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The potential of salivary albumin to degrade composite resin

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ABSTRACT

Albumin is a salivary enzyme capable of cleaving ester linkages and catalyzing degradation of resin-based dental materials. However, the effect of concentration-dependent esterolytic action on composite resins as yet remains unexplored. ***Aim:*** *The purpose of this study was to evaluate whether artificial saliva formulations with different concentrations of albumin affected the surface roughness, flexural strength and microhardness of a composite resin.* ***Materials and Method:*** *Specimens (25x2x2mm) of a nanofilled composite (Filtek Z350XT, 3M/ESPE) were prepared and analyzed for average surface roughness (Ra/µm). The specimens were then allocated to 6 groups (n=30), to be treated with different salivary albumin concentrations: 0, 10, 50, 100, 200, 400 µg/mL. The specimens were stored in their respective artificial saliva groups, half of them for 24 h and the remainder for 180 days (artificial saliva renewed weekly), after which they were submitted to a new Ra reading, and tested for three-point flexural strength (FS, MPa). The specimens stored for 180 days were analyzed for Knoop microhardness (KH, Kg/mm²). Data were submitted to two-way ANOVA (Ra and FS) and one-way ANOVA (KH).* ***Results:*** *Although Ra increased ($p < 0.001$) and FS decreased ($p < 0.001$) from 24 hours to 180 days of storage, the albumin concentration did not significantly affect Ra ($p = 0.168$), FS ($p = 0.477$) or KH ($p = 0.378$).* ***Conclusion:*** *The esterolytic action of albumin did not increase the artificial-saliva-induced hydrolytic degradation of the composite resin.*

Keywords: *composite resins - saliva - albumin - hydrolysis - aging.*

Albumina salivar: investigando seu potencial de degradar resinas compostas

RESUMO

Albumina, uma enzima encontrada na saliva, é capaz de clivar ligações éster e catalisar a degradação de materiais dentários à base resina. Apesar da ação esterolítica ser potencialmente concentração-dependente, a investigação desse efeito sobre resinas compostas ainda permanence inexplorado. ***Objetivo:*** *O objetivo deste estudo foi avaliar se formulações de saliva artificial contendo diferentes concentrações de albumina afetariam a rugosidade superficial, a resistência flexural e a microdureza de uma resina composta.* ***Materiais e Método:*** *Corpos de prova em barra (25x2x2mm) foram confeccionados a partir de uma resina composta nanoparticulada (Filtek Z350XT, 3M/ESPE) e foram submetidos à leitura de rugosidade superficial média inicial (Ra, µm), em rugosímetro. Então, as amostras foram divididas em 6 grupos (n=30) de acordo com a concentração de albumina na saliva: 0, 10, 50, 100, 200, 400 µg/mL. As amostras foram armazenadas nas formulações de saliva artificial correspondente ao seu grupo, metade por 24 h e as demais por 180 dias (com trocas de saliva semanais). As amostras foram submetidas a novas leituras de rugosidade (Ra final) e avaliadas quanto à resistência flexural de três pontos (RF, MPa). As amostras armazenadas por 180 dias foram avaliadas quanto à microdureza Knoop (KH, Kg/mm2). Os dados foram submetidos a análises de variância a dois critérios (Ra e RF) e a um critério (KH).* ***Resultados:*** *Apesar de haver aumento na Ra ($p < 0,001$) e uma diminuição da RF ($p < 0,001$) de 24 h para 180 dias, a concentração de albumina não afetou significativamente a Ra ($p = 0,168$), a RF ($p = 0,477$) ou a KH ($p = 0,378$).* ***Conclusões:*** *A ação esterolítica da albumina não aumentou a degradação hidrolítica da resina composta induzida pela saliva artificial.*

Palavras-chave: *resinas compostas - saliva - albumina - hidrólise - envelhecimento.*

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INTRODUCTION

Although direct composite resin restorations in anterior and posterior teeth have clinically acceptable failure rates[1], there is ongoing research to improve them by maximizing their surface biostability, bulk and interfacial integrity. This is partly motivated by the knowledge that saliva causes hydrolysis of the composite resin polymer matrix[2] by swelling and reducing the frictional forces between polymer chains, in a process known as plasticization[3]. This can affect the physicomechanical properties of the material by increasing surface roughness, and decreasing microhardness[4] and strength[5]. Moreover, there may be unconverted methacrylate groups in the polymer network, which may break down.

Besides the effects of salivary water content, enzymes in human saliva can catalyze hydrolysis and soften methacrylate polymers[6], possibly accelerating composite resin degradation[7]. Some studies have indeed demonstrated the detrimental effect of esterolytic enzymes, such as cholesterol esterase and pseudocholinesterase, on the biostability of resin-based restorative materials[8,9]. Although albumin has been identified as the main salivary esterase involved in monomer degradation[10], its effect on composite resin remains largely unexplored.

Since albumin is capable of cleaving ester linkages[11,12], composite resins containing methacrylate-based monomers such as Bis-GMA (bisphenol A-glycidyl methacrylate), TEGDMA (triethylene glycol dimethacrylate), Bis-EMA (bisphenol A ethoxylate dimethacrylate) and UDMA (urethane dimethacrylate) can be degraded. Albumin concentration varies widely among individuals[13], and is higher among patients with gingivitis and periodontitis[14], suggesting potential concentration-dependent esterolytic action of salivary albumin in the mouth.

The aim of this *in vitro* study was to assess the effects of artificial saliva formulations replicating the albumin concentrations of human saliva on some physicomechanical properties of a nanofilled composite. The null hypotheses were: 1) that artificial saliva containing different albumin concentrations would not affect the physicomechanical properties of a nanofilled composite; 2) that storage time would not affect the physicomechanical properties of a nanofilled composite.

MATERIALS AND METHOD

Experimental design and sample size calculation

This study adopted a completely randomized design in a 6x2 factorial arrangement, with six albumin concentrations (0, 10, 50, 100, 200 and 400 µg/mL, according to the range observed elsewhere)[13] and two storage periods (24 h and 180 days). The combinations among all the levels of each independent variable yielded 12 groups.

Sample size was calculated according to preliminary surface roughness data collected from three specimens per group, which showed an effect size of 0.28. G*Power 3.1.9.4 software (Heinrich-Heine Düsseldorf University, Düsseldorf, Germany) indicated that at $\alpha = 0.05$ and a power of 0.80, 180 samples would be needed, i.e., 15 samples per group. The dependent variables were surface roughness (µm) and flexural strength (MPa), measured after 24 h and 180 days, and Knoop microhardness (Kg/mm²), assessed after 180 days.

Specimen preparation

A total 180 specimens (25x2x2 mm, ISO 4049) were fabricated using a nanofilled composite (Filtek Z350XT, 3M/Espe, St. Paul, MN, USA). The composite was inserted in a Teflon mold, covered with a polyester strip and a glass microscope slide, and received a 500-gf axial load against the glass slide for 30 seconds. The glass was removed, and the composite was light-cured (Bluephase, Ivoclar Vivadent AG, Schaan, Liechtenstein) at three locations on the sample, for 20 seconds each, with an irradiance of 1000 mW/cm². A radiometer was used to monitor the irradiance after the preparation of every five specimens. Specimens were removed from the mold and any excess material was carefully removed with a scalpel blade.

Specimens were then randomly allocated to 12 groups (n = 15) of artificial saliva with different albumin concentrations (0, 10, 50, 100, 200, 400 µg/mL) and storage times (24 h or 180 days).

Baseline surface roughness measurement

Surface roughness was calculated as the arithmetic mean deviation of the profile (Ra, µm), and 0.25 mm cut-off value, using a roughness tester (Surftest SJ-210, Mitutoyo, Japan). It was measured at three locations on each specimen, and averaged.

Exposure to artificial saliva

Specimens were stored individually in an incubator at 37ºC for 24 h or 180 days in 1 mL of artificial saliva with one of the different albumin concentrations. For the samples stored for 180 days, the saliva formulations were renewed weekly.

The artificial saliva formulation (pH 6.75) used in this study was described by McKnight-Hanes and Whitford[15], and modified by Amaechi et al.[16]. It was composed of sodium hydroxymethyl benzoate, sodium carboxymethylcellulose, KCl, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, K_2HPO_4 and KH_2PO_4. Bovine albumin (Sigma-Aldrich, St. Louis, MO, USA) was added to artificial saliva to prepare albumin concentrations of 10, 50, 100, 200 or 400 µg/mL. The artificial saliva of the control group contained no albumin.

After completing the storage time (24 h or 180 days), the samples were rinsed in purified water for 20 seconds, and allowed to dry at room temperature (23ºC).

Post-storage surface roughness measurement

The post-storage surface roughness was measured as described above in the baseline measurement section. The change in surface roughness (ΔRa) was calculated as the difference between post-storage and baseline values.

Flexural strength testing

The specimens were placed in a three-point bending fixture mounted on a universal testing machine (Emic, São José dos Pinhais, PR, Brazil), which held the composite resin bar on two supports (20 mm apart). A crosshead placed at the center of the bar loaded the sample to failure at a crosshead speed of 0.5 mm/min, and the maximum load was recorded. Flexural strength (MPa) was calculated according to the following equation: $FS = 3PL/2wb^2$, where "P" is the maximum load (N); "L" is the distance between the two supports (in mm); "w" is the sample width (mm); and "b" is the sample height (mm).

Knoop microhardness measurement

The microhardness of the specimens stored for 180 days was assessed with a microhardness tester (HVS-1000, Pantec, São Paulo, SP, Brazil), using a Knoop indenter, a 50-g load and a 20-s load time. Five indentations were performed on each specimen and averaged.

Statistical analysis

All the data were assessed initially for the assumptions of normal distribution and homoscedasticity, using the Shapiro-Wilk and Levene tests, respectively. The surface roughness change and flexural strength data were evaluated by two-way analysis of variance (ANOVA) to investigate the effect of the different albumin concentrations and storage times. The data were tested using one-way ANOVA to compare the microhardness values among the groups after 180 days of storage. Statistical calculations were run using SPSS 23 (SPSS Inc., Chicago, IL, USA), at a significance level of 5%.

RESULTS

Two-way ANOVA indicated no significant interaction between the albumin concentration and the storage time, either for surface roughness change ($p = 0.061$), or for flexural strength ($p = 0.631$). Although these properties were not significantly affected by the albumin concentration (roughness change: $p = 0.618$; flexural strength: $p = 0.477$), surface roughness was significantly higher after 180 days than after 24 h ($p < 0.001$; Table 1). After 180 days, there was a statistically significant reduction in the flexural strength values ($p < 0.001$; Table 1). The Knoop microhardness values did not differ significantly among the groups stored for 180 days in the different albumin-containing artificial saliva formulations ($p = 0.378$; Table 1).

Table 1. Mean (standard deviation) for surface roughness change, flexural strength and Knoop microhardness according to albumin concentration in artificial saliva after 24 h and 180 days' storage.

Dependent variable	Albumin concentration (µg/mL)	24 h	180 days	Grand mean
Roughness change (post-storage – baseline, µm)	0	0.022 (0.041)	0.018 (0.030)	0.020 (0.035) A
	10	-0.003 (0.039)	0.041 (0.039)	0.019 (0.044) A
	50	0.005 (0.041)	0.045 (0.055)	0.025 (0.051) A
	100	0.003 (0.051)	0.074 (0.067)	0.039 (0.069) A
	200	0.008 (0.044)	0.052 (0.054)	0.030 (0.053) A
	400	0.016 (0.046)	0.034 (0.052)	0.025 (0.049) A
	Grand mean	0.008 (0.043) a	0.044 (0.052) b	-
Flexural strength (MPa)	0	130.6 (34.7)	76.0 (21.1)	103.3 (39.6) A
	10	126.6 (35.7)	88.5 (19.4)	107.5 (34.3) A
	50	121.9 (37.7)	77.7 (24.4)	99.8 (38.5) A
	100	105.1 (45.2)	77.2 (29.0)	91.1 (39.9) A
	200	125.7 (38.0)	71.7 (22.1)	98.7 (41.0) A
	400	119.0 (49.4)	70.4 (32.6)	94.7 (48.0) A
	Grand mean	121.5 (40.2) a	76.9 (25.2) b	–
Knoop microhardness (Kg/mm^2)	0	_	60.4 (6.0)*	-
	10	_	57.9 (5.0)*	–
	50	_	58.0 (4.5)*	–
	100	_	59.4 (4.8)*	–
	200	_	56.5 (4.8)*	–
	400	_	57.6 (2.7)*	–

Means followed by the same uppercase letters indicate no statistically significant difference among groups stored in saliva formulations containing different albumin concentrations. Means followed by different lowercase letters indicate statistically significant difference between the values obtained at 24 h and 180 days. Microhardness means followed by asterisks do not differ from one another significantly.

DISCUSSION

This *in vitro* study investigated whether the potential catalytic action of salivary albumin would affect physicomechanical properties of a nanofilled composite stored for 24 h and 180 days in saliva formulations with clinically established albumin concentrations. The results showed that the albumin esterase effect, if any, remained unnoticeable in the Filtek Z350XT nanofilled composite resin, regarding surface roughness change, flexural strength or microhardness at the concentrations and storage times tested. Therefore, the null hypothesis that artificial saliva containing different albumin concentrations would not affect the physicomechanical properties was accepted.

One possible explanation for these results is the restricted mobility of the proteins in the polymeric chain of the composite resin tested, which may have reduced the bulk damage to the composite[3], thereby avoiding softening or a detrimental decrease in flexural strength. The fact that the flexural strength was not reduced may have been reinforced by the effect of the Mylar strip on the resin, which caused a resin matrix-rich surface to form on the composite resin and a reduction in the diffusion pathways through the interface between the filler particles and the resin matrix. As a result, one can expect less detrimental effect on bulk properties of the composite resin. In future research, it would be interesting to test whether polished composite resins behave differently in response to albumin.

Although certain types of esterase have been associated with softening and reduction in the strength of composite resin restorations[6,17-19], previous studies have often used cholesterol esterase and pseudocholinesterase. The rationale for selecting albumin for the current study was to simulate a condition that would be as similar as possible to reality, since albumin plays a major role (if not the most important) in the esterolytic process of polymeric materials[10]. It should be noted

that bovine albumin replicates the effect caused by human albumin[20].

Although the Mylar-strip surface provides the maximum ester linkages required for albumin to catalyze hydrolytic degradation, the albumin did not significantly affect surface roughness at the concentrations and times tested. This could be attributed to the material-dependent nature of this degradative process[4]. In fact, enzymatic degradation may depend on the monomeric composition of each material[21]. However, the filler content also influences degradation, given that composite resins containing more than 80% filler by weight are more resistant to degradation[22]. In this respect, the highly filled (>80% by weight) composite resin used in this study (Filtek Z350XT; 82% filler by weight) may have resisted enzymatic degradation. The use of other composite resins in other studies may have led to different results, based on the dissimilarities of their matrix resin composition, conversion degree and filler content, a difference that warrants further research.

The lack of differences in surface roughness among groups may also be explained by the supersaturation of the artificial saliva, which may have led to the formation of precipitates. Although the saliva was renewed weekly, these precipitates could have remained on the surface of the specimens, occluding their micro-irregularities, and thereby equalizing their surface roughness values.

It is important to note that despite the variation in albumin concentration among individuals, the amount of 400 µg/mL used in the present study as the maximum concentration in whole saliva was based on the range measured by Delacroix et al.[13] (16-385 µg/mL). However, other studies show that albumin concentration in saliva can be up to 1,000 µg/mL[14], which may increase the risk of degradation. Since albumin is derived from plasma[14], cervical restorations, especially in patients with periodontal disease, would come into contact with albumin concentrations higher than those in whole saliva.

In relation to the storage time, the specimens stored for 24 h and 180 days differed significantly, with higher roughness and lower flexural strength after 180 days. Therefore, the second null hypotheses that storage time would not affect the physicomechanical properties of a nanofilled composite was rejected. A previous paper reported that a short-term storage period (28 days) did not alter composite surface roughness[23]. On the other hand, longer storage periods (12 months) in aqueous solutions have been associated with increased surface roughness of composite resins[4]. However, considering the 6-month period of this study, the composite resin used, and the enzyme concentrations tested, the specific role of albumin in accelerating composite resin degradation cannot be confirmed.

Regarding flexural strength values, the decrease observed in the present study has also been reported elsewhere[7], and may be ascribed to the effect of the water that enters the material through spaces formed between the polymeric chain linkages, resulting in plasticization[3]. When water enters the structure of the material, it degrades ester linkages[24], compromising the physicomechanical properties of the material.

It is also important to note, regarding the esterolytic action of salivary enzymes, that the adhesive layer may be more susceptible even though the composite resin may not present detectable changes at the tooth-restoration interface. Indeed, adhesive systems are susceptible to degradation by cholesterol esterase and pseudocholinesterase[25]. This effect could be even more substantial with albumin, which is the main esterase involved in the catalysis of hydrolytic degradation[10], compared to cholesterol esterase and pseudocholinesterase. This degradation may facilitate the development of secondary caries[9,24].

DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflict of interest regarding the research, authorship, and/or publication of this article.

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