. P-value for gingival surface= 0.005

Thus, the results obtained were statistically significant as P$\leq$ 0.05.

In 7^{th} Generation, minimum microleakage is observed in group 1, and maximum is observed in group 3. Amongst 8^{th} Generation, maximum microleakage is observed in 6 and the amount of microleakage in samples of 4 and 5 is almost same and less than that observed in blood. (Figures 1 and 2)

3.1. Occlusal surface

Score 0 (Minimum Microleakage) is seen in maximum samples of Group 1, Group 4 and Group 5.

Score 2 (Maximum Microleakage) is seen in maximum samples of Group 4.

Overall, the graph depicts less microleakage seen at the occlusal surface in the samples of Group 4, 5 and 6 than the samples of Group 1, 2 and 3, even in presence of contaminants like Blood and Saliva.

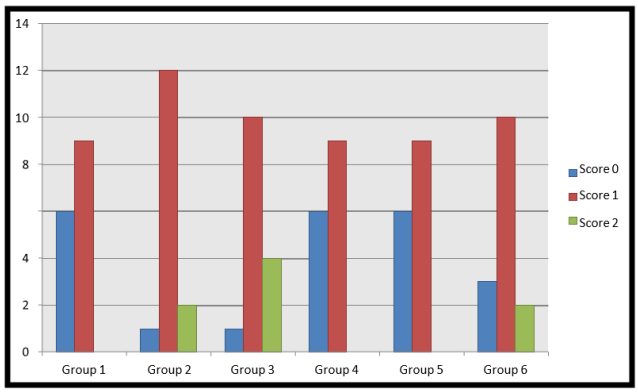

Fig. 1: Graphical presentation of result at occlusal surface

3.2. Gingival surface

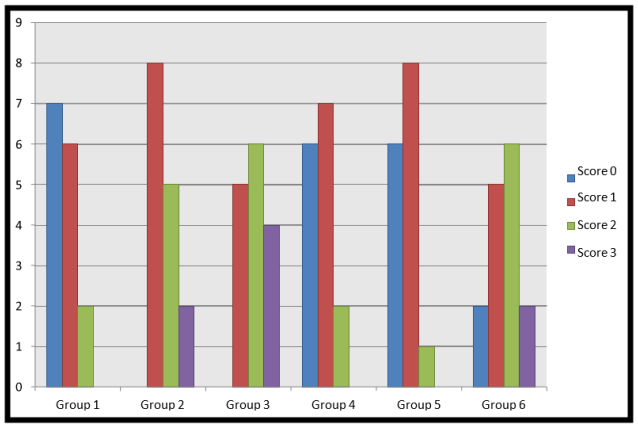

Fig. 2: Graphical presentation of the result at gingival surface

Score 0 (Minimum Microleakage) is seen in maximum samples of Group 1, Group 4 and Group 5.

Score 3 (Maximum Microleakage) is seen in maximum samples of Group 3.

Overall, the graph depicts less microleakage seen at the gingival surface in the samples of Group 4, 5 and 6 than the samples of Group 1, 2 and 3, even in presence of contaminants like Blood and Saliva.

4. Discussion

In last 45 years, evolution in dentin bonding systems has been noted with respect to their chemical composition, application, mechanism of action, technique and effectivity.[9]

Adhesion seems a challenge for the restoration of Class II and Class V cavities, due to the position of their cervical margin which is at Cemento-enamel Junction (CEJ), and is limited by Cementum.[10] Maintaining the marginal quality of the restoration, is the main clinical problem in these cavities.[11]

Moisture in any form (Blood or Saliva) must be prevented during clinical procedure because:[12] 1- It may affect the pulp while removing caries especially with pulp exposure. 2- Deteriorate the physical properties of the restorative material.[12]

Microleakage at the tooth-restoration interface is one of the biggest hurdles in attaining an ideal restorative material.[13]

Microleakage can be assessed by qualitative, semi-quantitative or true quantitative measurements of sealing effectiveness.[14]

Qualitative Measurement for evaluation of microleakage is used here as it is easy to use and simple to interprete.[15]

The factors that could probably be the cause for reduced bonding efficacy in saliva contaminated dentine are as follows:[16]

1. Glycoprotein adsorbed to the poorly polymerized adhesive surface which acts as a barrier and hinders complete wetting with the next increment of resin and thus leads to insufficient co-polymerisation.
2. The monomers cannot sufficiently penetrate the collagen network of dentin due to salivary proteins or the decreased bond strength may be attributed to increased contact angle.
3. Weak hybrid layer is produced due to dilution of primer with excessive saliva
4. Due to the removal of oxygen inhibited unpolymerized surface layer, co-polymerization with the subsequent resin layer may be compromised. However this hypothesis has been controversial with the other school of thought saying that it doesn't affect the bond strength.[16]

Before application of adhesive, when blood contacts the conditioned dentin surface, the infiltration of adhesive into treated dentin is hindered by the thin film formed by protein content and macromolecules of fibrinogen and platelets.[17]

In the present study, we have contaminated the samples after applying the bonding agent, but before light curing them.

Comparing the microleakage amongst 7^{th} Generation bonding agent, there is more microleakage observed under contamination of blood. This can be attributed to the composition of blood and its protein content.

Also in 7^{th}Generation, maximum microleakage at the gingival margin of the samples contaminated with blood. It may be due to the cavosurface bevel given at the occlusal surface which increases the surface area of a bondable margin. The enamel rods end on are exposed due to enamel surface bevel. The gingival surface of the cavities (below CEJ) observe increased microleakage, due to polymerization shrinkage in the direction of the tightly bonded enamel cavosurface margin.[8] One of the reasons for this suggests that the application of self-etch primer hydrolyzes blood on the enamel, at occlusal surface.[18]

Now, comparing the microleakage amongst 8^{th} Generation bonding agent, it can be seen that microleakage in the control group and the samples contaminated with saliva is almost similar. This could be because of the addition of a newer agent in the composition of G-Premio (GC), which is, MDTP Methacryloyloxydecyl dihydrogen thiophosphate (Thiophosphate ester monomer). Being, a functional monomer it helps in adhesion and initiates the interaction between the precious metal surfaces and the monomers (chemical adhesion).[19]

For the comparison of the microleakage between the samples of 7^{th} and 8^{th} Generation, from the figures of observation table, it depicts that the 8^{th} Generation does show better performance in terms of microleakage and especially in presence of saliva. Also the amount of microleakage due to blood contamination is less than that seen in 7^{th} Generation.

Both these products have a common ingredient which is considered to be one of the key ingredients in enhancing their performance. It is, 4-MET (4- metacryloxyethyl trimellitic acid), it is a Functional monomer which serves as, etchant, wetting agent and promotes adhesion. Also, dissolves the smear layer, and helps in micromechanical as well as chemical adhesion.[19]

Bacterial leakage can also be used to verify the result of research. Even occlusal loading has been effective in demonstrating microleakage values, so, this variable can be considered in future studies.[20]

5. Conclusion

Thus, within the limitations of this study, it can be concluded that, 8^{th} Generation Bonding Agent shows better performance than 7^{th} Generation Bonding Agent. Though further clinical studies need to be carried out, but the introduction of 8^{th} Generation Bonding Agent is a new ray of development in the field of adhesion.

6. Source of Funding

None.

7. Conflicts of Interest

There are no conflicts of interest.

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