



In Vitro Comparison of the Effect of Nano-Hybrid Composite Resin and Amalgam on the Adhesion of Streptococcus Mutans

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ABSTRACT

Background and Aim: Due to the increasing use of restorative materials, finding a suitable material with low adhesion rate and colonization of pathogenic Streptococcus mutans has a significant importance. The purpose of this study was the comparison of the adhesion rate of Streptococcus mutans to “Nano-hybrid composite” and “Amalgam” at 1, 3, and 7 day intervals.

Methods and Materials: In this experimental study, 72 samples of Amalgam and composite resin were placed in two equal groups and exposed to bacterial suspension holding 1×10^6 cell/ml and after three time periods of 1, 3, and 7 days, the restorative material samples were suspended in 1cc of physiologic serum, and 100µl of the suspension was cultured on Blood Agar medium. After 48 hours the number of Streptococcus mutans colonies were counted. The data were analyzed by T-test.

Results: The mean and standard deviation of Streptococcus mutans colonies adhered to Nano-hybrid composite resin at 1, 3, and 7 day intervals were measured 12.7 ± 2.3 , 1.5 ± 2.12 and zero colonies, respectively. Adherence of Streptococcus mutans to composite resin during these three days, showed a significant statistical difference ($p < 0.005$). The mean and standard deviation of colonies, which adhered to Amalgam at 1, 3 and 7 day intervals were 32 ± 7.01 , 18.8 ± 3.8 and zero colonies, correspondingly. The adherence of Streptococcus mutans to Amalgam during these three days showed a significant statistical difference ($p < 0.001$). The comparison between Amalgam and composite resin showed that the adherence of Streptococcus mutans to composite resin was lower during the first and the third day and the results were statistically significant ($p < 0.001$).

Conclusion: The result of this study showed that adhesion of Streptococcus mutans to Nano-hybrid composite is lower than the adhesion to Amalgam.

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Introduction:

Adherence of bacteria to smooth dental surfaces requires synthesis of insoluble polymers such as Glucan. Surface receptors of bacteria also have a role in this adherence. Acid producing bacteria adhere to the sticky substances in dental plaque and lay on the surface of dental enamel. These bacteria produce a large amount of acid by fermenting the sugar. The high concentration of acid in these areas dissolves the mineral contents of dental enamel and exposes the dentin to proteolytic enzyme activity of other oral bacteria which eventually leads to dental caries.⁽¹⁾

Among the bacteria available in dental plaque, Streptococci have the highest ability to adhere to the oral surfaces (mucosa and teeth).⁽²⁾

Materials used commonly in restorative dentistry including Amalgam, composite resin and glass ionomer show different degrees of Streptococcus mutans (*S. mutans*) adhesion and different results have been obtained in this regard. Different studies have shown that either the levels of *S. mutans* adherence to different restorative materials such as various types of composite resin, glass ionomer and Amalgam are similar or in some cases the level of adherence is higher for glass ionomer and even the fluoride release can not balance this higher level.⁽³⁻⁶⁾

Studies have shown that adherence of *S. mutans* to Silorane based Nano-hybrid composite is lower compared to other restorative materials and have related this to hydrophobicity of these materials.^(7, 8)

Moreover, other studies have shown that the level of *S. mutans* adherence to dental porcelains differs with different porcelain surface treatment methods.⁽⁹⁾

The level of adherence of other microorganisms such as *Candida albicans* to different dental materials has been evaluated in other studies.⁽¹⁰⁾

Considering that Amalgam is a restorative material frequently used in dental clinics and no other study has been performed on the adherence of *S. mutans* to this material in comparison with other restorative materials, the present study aimed to compare the level of adherence of *S. mutans* to the above mentioned materials.

Methods and Materials:

This experimental study was performed in-

vitro and specimens were prepared from Nano-hybrid composite resin (Herculite, Kerr, USA) and Tytin Amalgam (Spherical, Kerr, USA). 36 samples were assessed from each type of the mentioned restorative materials. A Plexi-glass sheet was used which contained 100 circles with the diameter of 5mm and 1mm height. Beneath this Plexi-glass sheet, a clean glass slab was placed to support the sheet and to ensure a proper condensing. After placing the composite resin in the mentioned circles, two celluloid tapes were placed on either surfaces, and each side was light cured for 40 seconds with the intensity of 400 mw/cm² using a light curing device (Coltene co).⁽⁶⁾

Afterwards, the composite resin samples were removed and were polished with fine, moderate and coarse sof-lex polishing discs (3MESPE).

Regarding the Amalgam samples, after condensing the Amalgam in the mentioned circles, both sides were burnished and then were polished after 24 hours. The mentioned samples were then washed in distilled water and autoclaved. Afterwards, the samples were placed inside wells and were exposed to 1×10^6 cell/ml concentration of bacterial suspension PTTC1683 which had been prepared with 0.5 Mcfarland (barium sulfate) solution in the microbiology laboratory of Milad hospital. 350µl of this suspension was added to 350µl human whole saliva and was exposed to the samples. The saliva had been obtained from a healthy woman not consuming any medications during the study with no periodontal and systemic diseases or active dental caries. Saliva samples were kept at 4 °C and the mentioned suspension along with the samples were incubated at 37 °C, at time intervals of 1, 3 and 7 days. For each sample a control consisting of 350 ml saliva and 350 ml physiologic serum along with the restorative materials was placed in the incubator. Then the samples were removed from the wells and were washed with normal saline for 2 seconds and each sample was suspended in 1cc of physiologic serum and was vibrated for 1 minute. 100ml of this new solution was taken and was linearly cultured on sterile blood agar culture medium and was incubated at 37 °C for 48 hours.

The colonies were counted with the naked eye and without using microscope.⁽³⁾ The data were analyzed by ANOVA and T-test.

Results:

72 Amalgam and composite resin samples in two groups of 36 at three time intervals of 1, 3 and 7 days were assessed regarding the adherence of *S.mutans*. 18 samples were exposed to bacteria and for each sample a control was designated. In composite resin samples, the level of adherence of *S.mutans* (number of colonies) was 12.7 ± 2.3 colonies on day 1 and 1.5 ± 2.3 colonies on day 3. No colonies were visible on the seventh day. The levels of *S.mutans* adherence during these three days were statistically different ($p < 0.005$). (Figure 1)



Figure1- Level of adherence of *S mutans* to



Figure2- Level of adherence of *S mutans* to Amalgam samples during the days of study

The number of adhered colonies on Amalgam equaled 32 ± 7.01 colonies on day one, 18.8 ± 3.8 colonies on the third day with no colonies on day seven, (Figure 2) which showed significant differences ($p < 0.001$). Comparing Amalgam and composite resin, the level of bacterial adherence on the first and third days were lower for composite resin. These results are statistically significant ($p < 0.0001$). No adhesion was detected in control samples. (Table 1)

Table 1- Adhesion level of *S. mutans* based on dental material and divided by assessment days

Day of assessment	First	Third	Seventh	Test result P Value
Amalgam N=18	32 ± 7.01	18.8 ± 3.08	0	$P < 0.0001$
Nano-hybrid composite N=18	12.7 ± 2.3	1.5 ± 2.3	0	$P < 0.0005$
Test result	$P < 0.0001$	$P < 0.0001$	-	-

Discussion:

Despite the extensive application of restorative materials in dentistry, little information is available regarding the adhesion level of *S mutans* to these materials. Because Amalgam is no longer being used in many countries, studies are scarce about this material but it is still commonly used in our country. In the present study saliva of a healthy person was used to simulate the clinical condition.

According to the present study, *S.mutans* adhered less to Nano-hybrid composite resin in comparison with Amalgam. Adhesion was higher on the first day compared with days 3 and 7. Pandi et al. showed that PANI-Ag-Au Nano-composites (Polyaniline) have high antibacterial activity against gram positive (streptococci) and gram negative (*E.coli* and *klebsiella*) bacteria compared with PANI-Ag Nano-composites, PANI-Au and natural Nano-composites.⁽¹¹⁾

Fang Li et al. experimented novel remineralizing and antibacterial restorations in rat tooth for the first time. The results showed that composites and adhesives containing NACP (Nanoparticles of Amorphous Calcium Phosphate) and DMADDM (dimethylaminododecyl methacrylate) showed milder pulpal inflammation and higher formation of tertiary dentin compared with the control. Therefore, novel composites and adhesives containing DMADDM and NACP herald an innovative restorative treatment system, which is effective against oral pathogens and biofilm acids and also facilitate the repair of dentin-pulp complex.⁽¹²⁾

In a study by Poggio et al. on five restorative materials, *S.mutans* showed higher adhesion to packable composites.⁽⁵⁾

No distinctive information is available regard-

ing the adhesion to the Amalgam among available references.

Osorio et al. assessed the impact of 3 types of composite resins (these three were then modified with polyacid and glass ionomer) on *S. mutans* adhesion in vitro and concluded that, despite the higher surface roughness of glass ionomer, no differences were detected among these materials.⁽⁶⁾ This can be attributed to the polish-ability of these materials.

In another study by Montanaro on 10 types of dental materials (Flow-able Micro-hybrid composite, glass ionomer, Ormocer and a control) the level of adhesion was measured with Turbidimeter. All the mentioned materials except glass ionomer showed similar sensitivity.⁽³⁾

Accumulation of bacteria on glass ionomer is influenced by the age of restoration, and until approximately one month after placement, fluoride release reduces the level of *S. mutans*. Therefore, in older restorations no reduction in the number of *S. mutans* colonies has been observed.⁽¹³⁾

In a study by Eigk et al. two types of Amalgam, four types of glass ionomer, three types of composite resin and 1 type of ceramic were selected and polished. Weight of the accumulated plaque, number of colonies and viability of the adhered bacteria were assessed with vital fluorescent technique. Weight of the synthetic plaque was higher on all materials compared with the ceramic and the highest level was related to two types of glass ionomer. The amount of dental plaque was correlated with surface roughness but no relation was found between surface roughness and number of colonies.⁽⁴⁾

Although Amalgam is not an antibacterial substance, it has shown different levels of antibacterial activity. This can be attributed to the release of elements such as copper, mercury, zinc, silver and chloride compounds. Some studies suggest the effectiveness of this material on cariogenic bacteria such as *S. mutans*, *actinomyces viscosus* and *lactobacillus*.⁽¹³⁾

It is known that *S. mutans* can secrete lactic acid in an acidic environment. In this condition, pH significantly decreases in the surface between Amalgam and tooth and demineralizes the den-

tal surface. In-vivo studies have shown that elements such as copper, silver and zinc (released from Amalgam) can block acid production in dental plaque.⁽¹³⁾ Therefore, less demineralization can be expected.

As mentioned in previous studies, the released monomers from composite resins stimulate bacterial growth due to the existence of unreacted double bonds in composite resins. On the other hand, the Nano filler particles reduce surface roughness and bacterial adhesion. Very small filler particles result in high polish-ability and the smoothest surface among available composites.⁽¹³⁾

Overall, it seems that composite resin samples have lower surface roughness and bacterial adhesion compared with Amalgam.⁽¹¹⁾

In Amalgam and composite resin specimens assessed separately, the amount of adhesion decreased during 1, 3 and 7 days. This descending process is in line with the growth course of *S. mutans* and the existence of autolytic enzymes which limit the viability of this bacteria to 7-10 days.⁽¹⁾ A study by Buerger can also justify the results of the present study. He stated that adhesion of *S. mutans* to silorane-based Nano-hybrid composites is lower due to hydrophobicity of composite.⁽⁸⁾ Maza et al. counted the number of *candida albicans* cells adhered to samples under fluorescent microscope and used saliva as an antimicrobial agent.⁽¹⁴⁾

In the present study, saliva was used to decrease bacterial adhesion. This study has been performed in-vitro and cannot perfectly simulate the in-vivo condition in oral cavity. Some of the advantages of the present study include quantitative counting of the colonies and assessing the impact of time on the level of *S. mutans* adhesion.

Further studies are suggested in order to assess the amount of *S. mutans* adhesion to other dental materials, assess the impact of antiseptics on the adhesion level and also to assess the effect of different polishing methods on decreasing the surface roughness of dental materials.

Conclusion: The results indicated that adhesion of *Streptococcus mutans* to Nano-hybrid composite is lower than the adhesion to Amalgam.

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