INFLUENCE OF ACID MEDIUMS ON DEGRADATION AND ADHERENCE OF *STREPTOCOCCUS MUTANS* TO A INDIRECT DIMETHACRYLATE-BASED POLYMERIC MATRIX

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ABSTRACT

Objectives: Dental biofilm can be formed in greater quantities and on rough surfaces. Biofilm maturation has clinical implications because the presence of pathogenic microorganisms. This study verified surface degradation and bacterial adherence to Resilab Master® after immersion in acid solutions. Material and Methods: The specimens (diameter=10 mm/ thickness = 2 mm) were stored in deionized water for 7 days, and divided into immersion groups (n=10): Coca Cola, G1; H2OH lemon refrigerant, G2; phosphoric acid, G3; and citric acid, G4; immersed for 7 days. Mass (g) was analyzed with a digital scale, and Ra roughness (μm) by readouts on specimen surfaces using a roughness meter. Three specimens of the groups were analyzed by SEM (deionized water – control). Five specimens of the groups were introduced into test tubes with *Streptococcus mutans* and glucose. Results: They were incubated at 37ºC/24h. Results: Increase in roughness (G1.T0=0.2209, G1.TF=0.2262; G2.T0=0.1705, G2.TF=0.1756; G3.T0=0.1848, G3.TF=0.2276; G4.T0=0.1694, G4.TF=0.1905) showing no significant differences (signal test, $p>0.05$). Loss of mass, being significant (Student's-t test $p>0.05$) for Groups G1 (G1.T0=0.3560, G1.TF=0.3535) and G3 (G3.T0=0.3594, G3.TF=0.3532). Adherence of *Streptococcus mutans* forming colonies was observed on the surface degraded (UFC/mg): 3.83×10^4, G1; 2.83×10^4, G2; 4.67×10^4, G3; 3.67×10^5, G4 showing no significant differences among the groups (Kruskal-Wallis test, $p>0.05$). Conclusions: The acid solutions caused damage to the material surface with loss of matter, intensifying bacterial colonization. Solutions with the same pH and type of acid, but different compositions, showed no significant differences on indirect polymeric matrix degradation.

Keywords: Microbial adherence, Composite resin, Surface Properties, Acids, Microscopy, Electron Scanning.

INTRODUCTION

The polymerizable resin matrix typically contains one or more monomers, such as bis-phenol-A-diglycidyl dimethacrylate (Bis-GMA), urethane dimethacrylate (UDMA), and triethylene glycol dimethacrylate (TEGDMA), inorganic filler particles coated with a methyl methacrylate-functional silane coupling agent to bond the filler to the organic matrix (DRUMMOND, 2008). Studies have been conducted with different polishing systems for microhybrid composites (GEDIK *et al.*, 2005). Although light-cured composites are excellent for aesthetics procedures, both the physical and chemical properties of filled resin composites are directly related to their degree of conversion (ALMEIDA, 2009).

In spite of the development of resin composites, polishing and polymerization systems, they are frequently subject to certain deleterious actions in the oral cavity, compromising surface quality. A rough surface favors the appearance of secondary caries, greater biofilm accumulation, increases

The physico-chemical alterations that occur in restorative materials are called degradation (PRAKKI *et al.*, 2005). The critical oral environment conditions, i.e., pH changes and humidity, may increase resin composite biodegradation over time (ATTIN *et al.*, 2006). This phenomenon is a complex process that may lead the composite polymer matrix to collapse (FERRACANTE *et al.*, 1998) Composite dental restorative materials can be modified mechanically by brushing, polishing, abrasion, erosion and microcracking gingival irritation, reduces esthetics and clinical longevity of the restoration (BAGHERI *et al.*, 2007; CARLEN *et al.*, 2001) processes, or chemically through an acid medium. The consumption of carbon dioxide-based beverages, such as popular soft drinks, and isotonic beverages frequently used by sports practitioners, teas and alcoholic drinks, have an erosive potential on teeth and restorative materials (ATTIN *et al.*, 2006; KON *et al.*, 2006).

Resin composite manufacturers allege that the properties of materials for indirect use are superior to those of materials for direct use, especially due to the type, size, shape and concentration of organic particles. The aim of this research was to assess the surface degradation and bacterial adherence in indirect dimethacrylate-based polymeric matrix after immersion in acid solutions with the same pH and type of acid, but with different chemical compositions.

MATERIAL AND METHODS

RESIN COMPOSITE TESTED
Indirect dimethacrylate-based polymeric matrix Resilab Master® (Wilcos Petrópolis, Brazil). Indicated for the fabrication of crowns and small dental bridges.

PROPERTIES OF THE RESIN COMPOSITE TESTED
Matrix: BisGMA, BisEMA, UDMA, TEGMA, methacrylate monomers, photoinitiators, inhibitors, and pigments.

Filler: 88% SiO_2, quartz, Ba-Al silicate, silicate dioxide high dispersion (0.05 mm).

PREPARATION OF SPECIMENS
Forty specimens of the material were fabricated in a Teflon matrix with 6 perforations, a 10 mm base, 2 mm high. The matrix was placed on a glass lamina and the resin was inserted in the perforations in a single increment, taking care to avoid trapping bubbles. After this a second glass lamina with the same thickness was used to cover the matrix. Screws were threaded into the matrix/material set interposed between the two glass laminas to promote better material accommodation.

The specimens were polymerized in the Duetto Linea Tempus polymerization appliance (Centrum Equipment, Sorocaba, Brazil) in accordance with the specification, by pre-polymerization for 60 seconds in QuicK mode, and final polymerization for 6 minutes in the LED shower mode. Polishing was performed with fine pumice stone powder at a speed of 10-15000 RPM, goat-hair disk and (Palapol, Bremen, Germany) resin polishing paste. The specimens were stored in deionized water for 7 days at room temperature and sheltered from light until they were submitted to the initial measurements.

PREPARATION OF THE ACID SOLUTIONS

The pH meter (model W3B Microprocessor pH/mV, Bel Engineering, Monza, Italy) calibrated for the pH ranges of 4 and 7 was used to verify pH. Coca Cola soft drink (Coca-Cola Co. Porto Real Brazil LTDA) presented pH 2.90 and H2OH lemon refrigerant (Pepsi Co. São Paulo Brazil LTDA), pH 3.74. The comparative solutions with the same pH values as those of the drinks were obtained by diluting phosphoric acid in deionized water (for comparison with Coca Cola) and citric acid (for comparison with H2OH lemon refrigerant).

CHEMICAL DEGRADATION MEASUREMENT

The specimens were dried with absorbent paper and submitted to initial mass analysis using a digital scale (Bell Engineering Monza, Italy), to 04 decimal places, standard deviation = 0.1 mg and error 1 mg. After the immersion period in the acid solutions, the specimens were washed in deionized water, dried with absorbent paper and then submitted to mass analysis again.

SURFACE ROUGHNESS MEASUREMENT

Four readouts were taken with the calibrated Roughness Tester (Model TR 200, Beijing, China), (cut-off: 0.8*5 mm, Ranger: AUTO and Fil: Gauss) on the non-marked surface of each specimen, totaling 40 measurements per immersion group. After the immersion period in acid solutions, the specimens were washed in deionized water and dried with a paper towel and again submitted to final roughness analysis, totaling 40 measurements per immersion group.

MATERIAL STORAGE IN THE SOLUTIONS

The specimens were divided into 4 groups (n = 10) according to the immersion solution G1 = Coca Cola, G2 = H2OH lemon refrigerant, G 3 = phosphoric acid solution with the same pH as Coca Cola, G4 = citric acid solution with pH equal to that of H2OH refrigerant. The specimens were immersed for seven days in acidic solutions, with the face to be analyzed by the roughness meter facing up, sheltered from light and heat, at room temperature.

SCANNING ELECTRON MICROSCOPY ANALYSIS

Three specimens of each group (Coca Cola, H2OH lemon refrigerant, phosphoric acid, citric acid, deionized water - control group) were fixed onto a conductive adhesive surface, covered with approximately 20 nm of gold in a (BAL-TEC, model SCD 005) sputter coater, in high mode SEM (FEI Quanta 400, Oregon, USA) represented in 4000 X magnifications.

BACTERIAL ADHERENCE ASSESSMENT

Microbial suspension of *Streptococcus mutans* (ATCC 25175) and three specimens of each immersion group were dispensed into 4 test tubes containing TSB broth with the addition of 1% glucose. They were incubated at 37ºC/24h. The specimens were removed from the tubes and washed in a saline solution (PBS). They were agitated for 60 seconds (Phoenix agitator Model AP 56, SPLABOR, São Paulo, Brazil) in test tubes containing glass micro-pearls + 1 ml of saline solution. The microbial suspension (100 µl) was diluted in 900µL of PBS in 24 well plates and plated in triple spots equivalent to 10µl on the surfaces of the TSA plates. The TSA plates were incubated at 37ºC/24h for later readout of the number of colony forming units per milligram (CFU/mg).

STATISTICS

Roughness meter data was verified using the Shapiro-Wilk test to verify normality of data; the Kruskal-Wallis test for comparison among all the solutions; Mann-Whitney test for the comparison between two solutions and the signal test for initial and final analysis of each solution. For initial and final mass analysis, the Shapiro-Wilk test was performed to verify the normality of data, and the Student's-t test for paired samples (H2OH lemon refrigerant and citric acid groups), and Wilcoxon paired t test (Coca Cola group) ($p > 0.05$) were used.

In the comparison of microbial adherence data among the immersion groups, Kruskal-Wallis test was used ($p > 0.05$).

RESULTS

Table 1 shows the parametric statistical description of the values found in the Initial and Final roughness (Ra), respectively, per immersion group.

Phosphoric acid showed the highest increase in surface roughness in comparison with Coca Cola, however, no statistically significant difference was found. Immersion groups H2OH lemon refrigerant and citric acid showed an increase in surface roughness, which was more evident for the citric acid group, however, no statistically significant difference was found ($p> 0.05$).

Table 2 shows the values found for initial and final mass in grams per immersion group. Loss of mass was observed in all groups, being statistically significant for the Coca Cola and phosphoric acid immersion groups ($p>0.05$).

Figures 1, 2, and 3 show the surface analyses by Scanning Electron Microscopy (4000 X). After seven days of immersion in deionized water, with neutral pH, no representative areas of degradation were observed on the material surface (Figure 1). In general, the specimens showed the smooth surface before immersion in acidic solutions. However, areas of matrix degradation were observed on the surface of the material immersed in acid solutions, being more representative in the Coca Cola (Figure 2) and phosphoric acid immersion mediums (Figure 3). Damage on composite surface was more evident especially for Coca cola (Figure 2), in which filler particles were observed protruding from the surface, as well as voids suggestive of particle loss.

Acidic solutions impairs the quality of the restorative material, causing increased formation of biofilm. The Table 3 shows the number of colony forming units per immersion group, in which microbial adherence can be observed on the surface of specimens of all the groups, being more representative for the phosphoric acid and citric acid immersion medium, but presenting no statistical differences ($p> 0.05$) in comparison with the Coca Cola and H2OH lemon refrigerant immersion medium, respectively.

DISCUSSION

This study was conducted under in vitro conditions, on the premise that acid drinks influence the integrity of the restorative material. However, the deleterious action is not influenced only by the acid, but also by the composition of the medium (ALIPING-MCKENZIE, 2004) solutions with different pH such as coffee and tea promote more expressive alterations than wine, although the pH value of wine has been shown to be lower (BAGHERI *et al.*, 2007). The erosive and degradation potential does not depend exclusively on its pH, but is also strongly influenced by titration, chelating properties of the acid, frequency and duration of intake. In addition to these factors, the temperature and form of exposure to acid food and drinks also influence alterations in the restorative material (LUSSI *et al.*, 1995).

The studied material showed damage after immersion in acid solutions (Tables 1 and 2). The acid medium acts primarily on the polymer matrix and the load-matrix interface. The TEGMA monomer present in the composite can reduce softening of the surface caused by acid, by increasing the degree of polymerization of resinous materials and improving their physical properties, therefore reducing their degree of abrasion.

The intake of acid drinks, such as soft drinks with pH 3.0 or less, for a long period lead to considerable degradation of tooth enamel and resin composites (HAN *et al.*, 2008), allowing greater production of acids in the dental biofilm. Medications with acid characteristics promote surface degradation in resin composites. The influence of low pH on the composites is explained by hydrolysis of ester group in the composition of the matrix, leading to the formation of free carboxylic acid ions (PRAKKI *et al.*, 2005). Surface degradation of the resinous material is related to the content, load distribution, and matrix composition (HAN *et al.*, 2008).

SEM showed that after seven days of immersion in deionized water, with pH tending to neutral, no representative surface degradation areas were observed (Figure 1). However, over time, the leaching of the components, the swelling and degradation of the

Table 1. Asessment of initial and final roughness in umm per immersion group
Initial roughness

Initial roughness

Solutions	n	Min	max	mean	s.d.	median	iq.i (*)
Coca-Cola	10	0.1493	0.5425	0.2209	0.0136	0.1899	0.0880
Phosphoric Acid	10	0.1353	0.3300	0.1848	0.0568	0.1690	0.0581
H2OH	10	0.1223	0.2080	0.1705	0.0294	0.1706	0.0447
Citric Acid	10	0.1353	0.2138	0.1694	0.0246	0.1629	0.0359

Final roughness

Solutions	n	Min	max	mean	s.d.	median	iq.i (*)
Coca-Cola	10	0.1593	0.3430	0.2262	0.0577	0.2139	0.0860
Phosphoric Acid	10	0.1630	0.3493	0.2276	0.0689	0.1984	0.1367
H2OH lemon	10	0.1373	0.2308	0.1756	0.0283	0.1704	0.0331
Citric Acid	10	0.1535	0.2495	0.1905	0.0367	0.1775	0.0754

(*) Interquartile interval

Table 2. Parametric description of the values found in initial and final mass in grams per immersion group

Initial mass

Solutions	n	Min	max	mean	s.d.	median	iq.i (*)
Coca-Cola	10	0.3833	0.4155	0.3980	0.01117	0.3960	0.0247
Phosphoric Acid	10	0.3516	0.3709	0.3594	0.0056	0.3581	0.0978
H2OH lemon	10	0.3381	0.3558	0.3485	0.0102	0.3460	0.0170
Citric Acid	10	0.3342	0.3760	0.3545	0.0126	0.3537	0.0179

Final mass

Solutions	n	Min	max	mean	s.d.	median	iq.i (*)
Coca-Cola	10	0.3562	0.4030	0.3843	0.0178	0.3917	0.0360
Phosphoric Acid	10	0.3358	0.3553	0.3532	0.0093	0.3550	0.1100
H2OH lemon	10	0.3307	0.3658	0.3482	0.0107	0.3452	0.0160
Citric Acid	10	0.3335	0.3730	0.3530	0.0136	0.3556	0.0251

(*) Interquartile interval

FIGURE 1

SEM Analysis of the microhybrid composite resin Resilab Master® (Wilcos Petrópolis, Brazil) surface after immersion in deionized water (4000X magnification).
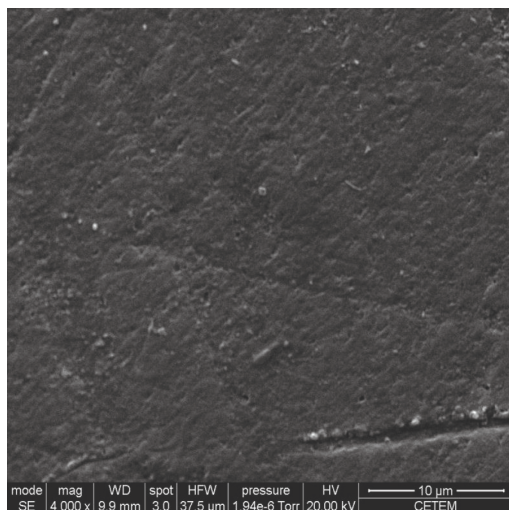

FIGURE 2

SEM Analysis of the microhybrid composite resin Resilab Master® (Wilcos Petrópolis, Brazil) surface after immersion in Coca Cola (4000X magnification).
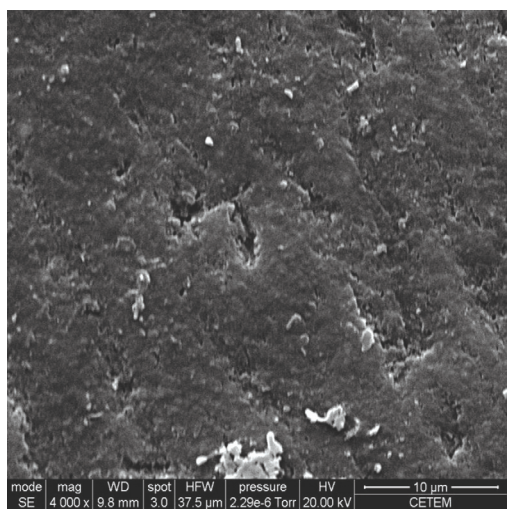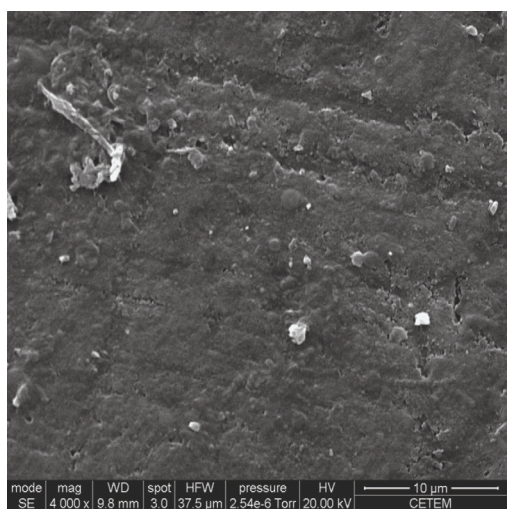

FIGURE 3

SEM Analysis of the microhybrid composite resin Resilab Master® (Wilcos Petrópolis, Brazil) surface after immersion in phosphoric acid (4000X magnification).

cross-linked matrix in the dental composite, and hydrolysis of the filler-matrix interfaces eventually lead to a decrease in mechanical properties (Figure 2 and 3). Other theories as to the cause of the degradation of the dental resin include the formation of microcracks through repeated sorption/desorption cycles, leading to hydrolytic degradation of the polymer (MUSTO *et al.*, 2002).

The influence of the immersion mediums that have phosphoric acid and citric acid in their composition can be observed, because they revealed areas of degradation on the material surface, which favors the highest form of dental biofilm. Surface alterations that occur in the restorative material become unfavorable by allowing greater impregnation of the biofilm (PRAKKI *et al.*, 2005).

Previous studies have shown that finishing, surface roughness, surface integrity, and physicochemical properties of the restoration material can influence plaque retention (DRUMMER, HARRISON, 1982; FERRACANTE *et al.*, 1998; LILJEMARK, BLOOMQUIST, 1992). The presence of subgingival terminals in composite resin restorations causes gingival inflammation (NOGUEIRA *et al.*, 2001). Even smooth and polished surfaces of microparticle resin composites are capable of retaining bacterial plaque. Studies are needed to determine which finishing techniques are best suited to clinical situations in which access is limited and restoration surfaces are complex (GEDIK *et al.*, 2005). Effectiveness of the polishers seems to be material dependent (ERGÜCÜ, OKAMOTO, 2007).

Surface roughness allows dental biofilm to be formed in greater quantities and more quickly on rough surfaces (Table 3), starting in the irregularities and afterwards expanding over the entire surface. Biofilm maturation has clinical implications because it intensifies the presence of pathogenic microorganisms. Different chemical properties and surface topographies of the various materials may play a role in biofilm formation and influence the differences in composition and general properties from one to another (AL-NAIMI *et al.*, 2007).

Table 3. Colony forming units (CFU/mg)

Solutions	mean	s.d.	median	iq.i (*)
Coca-Cola	3.83	0.764	4.0	0.750
H2OH lemon	2.83	1.258	3.0	1.250
Phosphoric Acid	4.67	1.155	4.0	1.000
Citric Acid	3.67	2.082	3.0	2.000

(*) Interquartile interval

Complete polymerization of composites is not achieved in most cases, and their degree of conversion is from 50 to 70% on average (IMAZATO *et al.*, 2001), releasing particles that can be incorporated into the tissues. The residual monomers are responsible for several problems, causing tissue irritation, promoting reduction of mechanical properties and toxic effects on pulp cells. Moreover, they can favor a structure for the development of bacterial biofilm (HEINTZE, 2006).

The objective of indirect dimethacrylate-based polymer matrix was increase the mechanical properties of the composites, and thereby lengthen their service life in the oral cavity. However, acidic solutions compromise the surface quality of indirect resin, suggesting a similar behavior of the direct and indirect systems before an acid challenge. This study confirmed the influence of low pH solutions on the surface quality of resinous materials, which may compromise the restoration and lead to the accumulation of biofilm on rough surfaces of the material.

CONCLUSIONS

Acid solutions cause damage to the restorative indirect dimethacrylate-based polymer matrix surface with loss of matter, favoring biofilm accumulation. No significant differences were observed in the deleterious actions caused on the resin composite surface when immersed in solutions that had a similar pH and the same type of acid, but with different chemical compositions.

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