

ANTIBACTERIAL ACTIVITY OF THREE TOOTH-COLORED RESTORATIVE MATERIALS AGAINST STREPTOCOCCUS MUTANS: AN IN VITRO STUDY

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ABSTRACT

Background: Antibacterial activity of restorative materials had an important role in preventing caries recurrence.

Aim of the study: to evaluate the antibacterial effect of three tooth-colored restorative materials.

Materials and Methods: Three commercially available tooth-colored restorative materials, GC Fuji (Group I), Riva self-cure (Group II) and Versa Comp (Group III) were evaluated. The test samples of restorative materials were manipulated in accordance with manufacturer's instructions. Eppendorf tubes were filled with constant measured amount of the three tested restorative materials; a conical cavity was created in the center of each material after curing. Bacterial suspension was prepared from fresh overnight growth of *Streptococcus mutans* on blood agar. 50 μ L of bacterial suspension was placed in the conical cavity and around the restorative material inside each eppendorf tube and then were incubated. At the end of incubation period, eppendorf tubes were removed from candle jar and viable bacterial count was estimated.

Results: It has been found that at day1, day 2 as well as day 3, there was decrease in bacterial count but, there was no statistically significant difference between the three groups. On studying each group separately, group I there was a statistically significant decrease in bacterial count at day 2 and group II and III showed statistically significant decrease from day 2 to day 3.

Conclusions: Fuji LC was more effective against *Streptococcus mutans*, than Riva self-cure and Versa comp, but with no significant difference.

INTRODUCTION

Dental caries still remains a major public health problem despite the widespread use of fluoride and

the decline in caries prevalence observed in the majority of highly industrialized countries.^[1] Long-lasting restorations are clinically attractive because they can reduce patients' pain and expense as well as

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the number of their visits to dental office. ^[2,3] Both restorative materials and oral bacteria are believed to be responsible for restoration failure. ^[3]

In an attempt to obtain restorative materials that could prevent marginal gaps colonization, materials capable of releasing fluoride and providing antimicrobial activity have been developed, such as; Glass Ionomer cements (GIC), "compomers" and fluoridated composite resins. ^[4,5]

Tooth-colored restorative materials are increasingly demanded by patients and applied by clinicians. In spite of routine use of posterior tooth-colored restorative materials, poor marginal adaptation of composite resins, mostly due to the polymerization shrinkage, is still considered a major reason for the failure of these restorations. ^[6-8]

Microleakage and recurrent caries are major clinical problem associated with direct posterior composite restorations. Bacterial penetration into the prepared cavity can lead to secondary caries, which is the main reason for failures in dental restorations. ^[9] Due to this potentiality of leakage, any restorative /base/liner material used in the mouth should have antibacterial properties in defense against bacterial migration. ^[10]

Effective and long lasting antibacterial property of composite resin-restorative material may eliminate bacterial biofilm formation at the interface and thus increase restoration longevity. ^[11]

Several species of bacteria has been isolated from dental plaque, such as Lactobacilli, Streptococcus mutans, Streptococcus sorbinus etc, ^[12] which may induce the formation of caries. Streptococcus mutans is one of the most frequent bacteria involved in dental caries. ^[13]

The aim of the present study was to evaluate the antibacterial effect of three tooth colored restorative materials.

MATERIALS AND METHODS

TABLE (1): Materials used in this study:

| Materials | Manufacturer |
|---------------------------------------|------------------------------|
| GC Fuji LC capsule VLC | GC corporation, Tokyo, Japan |
| Riva self-cure Glass Ionomer capsule | SDI Limitation, Australia |
| Versa Comp universal hybrid composite | Sultan |

Three commercially available tooth-colored restorative materials were used in this study; the details of these materials were shown in table (1).

GC Fuji (Group I), Riva self-cure (Group II) and Versa Comp (Group III).

The micro-organisms used in this study were four strains of Streptococcus mutans. Bacterial plaque was collected from different parts of the patient's mouth immediately cultured on Mitis Salivarius agar kept in an incubator at 37°C for 24 hours; afterwards isolated and identified. Identification was accomplished by colony morphology, gram stained films, catalase test, subculture on blood agar and bile esculin agar. Pure isolates were preserved till time of experiment.

The test samples of restorative materials were manipulated in accordance with manufacturer's instructions. The samples were prepared in strip crown form (Uniteck 3M Dental products division) for standardization as pre-fabricated mould. ^[10] Clean Eppendorf tubes were filled with constant measured amount of the three tested restorative materials; a conical cavity was created in the center of each material after curing by fissure stone bur number 5. The specimens were autoclaved afterwards at 121°C for 30 minutes.

Bacterial suspension equivalent to 0.5 McFarland was prepared from fresh overnight growth of Streptococcus mutans on blood agar. ^[14] 50 µL of bacterial suspension was placed in the conical cavity and around the restorative material inside each eppendorf tube. They were then incubated in candle jar for 24, 48 and 72 hours. At the end of incuba-

tion period, eppendorf tubes were removed from candle jar and viable bacterial count was estimated. Ten folds serial dilutions were done for each eppendorf tube (up to 1/10000) and counting was done on Mitis Slivarius agar. The number of colony forming unit (CFU) was calculated by this formula: $50 \times \text{No. of colonies} \times \text{Inverse of dilution}$.

Statistical analysis:

All data were collected and statistically analyzed. A logarithmic transformation (Log10 transformation) of each CFU count was performed because of the high range of bacterial counts. Data were explored for normality after Log10 transformation using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed non-parametric (non-normal) distribution. So, data were presented as median and range values. Kruskal-Wallis test was used to compare between the three groups. Friedman's test was used to study the changes by time in each group. Wilcoxon signed-rank test was used for pair-wise comparisons when Friedman's test is significant.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS:

It has been found that at day1, day 2 as well as day 3, as regards median and range there was decrease in bacterial count but, there was no statistically significant difference between the three groups as shown in table (2) & figure (1).

TABLE (2): Median, range values and results of comparison between Log10 CFU in the three groups

| | Group I | Group II | Group III | P-value |
|-------|--------------------|--------------------|--------------------|---------|
| Day 1 | 5.2 (5.0 – 6.1) | 5.4 (0.0 – 7.6) | 6.5 (5.9 – 6.9) | 0.388 |
| Day 2 | 0.0 (0.0 – 4.7) | 2.0 (0.0 – 6.0) | 4.7 (0.0 – 6.9) | 0.396 |
| Day 3 | 0.0 (0.0 – 0.0) | 0.0 (0.0 – 0.0) | 0.0 (0.0 – 3.2) | 0.368 |

*: Significant at $P \leq 0.05$

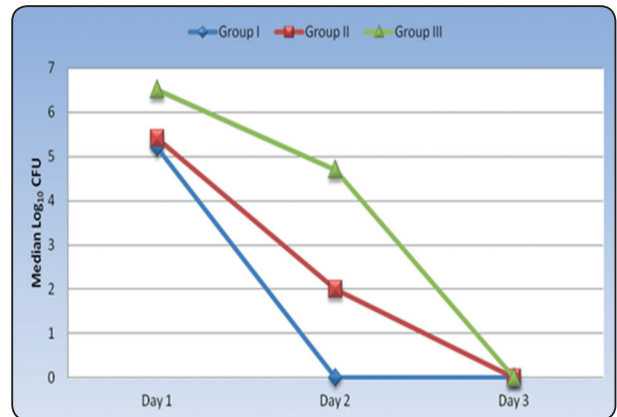


Fig. (1): Line chart representing median Log10 CFU in the three groups.

On studying each group separately, in group I there was a statistically significant decrease in bacterial count at day 2 and day 2 and 3 didn't show any bacterial growth, while in group II and III, there was no statistically significant decrease in bacterial count at day 2, but from day 2 to day 3 there was a statistically significant decrease in bacterial count as shown in table (3).

TABLE (3): Median, range values and results of comparison between Log10 CFU in each group at different time periods

| | Group I | Group II | Group III |
|---------|----------------------|----------------------|----------------------|
| Day 1 | 5.2 (5.0 – 6.1) a | 5.4 (0.0 – 7.6) a | 6.5 (5.9 – 6.9) a |
| Day 2 | 0.0 (0.0 – 4.7) b | 2.0 (0.0 – 6.0) a | 4.7 (0.0 – 6.9) a |
| Day 3 | 0.0 (0.0 – 0.0) b | 0.0 (0.0 – 0.0) b | 0.0 (0.0 – 3.2) b |
| P-value | 0.023* | 0.045* | 0.037* |

*: Significant at $P \leq 0.05$, Different superscripts in the same column are statistically significantly different

As regards the % reduction in bacterial count in the three groups results showed no statistically significant difference as shown in table (4).

TABLE (4): Median, range values and results of comparison between % reductions in bacterial counts in the three groups

| | <i>Group I</i> | <i>Group II</i> | <i>Group III</i> | <i>P-value</i> |
|--------------------|-----------------------|---------------------|---------------------|----------------|
| Day 1- Day2 | 100.0(45.0 – 100.0) | 98.8 (0.0 – 100.0) | 98.8 (0.0 – 100.0) | 0.446 |
| Day 1- Day3 | 100.0 (100.0 – 100.0) | 100.0 (0.0 – 100.0) | 100.0 (0.0 – 100.0) | 0.573 |

*: Significant at $P \leq 0.05$

DISCUSSION

Dental caries constitutes one of the most common infectious diseases. It is multi-factorial disease related to the presence of cariogenic bacteria embedded in dental plaque which is particularly *Streptococcus mutans*.^[15-16] Therefore several experiments have been conducted to incorporate antibacterial agents into dental filling materials as resin composites and glass-ionomers, in order to inhibit bacterial attachment and thus plaque accumulation.^[17-18]

The aim of the present study was to evaluate the antibacterial effect of three tooth colored restorative materials on four strains of mutans Streptococci.

In the present study, an assay was established to determine viable bacterial counts after incubation of small amount of bacterial suspension within the materials, designed to avoid dilution of components released from the materials.^[19] This method is more reliable than other methods as its most significant advantage is the direct contact between the bacteria and the restorative material, which simulates the oral cavity.^[19-20] Direct contact test has been widely used for evaluating antibacterial activity of different dental materials.^[20-23]

Streptococcus mutans was used in this study as it is known to be the primary etiological factor for carious lesions, and routinely used in testing the antimicrobial activity of restorative materials.^[24]

The antibacterial effect of the tested materials was recorded after 24, 48 and 72 hours as *Malton et*

al; 2004,^[20] found that none of the composite resins used in their study could affect the bacterial growth immediately after polymerization.

In the current study, there was no statistically significant difference between the three tested materials at day1, 2 and 3. On the contrary *Shirani et al 2008*,^[25] and *Łuczaj-Cepowicz et al 2014*,^[26] revealed that there were significant differences between different materials in all the incubation periods.

On the other hand the results of the present study of each material separately showed that, regarding **group I (Fuji LC)**, there was statistically significant decrease in bacterial count at day 2, but regarding **group II (Riva self-cure) and group III (Versa Comp)** there was no statistically significant decrease in bacterial count at day 2, although from day 2 to day 3 there was statistically significant decrease in the count.

The effects of glass Ionomer based restorative materials on cariogenic bacteria are known, probably resulting from release of fluoride,^[27-29] *Vemeersch et al 1993*,^[30] concluded that the low of bacteria, during setting may contribute more to the antibacterial properties than their fluoride leaching capabilities. *Yap et al 1999*,^[31] reported that there was no antibacterial activity despite the presence of fluoride around the set material. However, varying results could be obtained, as it is known that the diffusibility of an antimicrobial agent depends on its size, form of filler particles, and concentration in the material. In addition, the diffusibility of ions (F⁻,

Ca^{++} , Al^{+++} , OH^-) from glass ionomers depends on the pH of the environment.

In the current study the results of group III which represents the composite is nearly similar to group II which represents the glass Ionomer, as it showed antibacterial activity comparable to group II, these results are in contrast to a study carried out by *Matalon et al 2004*,^[20] who revealed that no antibacterial activity is attributed to polymerized composite resins, however the results of present study regarding the antibacterial activity of composite may be due to the presence of barium boron fluoro-alumino silicate. On the contrary, *Shirani et al 2008*,^[25] concluded that fluoride release from composite resins enriched with fluoride is less than that from glass Ionomer.

CONCLUSIONS:

Based on the results of the present study, it can be concluded that Fuji LC was more effective against *Streptococcus mutans*, than Riva self-cure and Versa comp, but with no significant difference, this activity appears to be variable and dependent on factors such as the chemical composition, low pH, release of F^- , and other ions from the restorative materials. Thus, it can be established with the continuous urge for novelties in dentistry, ranging from changing professional perceptions to altering demands from the patient and the enormous progress in material sciences, the quest for an ideal aesthetic biomimetic restorative material still continues. Further, research regarding these bioactive materials is still required.

RECOMMENDATIONS

- 1- More specific and more sensitive trials are required to determine the materials' constituents responsible for the antibacterial activity.
- 2- Further studies are suggested to be undertaken covering other materials and a larger number of cariogenic bacteria.

- 3- Further studies are suggested to be done covering a longer incubation period.

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