DEGRADATION OF ACIDIC SOLUTIONS AND BACTERIAL ADHERENCE ON THE SURFACE OF INDIRECT POLYMERIC MATRIX

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ABSTRACT

Background: Surface roughness allows dental biofilm to be formed in greater quantities and more quickly on rough surfaces. Biofilm maturation has clinical implications because it intensifies the presence of pathogenic microorganisms. Methods: The specimens (diameter = 10 mm/thickness = 2 mm) were stored in deionized water for 7 days, and divided into immersion groups (n=10): Coffee, G1; Coca Cola, G2; H2OH lemon refrigerant, G3; 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. Microbial adherence analysis was performed from a microbial suspension of Streptococcus mutans dispensed in test tubes containing TSB broth with the addition of 1% glucose. Five specimens of the each group were introduced into the mentioned test tubes and incubated at 37ºC/24h. The specimens were then washed in a buffered saline solution (PBS). After this, they were placed in test tubes containing glass micropearls +1 ml of PBS and agitated for 60 second (Phoenix agitator). The microbial suspension was diluted in PBS and plated in triple spots on the surfaces of TSA plates. Results: There was increase in roughness (G1. T0=0.1861, G1.TF=0.2091; G2.T0=0.2209, G2.TF=0.2262; G3.T0=0.1705, G3.TF=0.1756) showing no significant differences (signal test, p>0.05). There was loss of mass, being significant (Student's-t test p>0.05) for Group G2 (G2.T0=0,3980, G2.TF=0,3843). Adherence of Streptococcus mutans forming colonies was observed (UFC/mg): 7,17 x 104,G1; 3,83 x 104, G2; 2,83 x 104, G3, showing no significant differences among the groups (Kruskal-Wallis test, p>0.05). Conclusion: It was concluded that acidic solutions compromise the surface quality of indirect polymeric matrix, propitiating an irregular structure that compromises the quality of the restoration and favor bacterial plaque formation.

Keywords: Composite resin, acidic solutions, surface properties, microbial adherence

INTRODUCTION

The bacterial plaque is a specific biofilm formed on oral tissues, restorative materials, orthodontic apparatus or any other surface in the oral cavity (Perez CR, 2008). The association between the amount of dental biofilm and superficial roughness was observed on different dental materials such as ceramics, titanium and acrylic resins (Yamauchi M, Yamamoto K, Wakabayashi M, Kawano J, 1990, Rimondini L, 1997, Kawai K, Urano M, Ebisu S, 2000). The presence of imperfections in dental restorative materials promote microbial colonization, harbors the bacteria against the force of saliva flow, chewing, hygiene procedures and allows them to establish links less reversible (Rimondini L, 1997).

In spite of the development of resin composites, polishing and polymerization systems, they are frequently subject to deleterious actions occurring in the oral cavity. The critical oral environment conditions, i.e., pH changes and humidity, may increase resin composite biodegradation over time. This phenomenon is a complex process that may lead the composite polymer matrix to collapse, causing several problems such as filler-polymer matrix debonding (Söderholm KJ, Zigan M, Ragan M, Fischlschweiger W, Bergman M, 1984) release of residual monomers (Ruyter IE, 1995) and wear and erosion caused by food, chewing and bacterial activity (Oilo G, 1992) Attempts to produce resin composite with antibacterial properties by incorporation of an antibacterial agent such as chlorhexidine have been reported, but problems can arise due to release of the inhibitory agent from the composite. Such problems may include toxic effects, influence on mechanical properties, and loss of effectiveness (Imazato S, Toriil M, Tsuchitani Y, McCabe JE, Russell RRB, 1994). Biomaterials with anti-microbial properties are highly desirable in the oral cavity (Namba N, Yoshida Y, (et al 2009).

Acidic beverages impairs the quality of the restorative material, causing increased formation of biofilm. The clinical indication should take into account the patient's eating habits, in order to obtain better clinical performance of dental restorations. The aim of this investigation was to evaluate the effect of degradation acidic media with different composition and bacterial adherence on the surface of indirect polymeric matrix.

MATERIALS 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% SiO2, quartz, Ba-Al silicate, silicate dioxide high dispersion (0.05 mm).

PREPARATION OF SPECIMENS

Thirty 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 prepolymerization 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 pH 6.8 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. Coffee (PILÃO Sara Lee ® coffees from Brazil LTDA) presented pH 5.27, Coca Cola soft drink (Coca-Cola Co. Porto Real Brasil LTDA), pH 2.90 and H2OH lemon refrigerant- citric acid group (Pepsi Co. São Paulo LTDA Brazil), pH 3.74.

CHEMICAL DEGRADATION MEASUREMENT

The specimens were dried with absorbent paper and analyzed for initial mass on a digital scale (Bell Engineering, Monza, Lombardy, Italy), to 04 decimal places, deviation = 0.1 mg and 1 mg error. After the period of immersion in acidic solutions, the specimens were rinsed in deionized water, dried with absorbent paper and re-submitted to mass analysis.

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 SOLUTIONS

The specimens were divided into 3 groups (n=10) according to the immersion solution G1=coffee, G2=Coca Cola, G3=H2OH, and were immersed in these acidic solutions under light and heat, at room temperature, for seven days.

BACTERIAL ADHESION EVALUATION

A microbial suspension of Streptococcus mutans (ATCC 25.175) was dispensed into three test tubes containing TSB broth with added glucose 1%, together with three specimens of each immersion group. They were left to incubate at 370 C/24h. The specimens were removed from the tubes and washed in saline solution (PBS). After this, they were agitated for 60 seconds (Phoenix agitator Model AP 56, SP LABOR, Presidente Prudente, São Paulo, Brazil) in test tubes containing glass micro-pearls + 1 ml of saline solution. The

TABLE 1
Results of surface roughness means (Ra; µm) before and after immersion in acidic solutions

Initial roughness			
Solutions	mean	s.d.(*)	i.iq. (**)
Coffee	0.1861	0.0359	0.0549
Coca-cola	0.2209	0.0136	0.0880
H2OH lemon refrigerant	0.1705	0.0294	0.0447
Final roughness			
Solutions	mean	s.d. (*)	i.iq. (*)
Café	0.2091	0.0457	0.0831
Coca-cola	0.2262	0.0577	0.0860
H2OH lemon refrigerant	0.1756	0.0283	0.0331

(*)Standard Deviation
(**) Interquartile interval

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

Initial mass			
Solutions	mean	s.d.	iq.i (*)
Coffee	0,3560	0,0131	0,0192
Coca Cola	0.3980	0.0117	0.0247
H2OH lemon refrigerant	0.3485	0.0102	0.0170
Final mass			
Solutions	mean	s.d.	iq.i (*)
Coffee	0,3536	0,0121	0,0198
Coca Cola	0.3843	0.0178	0.0360
H2OH lemon refrigerant	0.3482	0.0107	0.0160

TABLE 3
Results of Colony forming units (x 104 CFU/mg)

Solutions	mean	s.d. (*)	iq.i (**)
Coffee	7,17	1,041	1,000
Coca Cola	3.83	0.764	0.750
H2OH	2.83	1.258	1.250

(*)Standard Deviation
(**) Interquartile interval

microbial suspension (100 µL) was diluted in 900 µL of PBS in 24 well plates and plated on the triple spots equivalent to 10 µl on the surfaces of TSA plates. The TSA plates were allowed to incubate at 370 C/24h and later the number of colony forming units per milligram (CFU/mg) was read.

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 ($p > 0.05$).

RESULTS

In general, the specimens showed the smooth surface before immersion in acidic solutions. Table 1 shows the parametric statistical description of the values found in the Initial and Final roughness (Ra), respectively, per immersion group. Coffee pH 5.27 showed the highest increase in surface roughness in comparison with Coca Cola pH 2.90 and H2OH refrigerant lemon pH 3.74, however, no statistically significant difference was found ($p > 0.05$).

Table 2 shows the values found in the initial and final mass in grams per group of immersion. A loss of mass was observed in all groups, G1.To = 0.3560, G1.TF = 0.3536; G2.To = 0.3980, G2.TF = 0.3843; G3.To = 0.3485, G3.TF = 0.3482 and was statistically significant for the Coca Cola immersion group ($p > 0.05$).

Table 3 presents the mean number of colony forming units per group per milligram of immersion, in which microbial adherence can be observed on the surfaces of specimens in all groups, which was more significant for group immersed in coffee when compared with the other groups, however, there was no statistical difference between the groups ($p>0.05$).

DISCUSSION

The Bacterial colonization in the dental structure or restorative materials is considered critical to the development of caries, gingivitis and periodontal disease (Löe H, Theilade E, Jensen SB, 1965, Gibbons RJ, 1989, Van Houte J 1980). Studies with scanning electron microscopy revealed that the initial adhesion of microorganisms, clearly, starts with irregularities and subsequently expands across the surface (Lie T, 1979, Lie T, 1977, Nyvad B, Fejerskov O 1987).

The studied material showed acidic solutions compromise the surface quality of indirect polymeric matrix. The acid medium acts primarily on the polymer matrix and the load-matrix interface (Wongkhantee S, Patanapiradej V, Maneenut C, Tantbirojn D, 2006). The citric acid in many fruits and drinks present a much greater risk of erosion that other acids (Sobral MAP, Luz Maac, Gama-Teixeira A, Garone Netto N, 2000). Nevertheless, in the present study, coffee, with the highest pH of the beverages studied, had the highest average roughness (Table 1). Coca Cola that has phosphoric acid in its composition, led to the greatest loss of mass (Table 2), being significant (Student's-t test $p>0.05$). Cola-based soft drinks that had a pH of 2.74, the lowest among the tested beverages, caused the greatest change in the surface of dental structures (Wongkhantee S, Patanapiradej V, Maneenut C, Tantbirojn D, 2006)

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 L, Okamoto A, Fukushima M, Okiji T, 2008).

Medications with acid characteristics promote surface degradation in resin composites (Valinoti AC, Neves BV, Silva EM, Maia LC, 2008).

Previous studies have shown that finishing, surface roughness, surface integrity, and physicochemical properties of the restoration material can influence plaque retention (Prakki A, Cilli R, Mondelli RFL, Kalachandra S, Pereira JC, 2005, Svanberg M, Mjor IA, Orstavik D, 1990, Dummer PM & Harrison KA, 1982, Liljemark WF & Bloomquist C, 1992).

Multiple species of bacteria colonize the human oral cavity, and the species most commonly associated with human caries is S. mutans (Loesche WJ, 1986). The type of

restorative dental material chosen and the type of finishing / polishing treatment used affects significantly the values of surface roughness and its subsequent colonization by Streptococcus mutans in the inicial biofilm (Perez CR, 2008). It was possible to observe the bacterial colonization of Streptococcus mutans in all areas degraded by acidic solutions (Table 3) being more representative the number of colony forming units on the surfaces immersed in coffee.

Surface roughness allows dental biofilm to be formed in greater quantities and more quickly on rough surfaces, starting in the irregularities and afterwards expanding over the entire surface. Biofilm maturation has clinical implications because it intensifies the presence of pathogenic microorganisms (Kantorski KZ, Pagani C, 2007).

The presence of subgingival terminals in composite resin restorations causes gingival inflammation (Nogueira Filho GR, Stefani CM et al 2001). Even smooth and polished surfaces of microparticle resin composites are capable of retaining bacterial plaque (Keenan MP, Shillinbourg HT. JR, Duncanson MG. JR, Wade CK, 1980). 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 R, Hürmüzlü F, Akin C, Bektas OO, Özdemir AK, 2005). Effectiveness of the polishers seems to be material dependent (Ergücü Z, Türkün LS, 2007).

The association between the amount of biofilm and roughness was observed on different dental materials such as ceramics, titanium and acrylic resins (Yamauchi M, Yamamoto K, Wakabayashi M, Kawano J, 1990, Rimondini L, 1997, Kawai K, Urano M, Ebisu S, 2000, Wise MD, Dykema RW, 1975). Studies with zirconia based ceramic concluded that when it presents itself with glazed surface, accumulates more biofilm and the brushing does not remove it completely, whereas, when with a polished surface has a low tendency to form biofilm (Scott R, Kantorski KZ, Monaco C, Valandro LF, Ciocca L, Bottino MA, 2007). On the titanium surface, was found lower results of bacterial adhesion on smooth surfaces compared to rough surfaces (Wu-Yan CD, Eganhouse KJ, Keller JC, Walters KS, 1995), and results showed no significant difference of adhesion between the two surfaces (Mioralli M, 2009). One feature of composites resins that must be considered is the degree of polymerization, ranging from 50 to 70% (Wongkhantee S, Patanapiradej V, Maneenut C, Tantbirojn D, 2006). Complete polymerization of composites is not achieved in most cases, and their degree of conversion is from 50 to 70% on average (Imazato S, McCabe JF, Tarumi H, Ehara A, Ebisu S, 2001) releasing particles that can be incorporated into the tissues. The residual monomers are responsible for several problems, causing tissue irritation (Heintze SD, 2006) promoting reduction of mechanical properties and toxic effects on pulp cells. Moreover, they can favor a structure for the development of bacterial biofilm (Takahashi Y, Imazato S, Russell RRB, Noiri Y, Ebisu S, 2004).

Researches has been dedicated to the development of restorative materials containing an antibacterial agent. The antibacterial effect of experimental resins containing 0, 1 and 3% cetylpyridinium chloride (CPC) against Streptococcus mutans. Demonstrated that the bactericidal molecule still had bacteriostatic activity when immobilized on the resin matrix (Lie T, 1979).

Despite smoother surfaces accumulate less plaque initially, over time all surfaces accumulate plaque. Therefore, the surface smoothness while important, is not sufficient to prevent plaque formation (Keenan MP, Shillinbourg HT. JR, Duncanson MG. JR, Wade CK, 1980). Biomaterials with antimicrobial properties are highly desirable in the oral cavity. Ideally, bactericidal molecules should be immobilized within the biomaterial to avoid unwanted side-effects against surrounding tissues. They may then however loose much of their antibacterial efficiency (Namba N, Yoshida Y et al, 2009).

In general, no isolated property can be used to measure the quality of a material and the success of a restoration. This depends on the interaction of its physical, mechanical and biologic properties (Gouvêa CVD, Couto CF, Brito ACR, 2008). 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 OT, Itota T, Hobson RS, McCabe JF, 2007).

However, under acidic conditions, restorative materials, including the composite resins analyzed in this study, may suffer degradation over time, which can be predicted by changes in surface topography and roughness, decrease in hardness and wear resistance and substance loss (Jaeggi T, Gruninger A, Lussi A, 2006, Silva RC & Zuannon ACC, 2002). One of the determinant factors in the clinical longevity of any restoration is its surface characteristic.

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. Acidic beverages impairs the quality of the restorative material, causing increased formation of biofilm. The clinical indication should take into account the patient's eating habits, in order to obtain better clinical performance of dental restorations.

CONCLUSION

The results show that the continual consumption of acidic beverages compromises the surface quality of restorative indirect polymer matrix, propitiating an irregular structure, which may result in a greater accumulation of bacterial plaque, favoring gingival inflammations and recurrent caries. The influence of the acidic medium on the quality of the restoration is not due to pH alone and to the composition of the acidic solutions, but also the surface properties of material.

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