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Original Article

Esthetic restorative materials and glass ionomer cements: Influence of acidic drink exposure on bacterial adhesion

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ABSTRACT

Objective: The purpose of this research was to evaluate and compare bacterial adhesion on five esthetic restorative materials, three glass ionomer cements (GIC), and two GIC with coat. All the materials were considered after acidic drink exposure. **Materials and Methods:** Thirty cylindrical sample of each of the 10 materials were prepared and then divided into three groups: group 1 (baseline), Group 2 (1 day in acidic soft drink), and Group 3 (7 days in acidic soft drink). Bacterial suspension of *Streptococcus mutans* was cultured and deposited onto each material, and the adhesion was evaluated through the colony-forming units determination. One-way ANOVA and Bonferroni's *post hoc* tests were applied to estimate significant differences between the esthetic materials. **Results:** The highest amount of *S. mutans* was recorded in Group 3 and the lowest in Group 1 (baseline). In general, the GIC showed bacterial adhesion values higher than the ones related to composites both in Group 2 than in Group 3. Acidic soft drinks lead a time-dependent degradation of restorative materials causing an increase of the surface rugosity. In fact, a general increase in *S. mutans* cells adhesion to treated samples was observed. **Conclusions:** The use of acidic soft drink resulted in a degradation of the surface layer of the restorative material with consequent increase of bacterial adhesion. The GIC can be considered a more friendly environment for bacterial adhesion. This is true in particular if acid substances have already deteriorated the surface.

Key words: Acidic drinks, esthetic restorative materials, glass ionomer cement, *Streptococcus mutans*

INTRODUCTION

The use of resins-based materials for direct and indirect restorations has now entered the daily clinical practice; parallel to restorative composite resins, only for direct restorations, glass ionomer cements (GIC) are used for their self-adhesive capacity and antibacterial ability.[1-4]

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One of the problems concerning adhesive restorative procedures is represented by the secondary infiltration of the restoration. This is due to the presence of a marginal gap between the filling and the tooth surface. This occurrence is more frequent

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among GIC. Moreover, it is one of the most likely causes of resurfacing of previous fillings.[5,6] One of the biggest problems for these materials is the marked susceptibility to the accumulation of bacterial plaque.[7,8] In the execution of direct restorations, due to the shrinkage stress of polymerization, it is possible the genesis of marginal discrepancies in which the cariogenic bacteria contained in plaque can give rise to a secondary infiltration.[9,10] An increase in plaque retention places patients at risk for secondary caries adjacent to the composite resin margins, and additionally, the formation of biofilm may result in gingival inflammation.[11] For indirect restorations, this issue, even if present, is limited to the cement adhesive layer.

The strong tendency to accumulate plaque for composite materials thus plays a central role in the formation of secondary caries.[12,13] The formation of oral plaque on the surface of restorative materials, and in general on the surface of the teeth, is a complex process that is determined both by the salivary and bacterial conditions of the oral cavity, but also by the eating habits and the surface on which it is formed.[14,15] Moreover, common acidic beverages (cola, energy drinks) can produce erosion of restorative materials.[16] The distribution and form of the fillers, the composition of the resin matrix, and the silane surface treatment of the filler particles significantly affect the surface degradation of the composite materials.[17] Composites are complex heterogeneous materials formed from a resin base in which are embedded the filler particles, coated with a bonding agent, of different origin with variable size and shape. This implies that the surface of such materials can be a heterogeneous interface of particles distributed on different physical-chemical phases: commercially, available restorative composite resins will present different surfaces in terms of roughness and polishing depending on the filler used.[18-21] At the same way, GIC are heterogeneous in terms of formulation, and in addition, the release of fluoride ions causes a further deterioration of the surface that over time will facilitate the adhesion of bacterial plaque.[1] However, GIC are generally used where additional protection against tooth decay is required, especially in children, since potentially reduce microleakage adhering to tooth structure, inhibit the growth of cariogenic bacteria, and neutralize the acids produced by these bacteria by releasing ions.[22,23] The surface morphology of the restoration therefore plays a crucial role in adherence of bacterial plaque, both for composite resins and GIC.[24-26]

The purpose of this research was to evaluate and compare bacterial adhesion on five esthetic restorative materials and three GIC after acidic drink exposure. The null hypothesis of the study was that there is no significant difference in bacterial adhesion values among the different restorative materials after exposure to acidic drink.

MATERIALS AND METHODS

Specimens' preparation

Five esthetic restorative materials and three GIC were evaluated in this study [Table 1].

Materials were polymerized into silicon rings (external diameter 9 mm, internal diameter 6 mm, thick 2 mm) to obtain identical specimens.[27] Cavities of these rings were slightly overfilled with material, covered with Mylar Matrix Strip (Henry Schein, Melville, NY, USA), pressed between two glass plates and polymerized for 40 s on each side using a curing unit (Celalux II, Voco, Cuxhaven, Germany). One light polymerization mode was used for each material standard: 1000 mW/cm^2 for 40 s. The light was placed perpendicular to the specimen surface, at distance of 1.5 mm or less.[27] The upper surface of each specimen was then polished

Table 1: Esthetic restorative materials specified for direct restorations used in this study

Material	Manufacturer	Type	LOT
Filtek Supreme XTE	3M ESPE, St Paul, MN, USA	Nanofilled composite	N801824
Ceram.X Universal	Dentsply De Trey, Konstanz, Germany	Nanohybrid composite with prepolymerized fillers	1608000937
Essentia	GC Corporation, Tokyo, Japan	Microfilled hybrid composite	1601121
Admira Fusion	Voco, Cuxhaven, Germany	Nanohybrid ormocer-based composite	1630296
Estelite Asteria	Tokuyama Dental Corporation, Tokyo, Japan	Supranano spherical hybrid composite	066E16
ChemFil Rock	Dentsply De Trey, Konstanz, Germany	Glass ionomer	1607000503
Equia Forte Fil	GC Corporation, Tokyo, Japan	Glass ionomer	150810A
IonoStar Plus	Voco, Cuxhaven, Germany	Glass ionomer	1631408
Equia Forte Fil + Equia Forte Coat	GC Corporation, Tokyo, Japan	Glass ionomer + multifunctional monomer coating	150810A
IonoStar Plus + Easy Glaze	Voco, Cuxhaven, Germany	Glass ionomer + nano-filled coating	1631408

with fine and superfine polishing disks (Sof-Lex Pop On; 3M ESPE, St. Paul, MN, USA) to simulate clinical conditions.

Thirty cylindrical specimens of each material were prepared in this manner. After polymerization and during the experimentation, the specimens were stored in distilled water at 37°C and 100% humidity. Each material was tested 4 weeks after polymerization.

Immersion in acidic drink

Each material is represented by 30 specimens. The 30 specimens of each esthetic restorative materials were randomly attributed to three groups (n = 10): specimens of Group 1 were used as control, specimens of Group 2 were immersed in 50 ml of acidic drink (Coca Cola/Coca Cola Company, Milano, Italy) for 1 day, and specimens of Group 3 were immersed in 50 ml of acidic drink (Coca Cola/Coca Cola Company, Milano, Italy) for 7 days. The specimen of the given material pertaining to a specific group was removed from the beverage using tweezers, sterilized in autoclave at 121°C and packed in dry plastic sterile bags before being tested with bacteria.[27,28]

Bacterial growth condition

A strain of *Streptococcus mutans* (CCUG35176) obtained from the culture collection of the University of Göteborg was used for the *in vitro* adhesion tests. *S. mutans* was cultured in Brain Heart Infusion (BHI, Difco, CA, USA) supplemented with 10% (v/v) heat-inactivated horse blood serum (Oxoid, Milan, Italy) to improve its growth. The culture of *S. mutans* was statically incubated under aerobic conditions for 16 h at 37°C. This culture, used as source for the experiments, was reduced at a final density of 1×10^{10} cells/mL as determined by comparing the OD600 of the sample with a standard curve relating OD600 to cell number.[29]

Assessment of bacterial adhesion

After extensive washing of each materials, 100 µL of an overnight growth culture (10^7 bacteria/mL) was seeded onto each sample test placed at the bottom of a 24-well plate (Celbio, Milan, Italy) and incubated at 37°C for 4 h in static conditions. The choice of this time of incubation is due to the fact that biofilm formation in the oral cavity normally occurs in 2–4 h. After incubation, loosely adhering bacteria were removed by gently washing the samples tests with PBS. Three sample tests of each experimental condition were used for total viable count (TVC). Briefly, the samples with bacterial cells were dispersed into 1 mL sterile Ringer solution (Oxoid, Milan, Italy) by vortex for 3 min.[10] Serial dilutions of the bacterial cells suspensions were prepared, and 0.1 mL of each dilution was deposited onto BHI agar (Bacto agar, Difco, CA, USA) plates. The plates were incubated for 24–48 h at 37°C and the number of colonies counted. Mean TVC values were calculated for each sample, and the results are expressed as colony-forming units (CFU) per mL.[30]

Statistical analysis

First, data were assessed to be normal by means of Shapiro–Wilk normality test. The analysis of variance (differences among substrates at each condition and differences about treatment, per substrate) was carried out using two-way ANOVA followed by Bonferroni's *post hoc* tests. Analyses were performed using Prism 4.0 (GraphPad Software, La Jolla, CA, USA). Two-tailed P = 0.05 were considered statistically significant.

RESULTS

To evaluate the *S. mutans* ability to adhere to different restorative materials with or without soft drink treatment, a TVC assay was performed. The results are shown in Table 2 and collectively represented in Figure 1.

Table 2: Colony-forming unit values of *Streptococcus mutans* cells adherent to restorative materials

Material	Mean of bacterial adhesion±SD		
	Control	After 1 day in soft drink	After 1 week in soft drink
Filtek Supreme XTE	$1.50 \pm 0.70 \times 10^2$	$3.450 \pm 0.21 \times 10^3$	$2.0325 \pm 0.75 \times 10^4$
Ceram.X Universal	$1.075 \pm 0.74 \times 10^3$	$2.625 \pm 0.41 \times 10^3$	$1.7425 \pm 0.26 \times 10^4$
Essentia	$4.050 \pm 0.14 \times 10^{3\S}$	$5.175 \pm 0.32 \times 10^{3\S}$	$1.3800 \pm 0.64 \times 10^4$
Admira Fusion	$1.50 \pm 0.42 \times 10^2$	$4.075 \pm 0.46 \times 10^3$	$1.1200 \pm 0.58 \times 10^4$
Estelite Asteria	$5.50 \pm 0.31 \times 10^2$	$8.300 \pm 0.60 \times 10^{3\S}$	$1.2600 \pm 0.78 \times 10^{4\S}$
ChemFil Rock	$2.550 \pm 0.35 \times 10^3$	$2.5375 \pm 0.45 \times 10^{4\S}$	$1.9925 \pm 0.24 \times 10^{4\S}$
Equia Forte Fil	$4.825 \pm 0.51 \times 10^3$	$1.3325 \pm 0.67 \times 10^4$	$4.1750 \pm 0.87 \times 10^4$
Iono Star Plus	$3.225 \pm 0.84 \times 10^3$	$1.8400 \pm 0.41 \times 10^{4\S}$	$1.3725 \pm 0.53 \times 10^{4\S}$
Equia Forte Fil + Equia Forte Coat	$1.00 \pm 0.23 \times 10^2$	$2.2075 \pm 0.75 \times 10^{4\S}$	$2.3125 \pm 0.75 \times 10^{4\S}$
IonoStar Plus + Easy Glaze	$1.100 \pm 0.49 \times 10^3$	$4.275 \pm 0.36 \times 10^3$	$1.4800 \pm 0.34 \times 10^4$

Results were expressed as mean of bacterial adhesion±SD. §No significant difference among data. SD: Standard deviation

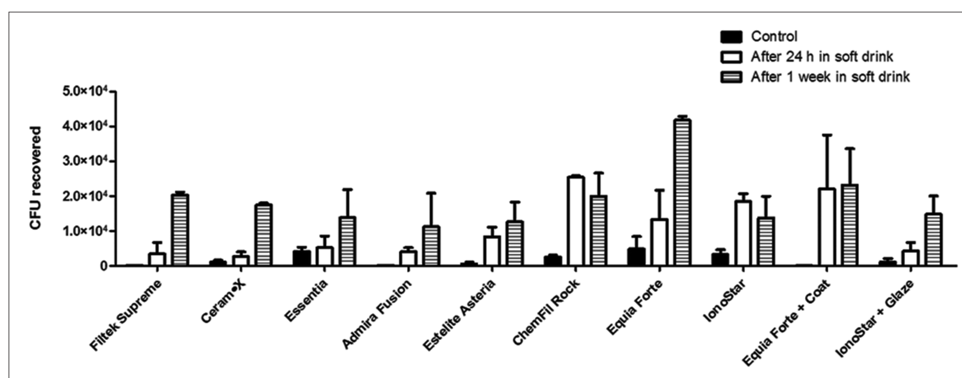

Figure 1: Colony-forming units values of *Streptococcus mutans* cells adherent to restorative materials. Results were expressed as colony-forming units/mL

The bacterial adherence values to the untreated materials (the control) are ranging from 10^2 to 5×10^3 CFU. Filtek Supreme XTE, Admira Fusion, and Equia Forte Fil + Equia Forte Coat exhibited the lowest bacterial adhesion values, whereas the CFU values for CeramX, Estelite Asteriam and Iono Star + coat were statistically significant ($P < 0.05$) and higher than the materials above mentioned. The highest bacterial adhesion values were showed for Essentia, ChemFil Rock, Equia Forte Fil, and IonoStar Plus ($P < 0.05$).

An increase in *S. mutans* cells adhesion to Coca Cola-treated samples was observed. Moreover, the treatment of Filtek Supreme XTE, Ceram•X Universal, Essentia, Admira Fusion, Equia Forte Fil, and IonoStar Plus + coat with acidic drink for long time (7 days) increases their susceptibility to be colonized compared to the same samples treated for a shorter time (24 h) ($P < 0.05$). After 24 h acidic drink treatment, Filtek Supreme XTE, Ceram•X Universal, Essentia, Admira Fusion, and IonoStar Plus + IonoStar Plus Coat showed the lowest values of bacterial adhesiveness (ranging from 2625 to 5175 × 10^3 CFU) ($P < 0.05$), without differences among themselves ($P > 0.05$). Instead, Estelite Asteria, ChemFil Rock, Equia Forte Fil, IonoStar Plus, and Equia Forte Fil + Equia Forte Coat showed the highest values (from 083 to 253 × 10^4 CFU) ($P < 0.05$), without differences among themselves ($P > 0.05$). No significant difference between samples treated for 7 days with acidic drink was observed ($P > 0.05$).

DISCUSSION AND CONCLUSIONS

Bacterial plaque is an aggregate of microorganism that adheres tenacely to each other and to the dental surfaces; it is a biofilm wrapped in an extracellular polymer matrix secreted by the bacterial flora of the oral cavity. After only few minutes of brushing, the teeth are covered with a film obtained from a layer of salivary mucoproteins that is rapidly colonized by the microorganisms present in the oral cavity: complex processes such as salivary pellicle formation, pellicle adsorption to the surface, passive transport of bacteria to the pellicle surface, coadhesion, and multiplication are the protagonists of oral plaque formation.[31] Modern restorative materials appear less affine to bacterial biofilm, consequently reducing and delaying oral plaque formation.[10]

During the initial stages of colonization, quantitatively, the bacterial adhesion to the restorative materials is related to the intrinsic physicochemical properties of restorative materials; however, also, the types of bacteria present in the biofilm and the active and passive adhesion mechanisms affect the development of oral plaque.[32,33] The growth and development of dental biofilm can be stimulated both *in vivo* and *in vitro*: to conduct the study analyzing simultaneously all the material in similar condition, it was decided to proceed by an *in vitro* research.[31] An *in vitro* biofilm model allows to the use of aseptic and removable samples, which should be discrete, representative, and reproducible. Extended exposure to soft drink was used to deteriorate and alters the surface of the materials tested in this study and creates a more suitable substrate for the formation and adhesion of bacterial plaque; the use of acidic soft drink is served to mimic the aging process of restorative materials that inevitably occur in the oral environment: the result is a degradation of the surface layer of the restorative material with consequent creation of a rougher surface.

Regarding the glass-ionomer group, no significant reduction in bacterial adhesion was recorded, even though they are known for their significant release of fluoride. On the contrary, all the GIC of the

study appeared as the most adhesive surfaces. This behavior can be explained by the reduced contact time between bacteria and GIC. As investigated in other studies, the fluoride released by these materials requires a variable time span (from 48 h to 7 days) to correctly express its antibacterial activity.[33,34] The objective of this *in vitro* study was to evaluate and compare bacterial adhesion in relation to the different types of materials and the timing of exposure of such materials to acidic drink. The results of this investigation suggest that surface morphology and roughness of restorative materials is critical for bacterial adhesion and a correlation can be established. In Group 1, differences among materials were recorded. the highest values were recorded for GIC that appear to be rougher than composites.[35] The use of coat reduces adhesion values in the control group, but the action of acids alters its surface very quickly, raising the values of adhesion to the amount of the other materials in Groups 2 and 3. All materials have demonstrated a statistically significant increase in terms of bacterial adhesion after exposure to acidic drink, which in most cases increases with exposure to harmful factor. Within the limits of the present *in vitro* study, bacterial adhesion seems to be related to erosion of restorative materials caused by acids.

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Conflicts of interest
There are no conflicts of interest.

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