Comparison of the Influence of a Packaged Fruit Juice on the Bacterial Adhesion on a Glass Ionomer Cement and an Esthetic Restorative Material In Vitro

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ABSTRACT

Objective: The aim of this research was to evaluate and compare the effect of a packaged orange juice on the two most commonly used restorative materials in pediatric dentistry.

Methodology: Fifteen samples each of 6 mm diameter and 2 mm thickness of a glass ionomer cement (GIC) and an esthetic restorative material were prepared using silicone rings. These were exposed to a packaged orange fruit juice and then placed in a standard culture of Streptococcus mutans. The bacterial adhesion to these samples was evaluated after exposure to the fruit juice for 1 day and for 7 days.

Results: Results from the study show that there is a decrease in the colony forming unit (CFU) after exposure to the packaged fruit juice as opposed to the studies using carbonated acidic drinks, which have shown a consistent rise in the CFU due to a change in the surface morphology.

Conclusion: Within the limitations of this study, it was seen that the consumption of fruit juice may not cause a deteriorating effect on the restorative materials considered. However, the results were not statistically significant and further research is necessary to come to a conclusion regarding the reduction in the bacterial count after exposure to the fruit juice.

Clinical implications: With further research, such studies can help in improving the diet counseling practices.

Keywords: Composite, Fruit juice, Glass ionomer cement, In vitro.

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INTRODUCTION

The practice of modern dentistry is based on the prevention, early detection, and treatment of dental caries. Various restorative materials have been developed over the past years which are being used routinely in the dental practice. GIC and composites are two of the most frequently used materials in pediatric dentistry. These are heterogeneous materials which may over time, on degradation enable plaque adhesion and promote secondary caries by increasing the bacterial adhesion on the teeth.

The formation of oral plaque is influenced by bacterial conditions of the oral cavity, salivary conditions, eating habits, and the roughness of the surface where it is formed. Secondary caries due to acid production from bacteria including S. mutans is one of the reasons for replacing dental restorations. The adhesion of these bacteria is increased when the surface roughness of the tooth or the restorative material is more.

Fruits are considered to be healthy and are a part of the routine diet of almost all children. A change in the lifestyle has resulted in a change in dietary practices and fruit juices and acidic beverages are now routinely consumed. These varied food habits can affect the teeth as well as the restorations. The longevity and prognosis of any restoration may be dependent on the diet of the child along with other factors. Various foods and beverages have been shown to cause an increase in surface roughness and a consequent increase in the microbial adhesion. Previous studies have shown more losses of surface roughness due to orange and apple juices as compared to carbonated drinks.

Patel et al. in their study concluded that acidic soft drinks caused a surface layer degradation of the restorative material which resulted in an increase of bacterial adhesion. However, no study has evaluated the effect of other beverages like fruit juices on the bacterial adhesion on a restorative material.

Citrus peel extracts have been known to have an antibacterial effect on the caries causing organisms in the previous studies. Even though there is literature showing the effect of citrus fruit juices on the surface hardness and roughness of various restorative materials, their effect on bacterial adhesion has not been evaluated.

Hence, this study evaluated the effect of a packaged fruit juice on the bacterial adhesion of the common caries causing bacteria S. mutans to the restorative materials that are commonly used in the clinical practice.
Influence of a Packaged Fruit Juice on Restorative Materials

**Methodology**

**Material (Company)**
- Type 2 Glass Ionomer Cement (GC India Dental)
- Filtek Z350 XT Universal Restorative (3M India)
- Real Fruit Power Preserved Orange Juice (Dabur India)

The Dabur Real Fruit Juice which was used in the study mainly contains the fruit juice concentrate and permitted acidity regulators. The acidity regulator helps in maintaining the pH of the Juice.

**Sample Size**
The sample size was estimated according to the previous studies. Fifteen samples were prepared for each group which were further divided into three subgroups.

**Experimental Groups**
The specimens of restorative materials were divided into three groups.
- Group I—control group included five samples.
- Group II—immersed in packaged fruit juice (50 mL) for 1 day which included five samples.
- Group 3—immersed in fruit juice (50 mL) for 7 days which included five samples.

**Preparation of the Specimens**
To obtain identical specimens, the materials were polymerized using silicon rings which had an external diameter of 9 mm, internal diameter of 6 mm, and thickness of 2 mm. The rings were slightly overfilled and after covering with Mylar Matrix Strip they were pressed between two glass plates for the GIC. For the composite material, polymerization was carried out for 40 seconds on each side using a curing unit using one polymerization mode. Manufacturer’s instructions were followed for the curing. Minimum 20 seconds of curing was done for every 2 mm increment. Polishing was done with fine and superfine polishing discs.

Artificial saliva at room temperature was used to store the specimens. Testing was done 4 weeks after the preparation of the specimens.

**Treatment with the Juice**
Equal quantity of the juice was taken in test tubes. The prepared samples were placed in these test tubes changing the juice everyday in case of the 7 days experimental group. The sample was removed from the juice using sterile tweezers and the microbial testing was carried out.

**Microbial Testing**
The culture of *S. mutans* at a density of $1 \times 10^{10}$ cells/mL was used. It was obtained by incubation for 16 hours at 37°C.

Each material was washed and 100 μL growth culture was seeded onto each sample test and incubated for 4 hours at 37°C in static conditions. The sample tests were gently washed with phosphate buffered saline (PBS) to remove the loosely adherent bacteria. For the total viable count, samples for each group were used. One milliliter of the sample was dispersed in 1 mL sterile Ringer’s solution using a vortex for 3 minutes. 0.1 mL of each serial dilution was deposited into mitis salivarius (MS) agar plates. The plates were incubated at 37°C for 24–48 hours and the number of colonies were counted.

**Statistical Analysis**
All the statistical analysis was performed using SPSS Version 19 software. Descriptive statistics such as mean and standard deviation were estimated. One-way analysis of variance (ANOVA) test was used to compare the bacterial count at different time intervals within each group followed by *post hoc* test for pairwise comparison. Intergroup comparison was done using independent *t*-test for time each interval. Level of significance was kept at $p \leq 0.05$ (Tables 1 and 2).

**Results**
The results showed that there was a decrease in the CFU count after the restorative materials were exposed to the packaged fruit juice. There was a sharp decrease in the group exposed to the orange juice for 1 day which increased in the group exposed to the juice for 7 days in the GIC group as well as the composite groups as can be observed from Tables 3 and 4. Table 5 shows the comparison of the change in

| Sl. no. | Sample marked as               | Parameter | Result          | Unit     |
|---------|--------------------------------|-----------|-----------------|----------|
| 1       | GIC (control)                  | A1        | $S. mutans$     | $2.45 \times 10^5$ | CFU per mL |
|         |                                | A2        |                 | $2.73 \times 10^5$ | CFU per mL |
|         |                                | A3        |                 | $1.36 \times 10^5$ | CFU per mL |
|         |                                | A4        |                 | $3.77 \times 10^5$ | CFU per mL |
|         |                                | A5        |                 | $4.03 \times 10^5$ | CFU per mL |
| 2       | GIC (1 day juice incubation)   | B1        |                 | $2.12 \times 10^3$ | CFU per mL |
|         |                                | B2        |                 | $4.5 \times 10^3$  | CFU per mL |
|         |                                | B3        |                 | $5.60 \times 10^2$ | CFU per mL |
|         |                                | B4        |                 | $2.04 \times 10^3$ | CFU per mL |
|         |                                | B5        |                 | $1.73 \times 10^3$ | CFU per mL |
| 3       | GIC (7 days juice incubation)  | C1        |                 | $1.43 \times 10^4$ | CFU per mL |
|         |                                | C2        |                 | $3.0 \times 10^4$  | CFU per mL |
|         |                                | C3        |                 | $2.145 \times 10^4$| CFU per mL |
|         |                                | C4        |                 | $4.0 \times 10^4$  | CFU per mL |
|         |                                | C5        |                 | $1.170 \times 10^4$| CFU per mL |
Table 2: Results for composite group

| Sl. no. | Sample marked as | Parameter       | Result       | Unit          |
|---------|------------------|-----------------|--------------|---------------|
| 1       | Composite (control) | A1   | S. mutans    | $2.47 \times 10^5$ | CFU per mL   |
|         |                   | A2   |              | $7.15 \times 10^5$ | CFU per mL   |
|         |                   | A3   |              | $4.5 \times 10^5$ | CFU per mL   |
|         |                   | A4   |              | $2.21 \times 10^5$ | CFU per mL   |
|         |                   | A5   |              | $1.365 \times 10^5$ | CFU per mL  |
| 2       | Composite (1 day juice incubation) | B1   |              | $3.44 \times 10^2$ | CFU per mL   |
|         |                   | B2   |              | $3.40 \times 10^2$ | CFU per mL   |
|         |                   | B3   |              | $3.84 \times 10^2$ | CFU per mL   |
|         |                   | B4   |              | $3.0 \times 10^3$ | CFU per mL   |
|         |                   | B5   |              | $2.04 \times 10^3$ | CFU per mL   |
| 3       | Composite (7 days juice incubation) | C1   |              | $3.5 \times 10^3$ | CFU per mL   |
|         |                   | C2   |              | $2.5 \times 10^3$ | CFU per mL   |
|         |                   | C3   |              | $2.73 \times 10^4$ | CFU per mL   |
|         |                   | C4   |              | $7.2 \times 10^4$ | CFU per mL   |
|         |                   | C5   |              | $8.45 \times 10^4$ | CFU per mL   |

Table 3: Comparison of CFU within GIC group

| Interval | Mean CFU     | Median CFU    | p-value | C vs 1 day p-value | C vs 7 days p-value | 1 vs 7 days p-value |
|----------|--------------|---------------|---------|--------------------|---------------------|---------------------|
| Control  | $2.428 \times 10^5$ | $2.73 \times 10^5$ | 0.053   | Diff: 2189.2       | Diff: 1465           | Diff: –724.2         |
| 1 day    | $2.388 \times 10^5$ | $2.04 \times 10^5$ | 0.134   | $p = 0.134$        | $p = 0.669$          | $p = 0.313$          |
| 7 days   | $9.63 \times 10^4$  | $1.17 \times 10^4$ |         |                    |                     |                     |

Repeated measure ANOVA test; post hoc Bonferroni test

Table 4: Comparison of CFU within composite group

| Interval | Mean CFU     | Median CFU    | p-value | C vs 1 day p-value | C vs 7 days p-value | 1 vs 7 days p-value |
|----------|--------------|---------------|---------|--------------------|---------------------|---------------------|
| Control  | $1.361 \times 10^5$ | $1.365 \times 10^5$ | 0.304   | Diff: 1100.6       | Diff: 619.6          | Diff: –481          |
| 1 day    | $2.604 \times 10^3$ | $3.40 \times 10^3$ | 0.265   | $p = 0.265$        | $p = 1.000$          | $p = 1.000$         |
| 7 days   | $7.414 \times 10^4$ | $7.2 \times 10^4$  |         |                    |                     |                     |

Repeated measure ANOVA test; post hoc Bonferroni test

Table 5: Comparison CFU among both the groups

| Interval | Groups | N | Mean     | Std. deviation | p-value |
|----------|--------|---|----------|----------------|---------|
| Control  | GIC    | 5 | 2428.0000 | 1608.88393     | 0.245   |
|          | Composite | 5 | 1361.0000 | 1012.40679     |         |
| 1 day    | GIC    | 5 | 238.8000  | 191.69429      | 0.846   |
|          | Composite | 5 | 260.4000  | 145.65988      |         |
| 7 days   | GIC    | 5 | 963.0000  | 919.30408      | 0.747   |
|          | Composite | 5 | 741.4000  | 1164.64471     |         |

Independent t-test
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It can be observed that the mean CFU was higher in the GIC in the control group as well as on the seventh day. It was slightly lower in the GIC on the first day. However, the intergroup as well as the intragroup differences were not statistically significant as shown by Figs 1 and 2.

**Discussion**

Bacterial plaque is a biofilm wrapped in an extracellular matrix. Within a few minutes of brushing, salivary mucoproteins create a film that covers the teeth which is then colonized by the microbes in the oral cavity.

The oral cavity offers various different surfaces for plaque accumulation. Quirynen and Bollen have stated the following statements regarding the effect of surface roughness on the plaque accumulation supragingivally: (1) Rough surfaces cause more accumulation and retention of plaque; (2) Plaque on rough surfaces which is undisturbed may give rise to inflammation as it is more mature.

The current practice of dentistry is based more on early detection or prevention of dental diseases. Various restorative materials have been developed which are routinely used in the clinical practice. However, these materials are susceptible to the detection or prevention of dental diseases. Various restorative materials have been developed which are routinely used in the clinical practice, parallel to the GICs. Numerous studies that evaluated effects of beverages on the restorative material’s surface roughness showed a statistically significant correlation between the prevalence of erosion and the consumption of these beverages. The acidic ingredients of these beverages have been shown to be erosive. Most of the fruit drinks contain citric acid as their main component typically in the concentration of 15–45 mmol. The most damaging storage medium for GICs was found to be citric acid, whereas pure composite resin in all the acid solutions remained relatively unaffected. Due to the carboxylic acid in the fruit juices, water-soluble complexes may be formed by producing chelation ions. Carbonated soft drinks, due to its phosphoric acid content cannot combine ions causing less harm.

However, the plaque accumulation is not only dependent on the erosion of restorative material. The effect of the other contents in the beverages, especially in the case of fruit juices will also play a role in the plaque accumulation on the restorative surfaces along with the intrinsic antibacterial activity of the restorative materials. The extract of Citrus sinesis peel has been shown to have antimicrobial activity against dental caries in vitro. Glass ionomer cement is biocompatible to dental pulp, has a property of chemical bonding and fluoride release, which can inhibit the bacterial growth and caries progression. This antibacterial property of the GIC could be the reason for lower S. mutans counts on the GIC samples as compared to the composite samples in the group tested on day 1. This finding was not consistent as the bacterial adhesion on the seventh day was less in the composite group as compared to the GIC group. The reason for this finding could be related to the surface degradation which needs to be evaluated on the first and the seventh day.

In this study, although not statistically significant, there was a decrease in the bacterial adhesion after an exposure to the orange juice. Preservatives in the juice buffering action of the artificial saliva.

In this study, the samples during experimentation were stored in artificial saliva which could have resulted in a buffering action as well as inhibitory effect on the S. mutans.

**Limitations**

This study evaluated only one bacteria that is S. mutans. However, a number of bacterial species have been implicated in the progression and causation of dental caries.

Only one brand of packaged fruit juice was considered. The composition of these juices varies according to the brands and hence can cause a difference in the results.

The tooth surface and restorative material interface was not taken into consideration.
**CONCLUSION**

Within the limitations of this study, observations are that the consumption of fruit juice even though it has a deteriorating effect on the restorative materials does not affect the bacterial adhesion.

However, the results are not statistically significant and further research is necessary to come to a conclusion regarding the reduction in the bacterial count after exposure to the fruit juice.

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