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Original research

Surface properties after chemical aging of chlorhexidine delivery systems based on acrylic resin

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ABSTRACT

Objectives: To evaluate the effect of chlorhexidine incorporation on the surface free energy and microtensile bond strength of three reline acrylic resins, after chemical aging.
Methods: For each of the studies, six experimental groups were set according to reline resin and chlorhexidine incorporation [Kooliner – 0% vs. 2.5% chlorhexidine; Ufi Gel Hard and Probase Cold – 0% vs. 5% chlorhexidine]. The specimens were submitted to a chemical aging process for 4 weeks (pH fluctuation in artificial saliva, cycles of 6 hours at pH=3 and 18 hours at pH=7). For the first study, 42 specimens were prepared (n=7) and, after chemical aging, the surface free energy was calculated. For the adhesive strength study, 36 denture base resin cubes were prepared and reline resin was applied to them according to the experimental group (n=6). Five sticks (1×1 mm section) were obtained from each specimen and submitted to chemical aging followed by microtensile test (1 kN; 1 mm/min). Data were analyzed with Kruskal-Wallis and Mann-Whitney non-parametric tests (α=0.05).
Results: Differences ($p<0.05$) were observed between resins, both in surface energy and in bond strength. The chlorhexidine incorporation did not affect the surface energy of neither of the resins and also did not affect the bond strength of Kooliner and Ufi Gel Hard ($p>0.05$). Incorporating 5% chlorhexidine (p=0.004) decreased the Probase Cold bond strength to the denture base resin.
Conclusions: After chemical aging, chlorhexidine incorporation only negatively affected the bond strength of Probase Cold. (Rev Port Estomatol Med Dent Cir Maxilofac. 2019;60(4):155-162)

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Propriedades de superfície após envelhecimento químico de sistemas de libertação de clorexidina à base de resina acrílica

R E S U M O

Objetivos: Avaliar o efeito da incorporação de clorexidina na energia de superfície e resistência adesiva à microtração de três resinas acrílicas de rebasamento, após envelhecimento químico.

Métodos: Para cada um dos estudos foram criados 6 grupos experimentais de acordo com resina de rebasamento e incorporação de clorexidina (*Kooliner* – 0% vs. 2,5%; *Ufi Gel Hard* e *Probase Cold* – 0% vs. 5%). Os espécimes foram submetidos a um processo de envelhecimento químico durante 4 semanas (variações de pH em saliva artificial, com ciclos de 6 horas em pH=3 e 18 horas em pH=7). Para o primeiro estudo foram preparados 42 espécimes (n=7) e após envelhecimento químico foi calculada a energia de superfície. Para determinação da resistência adesiva, foram preparados 36 cubos de *Probase Hot* e sobre estes aplicada a resina de rebasamento de acordo com o grupo experimental (n=6). Obtidos 5 palitos (secção 1×1 mm) de cada espécime, foram sujeitos a envelhecimento químico, seguido de teste de microtração (1 kN; 1 mm/min). Os dados foram avaliados estatisticamente com testes não-paramétricos segundo Kruskal-Wallis e Mann-Whitney (α=0,05).

Resultados: Observaram-se diferenças (p<0,05) entre as resinas, tanto na energia de superfície como na resistência adesiva. A energia de superfície de nenhuma das resinas foi afetada pela incorporação de clorexidina, que também não afetou a resistência adesiva de *Kooliner* e de *Ufi Gel Hard* (p>0,05). Incorporar 5% de clorexidina reduziu significativamente (p=0,004) a resistência adesiva de *Probase Cold*.

Conclusões: Após envelhecimento químico, a incorporação de clorexidina apenas afetou negativamente a resistência adesiva de *Probase Cold*. (Rev Port Estomatol Med Dent Cir Maxilofac. 2019;60(4):155-162)

Palavras-chave:
Resinas acrílicas
Clorexidina
Estomatite protética
Tensão superficial
Resistência à tração

Introduction

The physiologic progression of a residual ridge resorption can affect the adaptation and retention of a denture base and lead to loss of masticatory efficiency and comfort, as well as possible trauma of the underlying tissues.[1,2] In order to avoid denture rejection, periodic examination of the denture and underlying support tissues is advised to detect these changes and, if necessary, a relining procedure should be performed.[3,4]

Denture stomatitis is a highly prevalent chronic condition, usually asymptomatic, that manifests as a diffuse inflammation of the mucosa in contact with the denture.[5-8] Despite the evidence of fungal etiology, several factors have been suggested in a multifactorial etiology.[9] Even though other *Candida* species may contribute to this disease, *Candida albicans* is the main causative agent, and its adherence to the oral mucosa and the denture surface is considered the first step in the pathogenesis of denture stomatitis.[9]

Treatment usually involves topical or systemic antifungal therapy, good oral hygiene, denture cleaning procedures, adjustment of denture failures, discontinuation of night-time denture wear, nutritional restitution and relining or replacing of the denture.[7,10-17] Topical and systemic antifungal therapy is commonly applied, in spite of contributing to highly frequent relapse episodes of the disease, since the maintenance of the therapeutic dosage of the drugs is very dependent on complex regimes of patient compliance. Also, antifungal therapy does not provide the complete eradication of the microorganisms from the dentures surface.[16-19] Chlorhexidine (CHX), an antimicrobial agent that acts against a wide range of microorganisms, including *Candida* species, is another possible therapy.[14-17] However, its efficiency in topical solutions depends on the turnover of saliva and the cleansing action of the oral musculature.[14,20] In order to increase the availability of the agent in the target area at a therapeutic dosage, loading reline acrylic resins with CHX has been proposed as a therapeutic approach for denture stomatitis, to allow a slow and sustained releasing for at least 28 days, with a more effective antifungal activity than other drugs.[14-18,21,22]

Reline acrylic resins may have different chemical compositions and structural arrangements. Kooliner and Ufi Gel Hard, a non-crosslinking and a crosslinking relining material, respectively, are both poly(ethyl methacrylate)-based resins and are used in a direct technique, being polymerized in the mouth. On the other hand, Probase Cold, which is a poly(methyl methacrylate)-based reline material, is used in an indirect technique, being polymerized under laboratory conditions.[23,24]

The incorporation of CHX into polymeric materials may affect their mechanical and surface properties.[25] A CHX concentration of 2.5% for Kooliner and 5% for both Ufi Gel Hard and Probase

Cold was established as the minimal concentration effective against *Candida albicans*, without affecting mechanical properties, both immediately after acrylic preparation and after thermal aging.[17,26-31] However, the effect of the chemical aging process on the resin properties may also be relevant since, when in function inside the oral cavity, reline acrylic resins may biodegrade faster due to daily exposure to an acidic environment.[32-34]

The surface free energy of a solid consists in the sum of components arising from dispersive (apolar) and polar contributions. Changes in this property will impact the surface wettability of the material and, consequently, its interaction with saliva molecules, microbial molecules and other materials.[13,35] Also, an adequate bonding between the denture base resin and the reline material is essential since a failure can harbor bacteria, promote staining, decrease the strength of the denture and cause fractures.[11,16-39]

The objective of this study was to evaluate the impact of incorporating CHX in reline acrylic resins subjected to oral pH fluctuations on their surface free energy and their microtensile bond strength to the denture base resin. The following hypotheses were established: 1) no differences in surface free energy and bond strength are observed between the three resins; 2) CHX incorporation does not influence the surface free energy; and 3) incorporating CHX in the reline resins does not affect their bond strength to the denture base.

Materials and methods

Three auto-polymerizing reline acrylic resins were selected: two direct reline resins mainly formed by poly(ethyl methacrylate) – Kooliner and Ufi Gel Hard, and an indirect reline resin mainly composed by poly(methyl methacrylate) – Probase Cold.

The acrylic resins were manipulated according to the manufacturer's recommendations (Table 1). The liquid was measured using a graduated pipette. The powder of each reline acrylic resin and the chlorhexidine diacetate monohydrate (Panreac Applichem, Darmstadt, Germany) were weighted using a precision balance (A&D FZ-200i Company, Limited, Tokyo, Japan), to obtain a 2.5%powder weight (w/w) for Kooliner and a 5% powder weight (w/w) for Ufi Gel Hard and Probase Cold. They were then mixed with a mortar and a pestle until homogenization was achieved. Two groups were set for each material: one control group without CHX, and one experimental group with CHX incorporation.

For the surface free energy (γ) study, a metallic rectangular mold was used to prepare 42 specimens (n=7) with standardized dimensions (25x16x1 mm). After allowing the resin to polymerize under pressure, the specimens were polished with a 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL). They were then submitted to a chemical aging process in a graduated falcon tube filled with artificial saliva, with a ratio of 1 g / 5 mL, in an incubator (Memmert, Schwabach, Germany) at 37 ± 2ºC with constant gentle shaking (300 rpm).[40,41] The specimens were exposed to pH fluctuations in cycles of 6 hours in saliva at pH=3 and 18 hours at pH=7, for 28 days. Between each cycle, specimens were washed with distilled water and dried with absorbent paper (Figure 1).

The γ test was performed with a Tensiometer K12 (Kruss, Hamburg, Germany), where the specimen was suspended and immersed 4 mm in water (Merck Millipore, Germany) and 1,2-propanediol (1-2 Propanediol R.822324-1L; Merck, Germany) at a speed of 20 μms^{-1}. The contact angles were measured at 25 ± 0.1ºC using the Wilhelmy plate technique, and were used to estimate total surface free energy (γ), as well as its dispersive (γ^d) and polar components (γ^p), based on the harmonic mean method proposed by Wu.[42,43]

For the microtensile bond strength (μTBS) study, 36 cubic (10×10×10 mm) specimens of the heat-polymerizing denture base acrylic resin Probase Hot (Ivoclar Vivadent AG, Liechtenstein) were produced by a conventional flasking technique. These specimens were submitted to 2500 thermal cycles, alternating submersions of 20 seconds at 5ºC and 55ºC with a duel time of 5 seconds, on a thermocycling machine (Refri

Table 1. Materials under evaluation in the investigation.

Material	Composition P | Li		P/Li Ratio (G/Ml)	Curing cycle	Manufacturer	Batch number (Expiration date)
Kooliner	PEMA	IBMA	1.4/1	10 min 37ºC	GC America Inc., Alsip, IL., USA	P 1707271 (2020-07) Li 1704191 (2020-04)
Ufi Gel Hard	PEMA	1,6-HDMA	1.77/1	7 min 37ºC	Voco GmbH., Cuxhaven, Germany	P 1816582 (2020-09) Li 1804406 (2020-02)
Probase Cold	PMMA	MMA	1.5/1	15 min 40ºC 3 bar	Ivoclar Vivadent AG., Liechtenstein	P XT1222 (2022-10-24) Li X45991 (2022-10-11)
Chlorhexidine	Chlorhexidine diacetate monohydrate		–	–	Panreac Applichem, Darmstadt, Germany	8F015944 (10/2023)

P = Powder; **Li** = Liquid; **PEMA** = Poly(ethyl methacrylate); **IBMA** = Isobutyl methacrylate; **1,6-HDMA** = 1,6-hexanodioldimethacrylate; **PMMA** = Poly(methyl methacrylate); **MMA** = Methyl methacrylate.

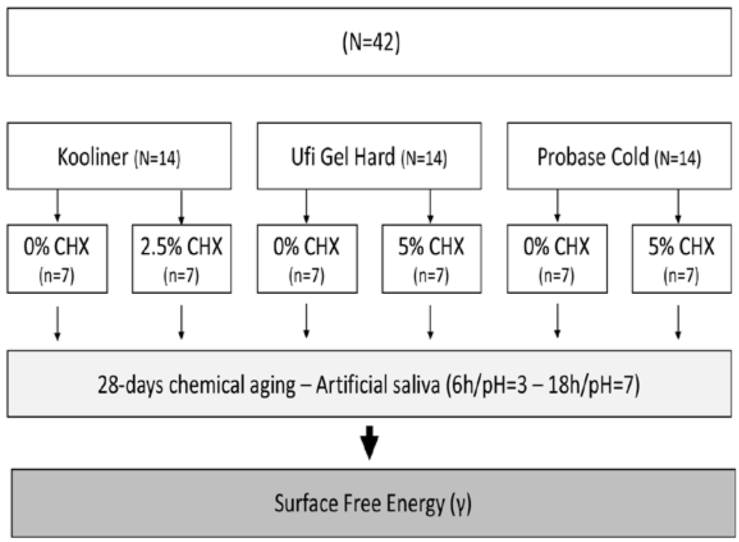

Figure 1. Experimental design for surface free energy (n=7). [CHX – chlorhexidine]

200-E, Aralab, Cascais, Portugal), in order to simulate a 3-month aging process inside the oral cavity.[44] The surfaces of the denture base specimens were finished to a 3-mm thickness in a rotational grinding and polishing machine (DAP-U, Struers, Denmark) with a 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL). Their thickness was confirmed using a digital micrometer (Mitutoyo Digimatic, MFG. Co., Ltd. Tokyo, Japan) with ± 0.01-mm precision.

Afterward, the relining procedure was performed. The surface of the Probase Hot was previously conditioned according to the reline resin used. For Kooliner and Probase Cold, the bonding area was scrubbed once with a microbrush soaked with the correspondent monomer. In Ufi Gel Hard groups, a specific conditioner was applied to the area and let dry for 30 seconds, as recommended by the manufacturer. Then, the freshly mixed reline resin was placed on the bonding area.

After polymerization, five sticks with a section of 1 mm^2 were obtained from each specimen, using an Isomet cutting machine 1000 Precision Saw (Serial No. 666-IPS-03518; Buehler, Lake Bluff, IL, USA), under constant water refrigeration. The sticks were identified, submitted to an aging process in artificial saliva by previously described methods, and tested. The μTBS test was performed with a universal testing machine (Instron model 4502, Instron Ltd., Bucks, HP 12 3SY, England) using a 1 kN load cell and a 1 mm/min crosshead speed, until fracture (Figure 2). The failure mode was assessed by two calibrated observers with

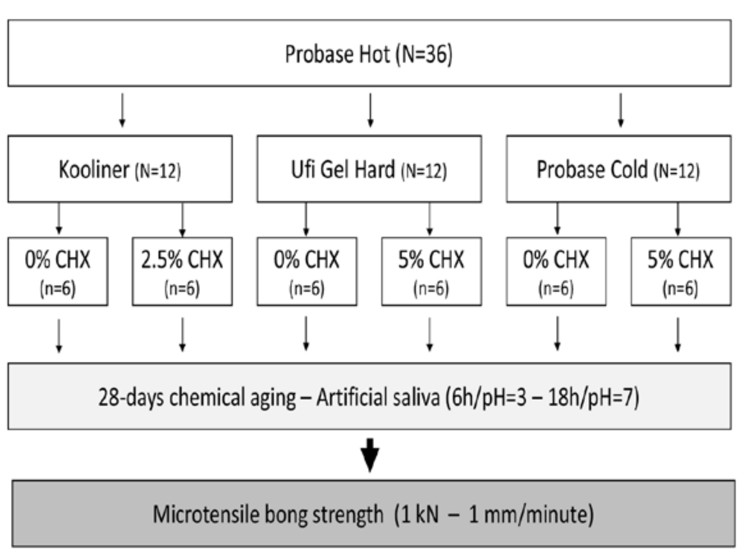

Figure 2. Experimental design for microtensile bond strength (n=6). [CHX – chlorhexidine]

a stereomicroscope and classified as adhesive, cohesive or mixed: adhesive if it occurred between the reline resin and the denture base resin, cohesive when it occurred exclusively within one of the resins, and mixed if it occurred in the interface of the two resins but included residues of reline resin.

Since normality was not verified (Shapiro-Wilk normality tests, $p<0.05$), γ and μTBS data were submitted to Kruskal-Wallis and Mann-Whitney nonparametric tests (α=0.05). Failure mode data were analyzed with chi-square tests (α=0.05).

Results

The total surface free energy values (Table 2) ranged between 41.9 mN/m, in the Ufi Gel Hard loaded with 5% CHX, and 32.1 mN/m, in the Kooliner without CHX.

The three reline acrylic resins presented statistically significantly ($p<0.001$) different total surface free energy values. The surface energy of the Kooliner specimens was lower than both Probase Cold ($p=0.046$) and Ufi Gel Hard ($p<0.001$) specimens. Also, Probase Cold showed higher surface energy values than Ufi Gel Hard ($p=0.035$).

Regarding the effect of the CHX incorporation, no statistically significant influence was detected in the three reline resins (Kooliner, $p=0.165$; Ufi Gel Hard, $p=0.383$; Probase Cold, $p=0.902$) (Figure 3).

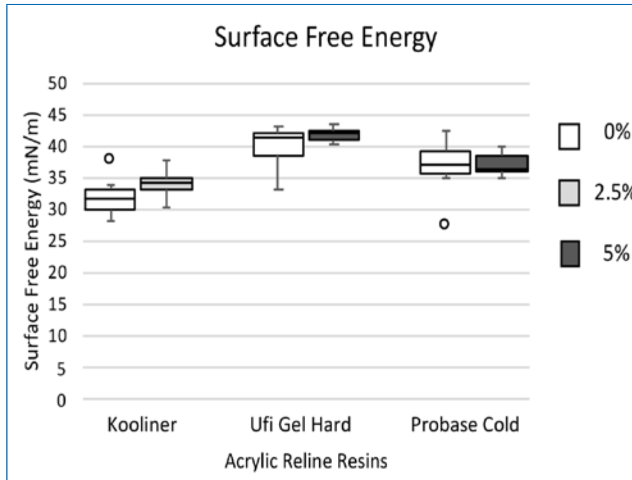

Figure 3. Boxplot of total surface free energy (mN/m) distribution among experimental groups after chemical aging [Kooliner – 0% vs. 2.5% (*p*=0.165); Ufi Gel Hard – 0% vs. 5% (*p*=0.383) and Probase Cold – 0% vs. 5% (*p*=0.902)].

The μTBS values (Table 3) ranged between 45.0 MPa, in the Probase Cold without CHX, and 13.0 MPa, in the Kooliner without CHX. Probase Cold specimens yielded a higher μTBS than Kooliner ($p<0.001$) and Ufi Gel Hard ($p=0.004$) specimens. No

Table 2. Descriptive statistics of the surface free energy according to experimental group (n=7)

Material	CHX %	Total Surface Free Energy (γ) (mN/m)		Dispersive Component (γ^d)		Polar Component (γ^p)	
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	MEDIAN (IQR)
Kooliner	0%	32.1 (3.19)	31.8 (4.20)	15.8 (6.64)	16.7 (4.30)	16.3 (9.23)	14.0 (7.60)
	2.5%	34.1 (2.25)	34.4 (2.30)	16.2 (3.04)	15.2 (5.00)	17.9 (4.79)	19.2 (4.20)
Ufi Gel Hard	0%	39.9 (3.48)	41.5 (4.40)	19.0 (2.39)	18.7 (2.40)	20.8 (5.48)	21.1 (6.70)
	5%	41.9 (1.09)	42.0 (1.80)	18.1 (2.69)	19.0 (4.70)	23.8 (2.92)	24.2 (3.80)
Probase Cold	0%	36.7 (4.60)	37.2 (4.40)	12.3 (5.50)	15.4 (9.50)	24.4 (6.78)	23.3 (10.40)
	5%	37.2 (1.75)	36.6 (2.80)	19.1 (3.74)	18.1 (2.90)	18.1 (4.59)	19.3 (3.50)

CHX % – chlorhexidine concentration; **SD** – standard deviation; **IQR** – interquartile range

Table 3. Descriptive statistics of the microtensile bond strength (μTBS) according to experimental group (n=6)

MATERIAL	CHX %	μTBS (MPa)		Failure mode		
		MEAN (SD)	MEDIAN (IQR)	Adhesive n (%)	Mixed n (%)	Cohesive n (%)
KOOLINER	0%	13.0 (3.74)	12.7 (5.88)	29 (96.7)	1 (3.3)	0 (0)
	2.5%	13.5 (3.64)	13.4 (6.01)	29 (96.7)	1 (3.3)	0 (0)
UFI GEL HARD	0%	22.6 (7.37)	22.2 (13.96)	19 (63.3)	5 (16.7)	6 (20.0)
	5%	18.3 (5.58)	16.8 (9.56)	12 (40.0)	5 (16.7)	13 (43.3)
PROBASE COLD	0%	45.0 (3.28)	44.0 (5.84)	27 (90.0)	2 (6.7)	1 (3.3)
	5%	33.7 (1.90)	33.1 (3.45)	27 (90.0)	3 (10.0)	0 (0)

CHX % – chlorhexidine concentration; **SD** – standard deviation; **IQR** – interquartile range

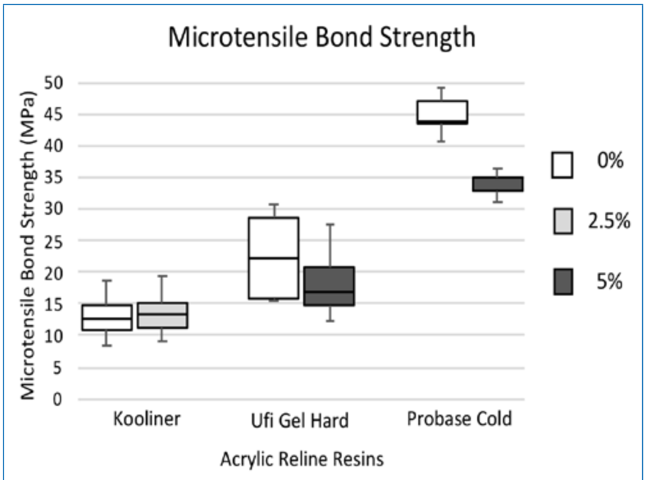

Figure 4. Boxplot of microtensile bond strength (MPa) distribution among experimental groups after chemical aging [Kooliner – 0% vs. 2.5% (*p*=0.818); Ufi Gel Hard – 0% vs. 5% (*p*=0.310) and Probase Cold – 0% vs. 5% (*p*=0.004)]

difference (*p*=0.144) was found between Kooliner and Ufi Gel Hard groups.

Neither Kooliner (*p*=0.818) nor Ufi Gel Hard (*p*=0.310) bond strength to the denture base was affected by CHX incorporation. However, loading Probase Cold with 5% of CHX resulted in lower (*p*=0.004) μTBS values than the control group (Figure 4).

The failure mode was predominantly adhesive (79.4%) and was not affected by CHX incorporation (Kooliner, *p*=1.000; Ufi Gel Hard, *p*=0.125; Probase Cold, *p*=0.549).

Discussion

Exposing reline acrylic resins to oral pH fluctuations may affect their physical and biomechanical properties.[40,45,46] Also, the maximum cumulative release of CHX reaches higher levels at pH=3 than at pH=7.[31,47] However, although all specimens in the present study were immersed in artificial saliva with a cyclic procedure of 6 hours at pH=3 interchanging with 18 hours at pH=7 for 28 days, the incorporation of CHX in the reline acrylic resins studied only affected the microtensile bond strength of the Probase Cold to the denture base.

Since differences were found between the surface free energy and microtensile bond strength of the three reline acrylic resins, the first null hypothesis is rejected. These differences may result from the distinct chemical composition and structural arrangement of the three reline acrylic resins.[23,24] Kooliner had lower total surface free energy values than Ufi Gel Hard and Probase Cold, probably because of its surface's porous structure. Air voids are entrapped when mixing the powder and liquid components as a consequence of a rapid polymerization reaction.[48,49] Regarding microtensile bond strength, Probase Cold presented higher values than the other tested resins, probably because its chemical composition is similar to the denture base resin: they are both based on polymethylmethacrylate (PMMA) polymer and have methyl methacrylate (MMA) as the monomer. This similarity allows a smooth diffusion of the reline monomers into the denture base resin and forms a complex and strong network in the interface. On the other hand, the lowest mean values of microtensile bond strength were obtained in both the control and the 2.5%-CHX loaded Kooliner. This result may be due to the composition of its monomer isobutyl methacrylate, a high-molecular-weight monomer that makes the dissolution of the PMMA in the denture base resin surface difficult and leads to a less effective penetration of the reline resin into the denture base.[24] Similar results were found in previous studies when the three resins were submitted to thermal aging conditions.[27-30]

Loading the three reline acrylic resins with CHX did not change their total surface energy after chemical aging. So, the second hypothesis is not rejected. Similar results were found in a previous study where the same percentage of CHX incorporation showed a null effect on total surface energy after thermal aging.[34] However, it seems that thermal aging in water leads to lower total surface free energy than chemical aging in saliva (the method used in the present study), probably because of the different aging environments.[34,35]

No differences were found in the microtensile bond strength values between control and experimental groups of Kooliner and Ufi Gel Hard. Probase Cold was the only resin whose microtensile bond strength decreased, since the 5%-CHX group had lower values than the control. This indirect acrylic resin is composed of pre-polymerized poly(methyl methacrylate) particles, and it is polymerized with an indirect method, under high temperature and pressure. Those results may be explained by the incorporation of CHX within the polymer matrix of the material, which probably led to higher distances between the molecules of the polymer net and less homogeneity in their structural arrangement, therefore weakening the bond strength.[16] Nevertheless, Probase Cold specimens loaded with 5% CHX presented higher values of μTBS than any of the other materials studied. Thus, the third null hypothesis is rejected.

Regarding the failure modes, the most observed failure in Kooliner was adhesive in both groups, justified by the distinct chemical composition of this reline resin and the denture base polymer, and proved by the lower μTBS values observed in the present study. Concerning Ufi Gel Hard, the most frequent failure mode was cohesive, probably caused by an increase of the bond strength promoted by the specific adhesive used before the relining procedure. Also, the cohesive failures on this resin were higher in the experimental group than in the control group. Thus, the presence of CHX may weaken the internal structure of the Ufi Gel Hard and promote failures in the polymer instead of in the bond interface. Both control and experimental groups of Probase Cold showed a predominance of adhesive failures. The robust and complex network formed by PMMA molecules and their strong internal structure can explain the absence of cohesive failures on Probase Cold.[50,51]

Although microhardness and flexural strength after chemical aging of chlorhexidine delivery systems have already been studied,[52] other properties should be evaluated, and more laboratory studies that mimic the oral environment and clinical trials are needed.

Conclusions

The incorporation of 5% chlorhexidine in Probase Cold resin negatively affected its microtensile bond strength to the denture base, after a chemical aging correspondent to 1 month of exposure to the oral environment. No other negative effects were observed on the surface free energy and bond strength of the three reline acrylic resins loaded with the studied percentage of chlorhexidine.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interest

The authors have no conflicts of interest to declare.

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