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RESEARCH ARTICLE

ADHESION OF *STREPTOCOCCUS MUTANS* BIOFILM ON THE SURFACE OF INDIRECT RESIN COMPOSITES RESTORATIONS.

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Key words:-

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Abstract

Objective: To evaluate and compare *streptococci mutans* adhesion on the surface of indirect resin composites compared to direct.

Materials and Methods: A total of forty standardized disk shaped samples of the four tested material were constructed in special mold and cured according manufacturer's instructions and finished and polished with sofex finishing system, then subjected to bacterial biofilm colonization and the adhered bacteria were calculated and, the collected data were subjected to statistical analysis.

Results: One way ANOVA showed that there was a significant difference between the tested resin composites, and the post hoc Tukey's test showed that the lowest bacterial adhesion recorded for SR Nexco and Ceram X one with statistically significant difference compared to Z 250 and Herculite classic (p 0.05)

Conclusion: Indirect resin composites have lower susceptibility to bacterial adhesion than do direct one in the same context the smaller the filler particle size the lowest the bacterial adhesion.

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Introduction:-

Tooth colored restorative materials are today preferred due to the patient's demand for esthetic restorations and minimal invasive treatment modes. Although resin composite can be applied directly to restore posterior teeth, it can be also indicated for indirect restorations. In particular for medium to large sized cavities on the condition that sufficient tooth structure remains for adhesive cementation.¹ This restoration strategy is highly required due to the need of marginal adaptation, proximal contacts, anatomic form, color match, polymerization shrinkage and wear resistance controlling.^{1,2}

Indirect composites now offer an aesthetic alternative to ceramics for posterior teeth as inlays and onlays. The mechanical properties of lab composites in comparison to ceramics are inferior, however the aim is not that indirect composites replace ceramics rather that they supplement and complement them dependent upon each individual patient situation.

A brand of indirect resin composites, SR Nexco (Ivoclar Vivadent, Schaan, Liechtenstein) was introduced in 2012. SR Nexo Paste is a purely light-curing laboratory resin composite with high content of inorganic microopal fillers.

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This affords optimum benefits in terms of abrasion, discoloration, processing and surface gloss. The balanced ratio between the matrix and filler components results in outstanding physical properties achieved with polymerization units.³ The combination of microfillers plus prepolymer enables a very high filling ratio and excellent physical properties. The use of the prepolymer allows the advantages of large filler particles to combine with those of microfillers. This technology allows for a superior strength of resin composite that if only inorganic microfiller were used.³

The initial adhesion and early colonizing of bacteria to the surface is an important step in the process of plaque formation on solid substrate surfaces such as teeth and restorative materials.^{4, 5} Resin composites have surface characteristics different from those of teeth. Unlike tooth surfaces, the surface properties of resin composite materials related to bacterial adhesion and biofilm formation are affected by a myriad of factors — such as mechanical surface properties, material components such as filler particles and resin matrix, and curing conditions. In particular, matrix monomers might influence the growth of some cariogenic bacterial species^{6,7}

Many studies have reported that composite resins tend to accumulate more bacteria or plaque than do other restorative materials *in vitro* and *in vivo*. Differences in the amount of bacterial adhesion can be explained by the diverse surface characteristics of each type of composite resin.⁸⁻¹⁰

The success of the esthetic restorations on a long-term basis depends on the quality and amount of adhered biofilm are. The initial adherence and subsequent colonization of bacteria on the surface of composite resins is the key of the pathogenesis of the secondary caries promoted particularly by *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*). *S. mutans* has been identified as the major etiological agent of human dental caries and composes a significant proportion of the oral streptococci in carious lesions.¹¹ *S. mutans* is harbored in mature plaque. Organic acids are trapped within the glucan barrier produced by these bacteria, resulting in a prolonged low pH around the enamel surface.¹²

Based on the facts described, the aim of this study was to evaluate *S. mutans* biofilm adherence on the surface of, nanohybrid, microfill direct resin composites, and nanofilled, microfill indirect resin composites.

Materials and methods:-

Materials:-

In this study these types of dental resin composite; SR NEXCO (microfill indirect composite Ivoclar Vivadent, Schaan, liechtenstein), Filtek Z 250 (Z 250) (microhybrid direct/indirect composite 3M Dental product, St Paul, MN, USA), Ceram X One Dentsply Deterey GmbH, De-Trey-Str. Germany and Herculite classic Kerr Italia S.r.l. via passanti, Scafati (SA) (microfill direct composite).

Methods:-

Specimens' preparation:-

A total of forty standard disc shape specimens were constructed from the four brands of the resin composite materials indirect microfill resin composite SR NEXCO, indirect microhybrid resin composite Z250 and direct microfill resin composite Herculite classic and nanohybrid resin composite Ceram X One (n=10).

A split Teflon mold was composed of an outer copper ring and split Teflon ring with an internal hole of 10mm diameter and 2mm thickness was used to construct the stranded disc shapes specimens, the mold can be split in two halves and reconstructed by the copper ring.

The selected shade was A3 shade to standardize the degree of polymerization. The filled split Teflon mold was assembled over a glass slide with a thickness of 2mm, covered with a 0.05mm transparent polyethylene film to standardize surface smoothness. The mold space was filled with each test material in a bulk pack technique, avoiding gross excess and entrapping of air, then covered with clear celluloid strip and another glass slide and pressed to remove excess material. The applied materials of the three tested groups was light cured for twenty seconds from each side using light emitting diode (LED) light-curing unit with an intensity of (900 MW/cm²). Then each specimens was light curried from each side for additional twenty seconds after removed of the glass slap and the polyethylene film. After that the indirect resin composite SR NEXCO there was subjected polymerization by special oven Lumamat-100* for ten minutes (Targis Power TP3 Upgrade, Ivoclar Vivadent AG Schaan,

Liechtenstein) using program P2 for 11 minutes in order to complete polymerization according to manufacturer's instructions

The surfaces were smoothed by rubber polishers and silicone polishing wheels, leather buff wheels and Universal Polishing Paste (Ivoclar Vivadent AG Schaan, Liechtenstein).³

Finally, all the specimens in the four groups were finished with flexible discs (Sof-Lex XT Pop On, 3M ESPE) following the recommended sequence of finishing and polishing discs (coarse, medium, fine and superfine) so as to have standardized a smooth surface.

Cultivation of the Microorganism and Preparation of Cell Suspensions:-

A standard suspension of *S. mutans* (ATCC 25175) containing 10^6 cells/mL was prepared. For this purpose, *S. mutans* was seeded onto blood (Oxoid, UK) and incubated for 24 hours. All incubation was carried out at 37°C in a CO₂ chamber. After incubation, the growth was suspended in sterile physiological solution (0.9% sodium chloride (NaCl)), and the number of cells in suspension was counted in a spectrophotometer (B582, Micronal, São Paulo, Brazil). The parameters of optical density and wavelength used were, respectively, 0.620 and 398 nm. These parameters were previously established by means of a standard curve with CFU vs. absorbance.

Biofilm Adhesion:-

Adherence testing was performed in an aseptic environment in a laminar airflow chamber. The broth used for adherence was trypticase soya broth, dissolved in 1000 mL of distilled water. The broth was sterilized by autoclaving at 121°C for 15 minutes. In each well of Sterile 24-well polystyrene tissue culture plates was placed one specimen, 1.5 mL of broth, and 0.1 mL of standardized *S. mutans* suspension. The plates were sealed and incubated at 37°C for 24 hours in a CO₂ chamber. Samples were then removed and washed twice with sterile physiological solution (0.9% NaCl) in order to remove loosely bound material. Following this, the samples were placed in tubes with 3 mL of sterile physiological solution (0.9% NaCl) and sonicated (Sonoplus HD 2200, 50 W, Bandelin Electronic, Berlin, Germany) for 30 seconds to disperse the biofilms. The suspension obtained was diluted 10, 100, and 1000 times, and aliquots of 0.1 mL were seeded in duplicate onto BHI agar and incubated for 48 hours at 37°C in a CO₂ chamber. After the incubation, the plates with 30 to 300 typical colonies of *S. mutans* were counted in a colony counter (Phoenix CP-600, São Paulo, Brazil), and mean values of CFUs were obtained (CFU/mL). Mean values of CFU/mL were converted into logarithmic (log₁₀) values and analyzed by ANOVA and the Tukey test. p value, 0.05 was considered to indicate a statistically significant difference.

Scanning Electron Microscopy (SEM) Evaluation:-

All the specimens were subjected for SEM evaluation. One specimen of each subgroup was prepared for the SEM (JSM-5310LV JEOL, Tokyo, Japan) evaluation. The specimens were coated with silver in a vacuum evaporator. The "retained biofilms" on the specimens after sonication were observed for each group. The samples were rinsed with PBS buffer and fixed in 4% paraformaldehyde with 1% glutaraldehyde in PBS for one hour. The samples were rinsed with PBS three times for two minutes each, and finally rinsed with deionized water three times for two minutes each. They were then dehydrated through an ethanol series (50, 70, 80, 95, and 100%) for 15 minutes each, desiccated, sputter-coated, and observed by a SEM. Photographs of representative areas of the polished surface were taken at 3000× magnifications. The results were analyzed by calculating the mean and standard deviations for each group. The data of each material were subjected to analysis of variance (ANOVA) followed by Tukey's high significant difference (HSD) test at a p-value of 0.05

Results:-

Mean and standard deviation values of the CFU/mL (log₁₀) of *S. mutans* within the biofilms formed are displayed in Table 1. The one way ANOVA test revealed that there was significant difference in terms of the level of adhesion between the tested the four groups (P=0.01); the highest value of bacterial adhesion were recorded for Herculite classic followed by Z 250, Ceram X One and, finally SR NEXCO. There was no statistically significant difference between SR Nexco and Ceram X one and, there was no statistically significant difference between Z 250 and Herculite classic. However, there was statistically significant difference between SR Nexco and Ceram X one in one side and, Z 250 and Herculite classic in the other side (p 0.05).Table 1

Type of Material	Mean Adhesion Level (colonies/mm ²)	P value
SR NEXCO	4.6±0.09 ^B	0.001
Ceram X One	4.7±0.08 ^B	
Z 250	4.97±0.06 ^A	
Herculite classic	4.99±0.06 ^A	

Table 1:- Mean bacterial adhesion in different groups

Scanning Electron Microscopy (SEM) Evaluation:-

SEM showed that *S. mutans* was observed to be accumulated on the surface of Herculite classic and Z 250 much more than SR NEXCO and Ceram X One. Figure 1&2 and 3.

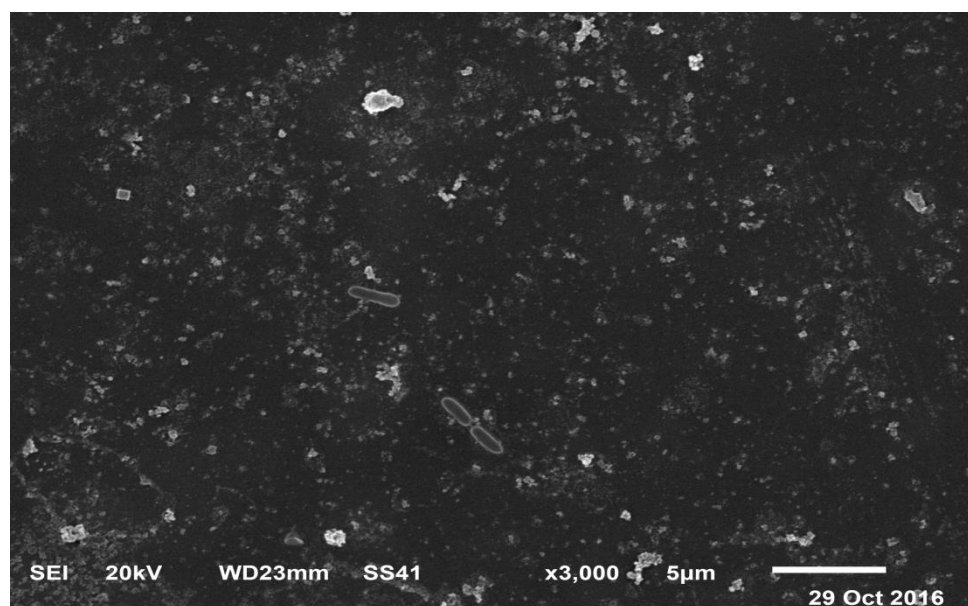


Figure 1:- showing SEM picture of *S. mutans* on the surface of SR Nexco.

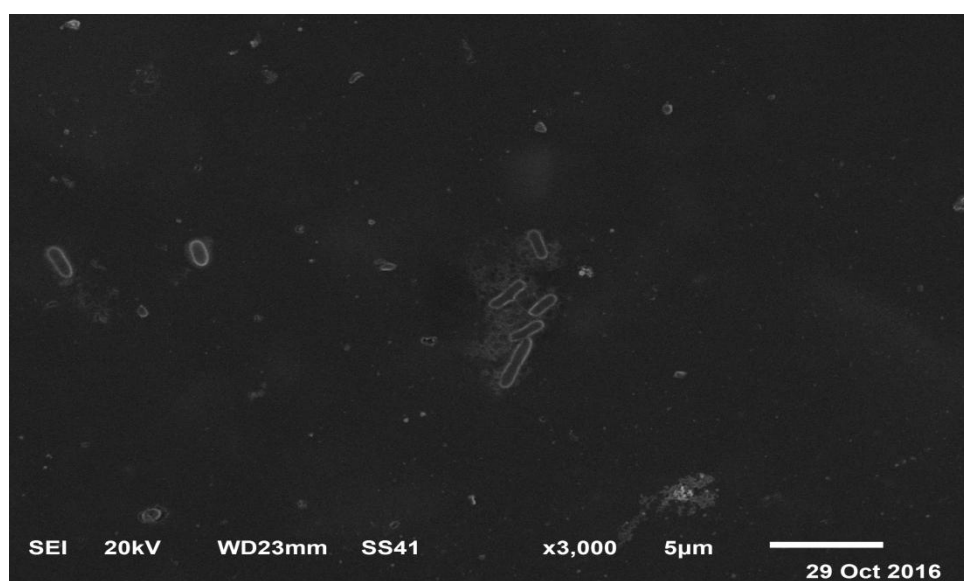


Figure 2:- showing SEM picture of *S. mutans* on the surface of Ceram X One.

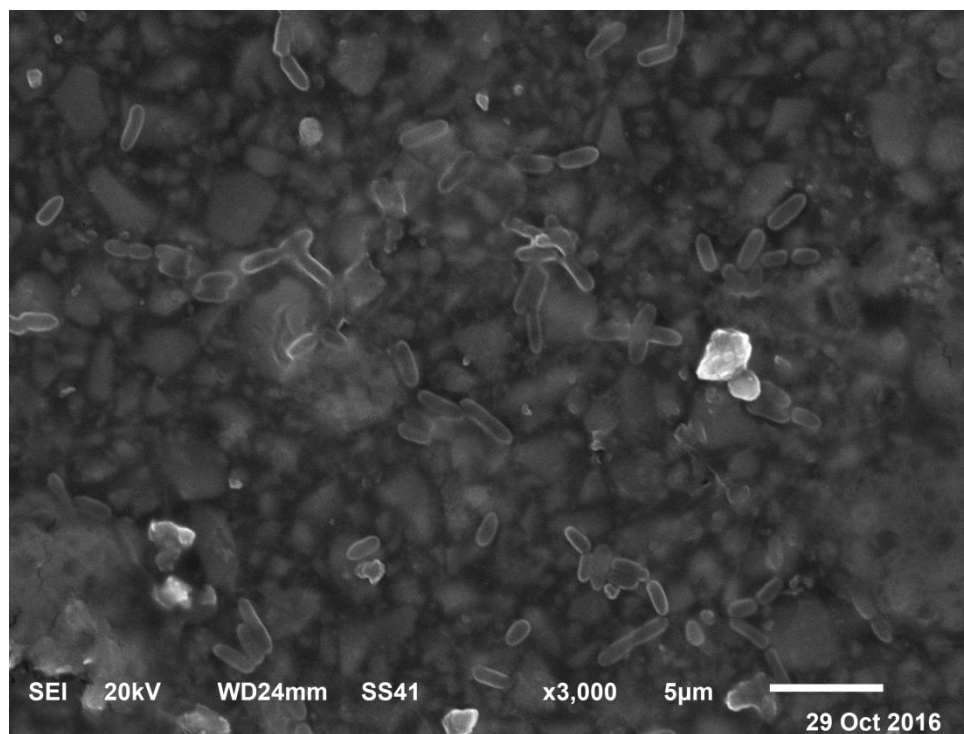


Figure 3:- showing SEM picture of *S. mutans* on the surface of Herculite classic.

Discussion:-

The quality and amount of adhered biofilm are important to the success of the esthetic restorations on a long-term basis. The initial adherence and subsequent colonization of bacteria on the surface of composite resins is the key of the pathogenesis of the secondary caries promoted particularly by *S. mutans* and *S. sobrinus*. *S. mutans* has been identified as the major etiological agent of human dental caries and composes a significant proportion of the oral streptococci in carious lesions.¹¹ *S. mutans* is harbored in mature plaque. Organic acids are trapped within the glucan barrier produced by these bacteria, resulting in a prolonged low pH around the enamel surface.¹²

Restorative materials may initiate plaque and secondary caries formation in the oral cavity. Therefore, it is important to observe for initial bacterial adhesion on restorative material surface, which is the first step in plaque formation. Initial bacterial adherence to solid surfaces is facilitated by several factors, namely electrostatic¹³⁻¹⁵ and hydrodynamic interactions¹⁶, thermodynamic binding parameters¹⁷, specific binding mechanisms including adhesion receptor interactions by which bacteria bind selectively to the surface, and cementation by polysaccharide matrices or glucans.^{5,17-19}

Although polyester matrix strips promote the smoothest surface most clinical situations require bulk removal of excess composite. Additionally, finishing and polishing techniques are necessary to remove the monomer rich surface layer of the composite material.²⁰ This eventually eliminates the organic matrix, exposing and dislodging the filler particles, thus increasing the surface roughness of polymer-based materials.¹⁶ Among finishing and polishing techniques, it has been reported that the Sof-LexTM aluminum oxide, discs technique provides a smoother surface on composites compared to carbide bur finishing followed by the Astrobrush TM technique.²¹

For this investigation, the samples were not coated with saliva because previous studies^{22,23} have found that saliva coating did not significantly alter the adhesion patterns of *S. mutans* and *S. sobrinus*. This phenomenon is consistent with other investigations showing that saliva coating does not significantly alter the adhesion of Streptococci to the underlying materials.^{24,25} However, other authors suggest emulating the conditions of the oral cavity, such as temperature, constant movement and saliva coating, which could represent an environment closer to reality.^{26,27}

In the present study it was found that SR Nexco and Ceram X one have the lowest bacterial adhesion with no significant difference between them and significant difference with the other two resin composite which may be in SR Nexco due to the enhanced polymerization of all resin composite by the furnace enhanced polymerization with

no or fewer double bond with subsequent enhancement of the surface characteristics minimizing bacterial adhesion which is in agreement with Derchi G who found that Indirect dental restorative composite resins less prone to biofilm adhesion than direct composite resins.²⁸, while in Ceram X one the lower bacterial adhesion may be due to the nano-ceramic filler used in its construction giving it a higher filler loading and smoother surface which yields a smoother surface with subsequent lower adhesion of bacteria, and this in agreement with the study conducted by

Ionescu A who concluded that the proportions of resin matrix and filler particles on the surface of resin-based composites strongly influence *S. mutans* biofilm formation in vitro, suggesting that minimization of resin matrix exposure might be useful to reduce biofilm formation on the surface of resin-based composites.²⁹

Both Z250 and Herculite classic have nearly the same tendency for bacterial adhesion also Z250 used as indirect resin composite it was cured by the direct LED without any furnace which may yield degree of conversion similar to that of Herculite, also they have similar filler size so under the same finishing and polishing procedure they gave nearly equal surface characters which explains the similarity in bacterial adhesion and this in accordance with Celik C et al who studied the effect of finishing and polishing procedures on surface roughness of tooth-colored materials and found that the smoothest surface was generated with Sof-Lex Pop-on disks for all the materials tested.³⁰

The surface micromorphology of resin composites after finishing and polishing has been shown to be influenced by the size, hardness, and amount of filler particles. Harder filler particles are left protruding from the surface during polishing, as the softer resin matrix is preferentially removed in hybrid composites. Filler particles should be situated as close together as possible in order to protect the resin matrix from abrasives.³¹ A nanocluster filler particle consists of loosely bound agglomerates of nano-sized filler particles. Eventually, the surface has smaller defects and better polish retention.³² Filtek Z250 showed the least surface smoothness compared with Ceram X and **SR NEXCO** composite restorative materials, probably because it contains large glass filler particles which can be plucked away, leaving voids or rougher surface after being polished.^{31,33} For these responses the *S. mutans* less attached to **SR NEXCO** and Ceram X than Filtek Z250 and **Herculite classic**.

Under the limitation of this study it was concluded that SR Nexco is a good choice for clinical situation that requires indirect esthetic restoration in regard to bacterial adhesion. Ceram X one is a good choice for clinical situation that requires direct esthetic restoration in regard to bacterial adhesion.

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