Universidade de Lisboa

Faculdade de Medicina Dentária

Effect of Chlorhexidine Loading on Surface Properties of Acrylic Reline Resins after Chemical Ageing

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Dissertação

Mestrado Integrado em Medicina Dentária

2019

Agradecimentos

A realização desta dissertação assinala o final de uma etapa muito importante da minha vida. Muitas foram as pessoas que me acompanharam durante este percurso académico, a quem não posso deixar de agradecer.

À minha orientadora, Professora Doutora Maria Cristina Bettencourt Neves, Professora Auxiliar com Agregação do departamento de Prostodontia Removível da Faculdade de Medicina Dentária da Universidade de Lisboa, um agradecimento pela oportunidade de conhecer o mundo da investigação laboratorial e por me permitir dar continuidade a um projeto que lhe é tão querido. Obrigada pela constante disponibilidade e confiança transmitida, assim como pela exigência e transmissão de saberes que a distinguem como docente.

À minha co-orientadora, Professora Doutora Ana Francisca Bettencourt, Professora Auxiliar do Departamento de Ciências Toxicológicas e Bromatológicas da Faculdade de Farmácia da Universidade de Lisboa, pela disponibilidade das instalações e de todos os recursos necessários durante a execução prática deste trabalho. Agradeço a sua ajuda sincera e dedicada, preocupação, interesse e auxílio teórico e prático. Foi um prazer conhecê-la.

À Professora Doutora Sofia Arantes e Oliveira, Professora Associada, regente da unidade curricular de Odontogeriatria e docente de Biomateriais Dentários, pela disponibilização das instalações e equipamentos do Laboratório de Biomateriais da Faculdade de Medicina Dentária, assim como pelas sugestões e auxílio prestado.

Ao Professor Doutor Jaime Portugal, Professor Catedrático e Regente das unidades curriculares de Biomateriais da Faculdade de Medicina Dentária da Universidade de Lisboa, pelas sugestões, auxílio prestado e pelos conhecimentos transmitidos na área de Biomateriais.

À Dra. Joana Costa, um agradecimento especial pela enorme paciência, compreensão e preocupação ao longo de todo o percurso. Todas as dicas, ensinamentos e tempo despendido a ajudar-me na minha luta contra o tempo são guardados com carinho, assim como a simpatia.

Ao Sr. Tomás, Técnico de Prótese Dentária, por toda a ajuda e dicas transmitidas.

A todos os docentes e funcionários da Faculdade de Medicina Dentária da Universidade de Lisboa que, direta ou indiretamente, contribuíram para a minha formação pessoal e profissional.

Ao meu colega, amigo e companhia de todas as horas de laboratório, Luís Nepomuceno, foi um prazer não só partilhar contigo este projeto como todos os anos de curso. Obrigada pela

paciência, amizade, músicas motivacionais, passeios a Farmácia, por ficares até tarde… Obrigada!

À minha dupla e eterna amiga Joana Martins, a que mais me aturou, um agradecimento especial e de coração cheio, por todo o apoio e amizade, mesmo nos momentos mais difíceis e cansativos. Obrigada por teres sempre a palavra certa e reconfortante a dizer, pela aprendizagem, por tornares a clínica mais fácil e alegre e por assumires este "compromisso" comigo, será impensável não continuar assim por largos (e largos…) anos.

Ao grupinho de todas as horas, de sempre e para sempre, Anita, Nepo, Inês Craveiro e Super, foram uma marcante parte deste meu percurso que não irei esquecer e que farei questão de levar daqui em diante. Obrigada pelo companheirismo, partilha e amizade!!!!

À minha Cá, a primeira amiga com que me cruzei na Faculdade e que me acompanhará para o resto da vida. Obrigada por seres única, por só tu me contares coisas random e super divertidas, e por te manteres pertinho, és uma força da natureza!

Agradeço ainda a todos os meus colegas deste percurso de 5 duros e longos anos, pelo convívio e cumplicidade diária.

À minha madrinha e amiga, Sara Tomé, por todo o apoio e motivação transmitida, nunca será possível agradecer o suficiente.

Ao meu namorado e melhor amigo, Tiago, obrigada por todo o amor, carinho, e apoio incondicional. Obrigada pela paciência infindável para as minhas crises, planos abortados e, acima de tudo, por estares a meu lado, sem ti era impossível cumprir esta etapa. Amo-te!

Por último, mas sempre em primeiro, agradeço aos meus pais, por TUDO. Pelo apoio incondicional, compreensão e incentivo ao longo de toda a minha vida, por serem os lutadores que são e nunca desistirem… Se aqui cheguei, a vocês devo! Esta vitória é também vossa, por todo o empenho para que eu fosse mais e melhor, para que fosse feliz. Amo-vos!

À minha querida irmã, Vânia, de quem tenho muito orgulho, que tanto me ensinou e mostrou que com empenho e paciência é possível. Por me ter dado o exemplo a seguir, por todo o apoio, por ser a mana mais velha e por me ter dado o presente mais especial, o meu Gonçalinho que consegue alegrar até os meus dias mais stressantes.

Por último, à minha família, um eterno agradecimento por estar sempre presente.

Resumo

Devido a processos fisiológicos decorrentes da perda de peças dentárias, como a reabsorção óssea contínua e progressiva do rebordo alveolar, ocorre inevitavelmente desadaptação da prótese dentária com perda de retenção e estabilidade. A readaptação desta aos tecidos pode ser conseguida através de rebasamento com resinas acrílicas, um procedimento que pode ser realizado pelo método direto (diretamente na cavidade oral) ou indireto (por intermédio de procedimentos laboratoriais). As resinas acrílicas são constituídas por polímeros obtidos através de uma reação de polimerização, durante a qual o monómero é convertido, mas não na sua totalidade. O monómero residual não só pode ter efeitos citotóxicos nos tecidos biológicos,como efeitos inconvenientes na estrutura da resina, possibilitando a formação de porosidades. A porosidade permite a colonização de *Candida albicans*, devido à aderência deste agente à resina acrílica, sendo este considerado o primeiro passo da patogénese da Estomatite Protética.

A Estomatite Protética é uma condição crónica observada em 45-70% dos utilizadores de prótese removível. Em geral, manifesta-se como uma inflamação difusa na mucosa do palato, delimitada pela região de contacto com a prótese, e pode ser provocada por vários fatores, entre os quais uma higiene oral insatisfatória, baixo pH salivar e uso contínuo da prótese. A terapia com antifúngicos tópicos e sistémicos tem sido considerada como a opção mais frequente, mas depende da adesão do paciente ao tratamento e não erradica os microrganismos presentes na prótese removível. Assim, a Clorexidina (CHX) surge como um agente antimicrobiano de elevada substantividade, com capacidade de suprimir a aderência de *Candida albicans* na prótese e na mucosa, através da sua ação anti-biofilme. Para garantir a disponibilidade da dose terapêutica na área pretendida, é sugerido um sistema de libertação de CHX que passa pela sua incorporação em resinas de rebasamento. Estudos microbiológicos prévios evidenciaram uma atividade antifúngica ideal com uma concentração de 2,5% na resina Kooliner (K) e 5% nas resinas Ufi Gel Hard (UG) e Probase Cold (PC), no entanto é importante avaliar o comprometimento desta incorporação nas propriedades físicas e mecânicas destes biomateriais dentários. A literatura existente estuda a influência da incorporação de CHX nas resinas de rebasamento sujeitas a envelhecimento térmico, contudo não contempla a submissão a processos de biodegradação química.

Como tal, o objetivo deste estudo foi avaliar o efeito da incorporação de uma concentração específica de CHX na energia de superfície, na resistência adesiva à microtracção

e no tipo de falhas obtidas com a sua fratura, de três resinas acrílicas de rebasamento, após serem sujeitas a um processo de envelhecimento químico.

Para o teste de energia de superfície, quarenta e dois espécimes (25×16×1 mm) das três resinas acrílicas de rebasamento (*n*=7) foram elaborados com recurso a moldes de aço (125×25×1 mm), para as quais foram realizados dois grupos: controlo (sem incorporação de CHX) e experimental, com a incorporação das seguintes concentrações de CHX - Kooliner 2,5%, Ufi Gel Hard 5% e Probase Cold 5%. Os espécimes foram imersos em saliva artificial num rácio 1g/5mL e incubados a 37ºC com agitação de 300 rpm, respeitando ciclos alternados de 6h em pH=3 e 18h em pH=7 até perfazer um total de 28 dias. Posteriormente foram testados com recurso a um tensiómetro de *Kruss*, imergindo cada espécime em água e 1,2-propilenoglicol. Os ângulos de contacto foram obtidos através da técnica da placa de *Wilhelmy* para cada líquido e usados para determinação da energia de superfície (γ) pelo método de *Wu*.

No caso da resistência adesiva à microtração (μTBS), primeiramente foram elaborados trinta e seis espécimes (*n*=6) com forma quadrangular (10×10×10 mm) de resina termopolimerizável de base de prótese (Probase Hot) e submetidos a 2500 ciclos de termociclagem (alternadamente submersos a 5 e 55ºC durante 20 segundos). Em seguida procedeu-se ao rebasamento de todos os espécimes com as resinas em estudo incorporadas com as concentrações de CHX específicas (Kooliner – 0% e 2,5%; Ufi Gel Hard – 0% e 5%; Probase Cold – 0% e 5%). Os cubos rebasados foram sujeitos à máquina de corte *Isomet* por forma a obter cinco palitos uniformes (1mm^2) de cada um, sendo estes posteriormente submetidos ao processo de envelhecimento químico. Seguidamente, os espécimes foram sujeitos a uma máquina de testes universal *Instron* e efetuou-se o teste de resistência adesiva à microtração, com uma carga de célula de 1kN e uma velocidade de 1mm/min, até ocorrer fratura. As superfícies previamente aderidas foram observadas num estereomicroscópio e classificadas consoante o tipo de falha: adesiva, coesiva ou mista.

A unidade experimental considerada para efeitos estatísticos na γ e na μTBS foi o cubo, enquanto na avaliação do tipo de falha foi considerado o palito. Assim, no primeiro caso a normalidade foi testada pelo teste de normalidade *Shapiro-Wilk* e os resultados foram analisados estatisticamente com recurso a testes não paramétricos de acordo com testes de *Kruskal-Wallis* e correções de *Mann-Whitney*. No segundo caso, os testes qui-quadrado e o teste exato de *Fisher* foram aplicados. Considerou-se nível de significância de 5% em todos os testes.

No que diz respeito à energia de superfície, não foram encontradas diferenças estatisticamente significativas em qualquer um dos grupos de cada material estudado. A única

diferença significativa diz respeito ao aumento da componente dispersiva do grupo 5% da Probase Cold em relação ao controlo, no entanto sem diferenças na energia de superfície total.

Quanto à resistência adesiva, não se verificaram diferenças estatisticamente significativas nos grupos experimentais de Kooliner e Ufi Gel Hard quando comparados com o controlo. Em contraste, o grupo Probase Cold com 5% de CHX apresentou valores inferiores comparativamente com o grupo controlo (p=0,004).

Na análise do tipo de falha, não foram observadas diferenças significativas entre os grupos experimental e controlo de cada material. Observou-se que o tipo de falha predominante no estudo foi adesiva (79,4%). Em ambos os grupos Kooliner, 90% das falhas foram adesivas e nenhuma falha coesiva foi observada. No que respeita Ufi Gel Hard, foi observada uma diminuição das falhas adesivas e um aumento das falhas coesivas com a incorporação de 5% CHX, sendo que neste grupo a falha coesiva foi predominante (43,3%). No caso da Probase Cold, em ambos os grupos 90% das falhas foram adesivas, no entanto verificou-se uma eliminação das falhas coesivas e um aumento das falhas mistas com a incorporação de 5% de CHX.

Os resultados obtidos podem ser explicados pela diferença de método de polimerização e pela composição inerente a cada resina de rebasamento, influenciando a formação de mais ou menos porosidades na superfície da resina e interferindo no grau de difusão do monómero na resina da base da prótese. Apesar de importantes noções poderem ser retiradas deste estudo, o processo multifatorial inerente à cavidade oral deverá ser recriado em necessários futuros estudos, sendo sugerida a simulação de forças mastigatórias repetidas até a ocorrência de fratura e a observação das falhas obtidas com microscopia electrónica de varrimento.

Em conclusão, a incorporação das referidas concentrações de clorexidina, após um processo de envelhecimento químico não afeta a energia de superfície total dos três materiais estudados nem a resistência adesiva à microtração nos grupos de Kooliner e Ufi Gel Hard. No entanto, parece influenciar negativamente a resistência adesiva à microtração nos espécimes de Probase Cold com incorporação de 5% de CHX. O tipo de falha apresentado após fratura não foi influenciado pela incorporação de CHX nas três resinas acrílicas de rebasamento em estudo.

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

Abstract

Denture stomatitis is a chronic condition for which a release system of Chlorhexidine (CHX) loaded on resins has been suggested as a promising treatment.

The purpose of the present study was to evaluate the effect of loading three acrylic reline resins, with a specific concentration of CHX, in the surface free energy, microtensile bond strength and type of bonding failure after a chemical ageing procedure, compared to a control group (0% of CHX).

Surface free energy (γ) was evaluated by immersing specimens of acrylic reline resins loaded with specific percentages of CHX (n=7) into water and 1,2-propanediol. Contact angles were obtained by the Wilhelmy plate technique and used to estimate the γ values through the Wu method.

Microtensile bond strength (μTBS) test was conducted testing sticks obtained from each specimen of denture base resin linked to a reline resin loaded with specific concentration of CHX (n=6) in a Instron universal machine, with 1kN load cell and crosshead speed of 1mm/min. Afterwards, the failure mode was assessed with a stereomicroscope and classified as adhesive, cohesive or mixed.

Data from γ and μTBS was submitted to the nonparametric tests according to the Kruskal-Wallis and Mann-Whitney tests, while failure mode data was submitted to the chi-square and the Fisher's exact tests, considering the 5% level of significance (α=0.05).

No statistical differences were observed in the γ between groups in all three reline resins, as well as in the μTBS between experimental K and UG. However, 5% CHX PC group presented lower μTBS values than the control. For all three reline resins, no statistical significant differences were found between the type of failures observed and CHX loading.

In conclusion, after a chemical ageing procedure, loading PC with 5% CHX seems to negatively influence bond strength, without other undesirable effects in the studied properties.

Keywords: Denture stomatitis; Acrylic resins; Chlorhexidine; Surface tension; Tensile Strength.

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1,6-HDMA	1,6-hexanedioldimethacrylate
CHX	Chlorhexidine
HEMA	2-hidroxyethylmethacrylate
IBMA	Isobutylmethacrylate
IR	Interquartile Range
ISO/TS	International Organization for Standardization/Technical Specification
K	Kooliner
L	Liquid
M	Mean
m	Median
Max	Maximum
Min	Minimum
min	Minutes
MMA	Methylmethacrylate
P	Powder
PC	Probase Cold
PEMA	Polyethylmethacrylate
PMMA	Polymethylmethacrylate
SD	Standard deviation
UG	Ufi Gel Hard
γ	Surface free energy
γ^d	Dispersive component of surface free energy
γ^p	Polar component of surface free energy
μ**TBS**	Microtensile Bond Strength

1. Introduction

Over the last years, the adult population has been experiencing an improvement in oral health, leading to a decrease of edentulism. However, the demographic trend for an elderly population leads to a still significant number of patients needing treatment with complete or partial dentures, which is believed to rise steadily for the next two decades.(1-3) This rehabilitation allows the reestablishment of function, vertical dimension, improves aesthetics and speech, as well as decreases psychological consequences.(2,4)

Due to physiologic progression of residual ridge resorption after tooth loss, adaptation of the denture base is affected, resulting in loss of retention, comfort, trauma in the underlying mucosa and consequent rejection of the denture.(5-6) The denture and the ridges should be examined periodically to detect these changes and if a situation like this is presented, a relining procedure should be done.(7-8) With this procedure, retention and stability improve significantly and an effective distribution of the masticatory load in the denture is achieved. This is a time-saving, convenient and relatively inexpensive prosthodontic treatment when compared to the cost and time-consuming of making new dentures.(9-12)

The relining procedure can be carried out with chairside relining materials, which means that the relining is directly performed inside the mouth of the patient, or laboratory relining materials, used in the indirect method.(5,7) Acrylic resins for relining procedures, alike to the denture base, consist of polymeric biomaterials composed by chains of monomers, where a maximum conversion of monomer is necessary.(13-14) The residual monomers can be trapped on the polymer matrix, affecting the mechanical and physical properties of the biomaterial, and can be diffused into the surrounding medium causing undesirable biological reactions, including local chemical irritation, hypersensitivity, ulceration, systemic allergic reactions and development or oral diseases, like denture stomatitis.(15-17)

Denture stomatitis is a chronic condition observed in 45-70% of denture wearers and manifests as a diffuse inflammation of the palatal mucosa that is delimited by the borders of the denture, usually asymptomatic.(2,18-21) It is considered a clinical finding of Erythematous Candidiasis or Chronic Atrophic Candidiasis, a subgroup of Oral Candidiasis.(22,23) Even though other *Candida* species may contribute to this disease, *Candida albicans* is the principal causative agent and its adherence to oral mucosa and denture surface is considered the first step in the pathogenesis of denture stomatitis.(21,24-28)

Despite the evidence of fungal etiology, several factors have been suggested in a multifactorial etiology, such as acid salivary pH or reduced saliva secretion, poor hygiene, continuous denture wear, trauma from ill-fitting dentures, nutritional deficiency, long-term antibiotic therapy, immune suppression and xerostomia.(19,23,26,28-30)

Treatment of denture stomatitis usually evolves 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 the denture.(23,25) Systemic antifungal like fluconazole is commonly used because is well tolerated and it has low toxicity, but they do not eradicate the microorganisms from the denture surface. Clinical effectiveness of topical antifungals is dependent upon its delivery and retention at a specific site, as well as patient compliance. One example is nystatin, which is a highly effective topical antifungal that has few drug interactions, but its four times daily dosage is a significant challenge for patient compliance.(21,25,28) Nevertheless, they are associated to relapses, since *Candida albicans* seem to penetrate the denture acrylic and some *Candida* species are azole-resistant.(6,21,31,32)

Chlorhexidine (CHX) is an antimicrobial agent widely prescribed as an antiseptic mouthwash in dentistry due to its activity against a wide range of microorganisms, including *Candida* species.(22,27,31) CHX has been showing low concentration efficiency, high substantivity, capacity to reduce biofilm formation and disorganize pre-formed biofilm.(29) However, its efficiency is influenced by not only its concentration but also its exposure time, which can be associated to a therapeutic failure caused by the turnover of saliva and the cleansing action of the oral musculature.(25,27,33) Immersion of acrylic dentures in CHX have been shown to suppress adhesion of *Candida* to the dentures for longer than with antifungal agents.(27)

Considering the disadvantages of some forms of treatment proposed in the literature to date and joining the best of these ideas, a new form of treatment for denture stomatitis was proposed, a novel drug release system. A release system of CHX loading on resins has been investigated in several studies, the general principle is the incorporation of resin dentures with CHX that releases from the device and inhibits microbial adherence and growth.(20,31,34,35)

By loading antimicrobial agents into resin-based denture relining materials, it is possible not only to create a drug delivery system, but also guarantee availability of the agent in the target area at a therapeutic dosage.(22,27,31,35) Some studies have evaluated the CHX release from acrylic resins and concluded that there is a high initial rate of delivery from the material, followed by a controlled slow and constant release for at least twenty eight days, being more

effective than the mouth rinse.(22,27,31,32) Direct delivery of the drug to the site of infection reduces the risk of systemic side effects or drug interactions.(21,31)

Most of the previous studies assessing the effect of CHX showed that a concentration of 10% was the most effective against *Candida* albicans and the maximum dose that could be safely incorporated in acrylic resins without interfering with the mechanical properties of the Ufi Gel Hard but affecting flexural strength in both Kooliner and Probase Cold.(21,22,27,31) However, recent preliminary results established minimal concentrations of CHX in order to assure proper antifungal activity against *Candida* albicans. Thus, 2.5% for the reline acrylic resin Kooliner seems to be enough to prevent the appear and the development of the fungus, while for Ufi Gel Hard and Probase Cold an incorporation of 5% is required.(36)

It is still uncertain the effects of these loading techniques on the mechanical properties of acrylic resins over time.(37,38) Surface free energy is a property that strongly influences the wettability of relining materials, which is one of the most important factor that influences the denture retention to the mucosa and in another field may contribute to the adherence, bonding and colonization of *Candida* species.(30,39) Bond strength between the base of the denture and the relining material is also important, since a weak bond encourages a gap formation with ingress of bacteria and fungus and promote staining.(7,40-44) Microtensile bond strength test (μTBS) was first introduced by Sano and colleagues, in 1994, and since then it has been used to test adhesion between several dental materials as a promising way to evaluate the adhesion between acrylic resins due to his reduced area, 1mm^2 by ISO/TS 11405:2015.(45-48)

Previous studies showed the influence of loading different concentrations of CHX in the microhardness, flexural strength, surface free energy and shear bond strength properties of acrylic reline resins immediately after being prepared and submitted with thermal ageing.(49-51) However, other studies that simulate other biodegradation processes of the oral medium have not been performed yet, like chemical ageing. The oral cavity is expose to endogenous and exogenous acids, such as dietary changes, that include a fluctuation of the pH present.(52,53) It is also known that the oral cavity pH in individuals with denture induced stomatitis is lower (pH≈5.2) and that an individual with a cariogenic diet is subjected to approximately 6h of acid environment per day.(54)

Thus, this investigation seeks to clarify the impact of CHX-loaded acrylic resin subjected to oral chemical fluctuations on the surface free energy of acrylic reline resins and microtensile bond strength between acrylic reline resins and denture base.

2. Objectives

The first objective of this study was to evaluate the effect of loading three different acrylic reline resins with a specific concentration of CHX on surface free energy, after a chemical ageing process, according to the following hypotheses:

$H0_1$: Loading Kooliner with 2.5% of CHX does not affect the surface free energy.
$H1_1$: Loading Kooliner with 2.5% of CHX affects the surface free energy.

$H0_2$: Loading Ufi Gel Hard with 5% of CHX does not affect the surface free energy.
$H1_2$: Loading Ufi Gel Hard with 5% of CHX affects the surface free energy.

$H0_3$: Loading Probase Cold with 5% of CHX does not affect the surface free energy.
$H1_3$: Loading Probase Cold with 5% of CHX affects the surface free energy

The second objective was to evaluate the effect of loading three different acrylic reline resins with a specific concentration of CHX on microtensile bond strength to denture base resin, after a chemical ageing process, according to the following hypothesis:

$H0_4$: Loading Kooliner with 2.5% of CHX does not affect the microtensile bond strength to the acrylic base resin.
$H1_4$: Loading Kooliner with 2.5% of CHX affects the microtensile bond strength to the acrylic base resin.

$H0_5$: Loading Ufi Gel Hard with 5% of CHX does not affect the microtensile bond strength to the acrylic base resin.
$H1_5$: Loading Ufi Gel Hard with 5% of CHX affects the microtensile bond strength to the acrylic base resin.

$H0_6$: Loading Probase Cold with 5% of CHX does not affect the microtensile bond strength to the acrylic base resin.

$H1_6$: Loading Probase Cold with 5% of CHX affects the microtensile bond strength to the acrylic base resin.

The third objective was to evaluate the influence of loading three different acrylic reline resins with a specific concentration of CHX in the type of bonding failure to the denture base resin, according to the following hypothesis:

$H0_7$: Loading Kooliner with 2.5% of CHX does not affect the type of bonding failure to the denture base resin.

$H1_7$: Loading Kooliner with 2.5% of CHX affects the type of bonding failure to the denture base resin.

$H0_8$: Loading Ufi Gel Hard with 5% of CHX does not affect the type of bonding failure to the denture base resin.

$H1_8$: Loading Ufi Gel Hard with 5% of CHX affects the type of bonding failure to the denture base resin.

$H0_9$: Loading Probase Cold with 5% of CHX does not affect the type of bonding failure to the denture base resin.

$H1_9$: Loading Probase Cold with 5% of CHX affects the type of bonding failure to the denture base resin.

3. Materials and Methods

3.1. Materials

Materials assessed in the present study evolve three auto-polymerizing acrylic reline resins, which were selected for their differences in chemical composition. Two of them consist in direct reline resins: **Kooliner** (GC America Inc., Alsip, Illinois, USA) (Appendix 2, Figure 1) a non-crosslinking poly(ethyl methacrylate)-based resin, and **Ufi Gel Hard** (Voco GmbH, Cuxhaven, Germany) (Appendix 2, Figure 2), a crosslinking poly(ethyl methacrylate) (PEMA)-based resin. The other material consists of an indirect reline resin: **Probase Cold** (Ivoclar Vivadent AG, Liechtenstein) (Appendix 2, Figure 3), a poly(methyl methacrylate) (PMMA)-based resin.

The name, composition, power/liquid ratio, polymerization condition, batch number and expiration date of which one of them are presented in Table 3.1.

Table 3.1 – Materials used in the study.

Product	Composition P	Composition L	P/L Ratio (g/mL)	Curing Cycle	Batch Number (Expiration Date)
Kooliner (K)	PEMA	IBMA	1.4/1	10 min 37ºC	**P** 1707271 (2020-07) **L** 1704191 (2020-04)
Ufi Gel Hard (UG)	PEMA	1,6-HDMA	1.77/1	7 min 37ºC	**P** 1816582 (2020-09) **L** 1804406 (2020-02)
Probase Cold (PC)	PMMA	MMA	1.5/1	15 min 40ºC 3 bar	**P** XT1222 (2022-10-24) **L** X45991 (2022-10-11)

P = Powder, L = Liquid; PEMA = Poly(ethyl methacrylate), IBMA = Isobutyl methacrylate

HDMA = Hexanedioldimethacrylate, PMMA = Poly(methyl methacrylate), MMA = Methyl methacrylate

Chlorhexidine Diacetate Monohydrate (Panreac AppliChem, Darmstadt, Germany) (Appendix 2, Figure 4) was incorporated in the previous listed acrylic reline resins with a specific concentration presented in Table 3.2.

Table 3.2 – CHX Concentration and number of specimens for each material.

	% CHX loaded	Surface Free Energy	Microtensile Bond Strength
Kooliner (K)	0% (control group)	n = 7	n = 6
	2.5%	n = 7	n = 6
Ufi Gel Hard (U)	0% (control group)	n = 7	n = 6
	5%	n = 7	n = 6
Probase Cold (PC)	0% (control group)	n = 7	n = 6
	5%	n = 7	n = 6
		N = 42	N = 36

The powder of acrylic reline resin and CHX (Figure 3.1.1-a) was weighted using a precision balance with internal calibration (A&D FZ-200i) (Appendix 2, Figure 5) and the liquid was measured using a graduated pipette. The mixture of the two materials was done according to the acrylic reline resin weight (w/w) and mixed with a mortar and a pestle until homogenization was achieved (Figure 3.1.1-b). Then the mixture was blend with the correspondent amount of liquid and the polymerization was taken by the recommendations of the manufacturer. Concerning the direct reline resins, specimens were maintained under compression in an incubator at 37±2ºC (Appendix 2, Figure 6), in order to simulate the intraoral polymerization conditions of the materials (Ehret, Mahlberg, Germany). Otherwise, for the indirect acrylic Probase Cold the relined specimens were placed inside an Ivomat pressure device (IvoclarVivadent, Liechtenstein) (Appendix 2, Figure 7) during 15 min at a temperature of 40ºC and 3 bar.

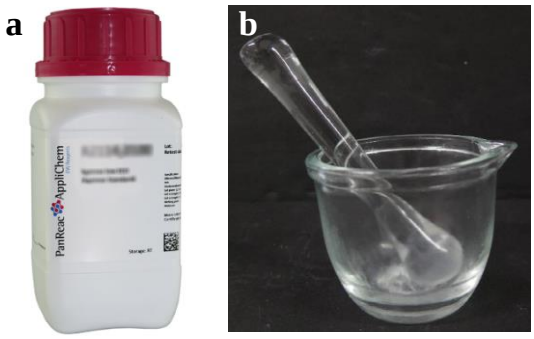

Figure 3.1.1 – Chlorhexidine diacetate monohydrate: a) Package;
b) Incorporation and homogenization.

3.2. Surface Free Energy

Preparation of the specimens

For each material two groups of seven specimens (*n*=7) were produced (one control group without CHX and one experimental group with the CHX percentages mentioned before), resulting in fourteen specimens per material and a total of forty-two specimens (Table 3.2).

Specimens were obtained by placing the mixed material into metallic rectangular shapes (125×25×1 mm) and then clamped together in order to spread the excess of the material (Figure 3.2.1-a). After polymerization with specific conditions according to the manufacturer's instructions and cited before (Table 3.1), all the samples were removed from the molds material (Figure 3.2.1-b) and cut with a turbine cylindrical drill to the dimensions of approximately 25 mm width, 16 mm height and 1 mm thickness. The edges of each sample were polished manually with 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL) (Appendix 2, Figure 8) in order to remove irregularities.

At this point, the specimens were submitted to chemical ageing procedure.

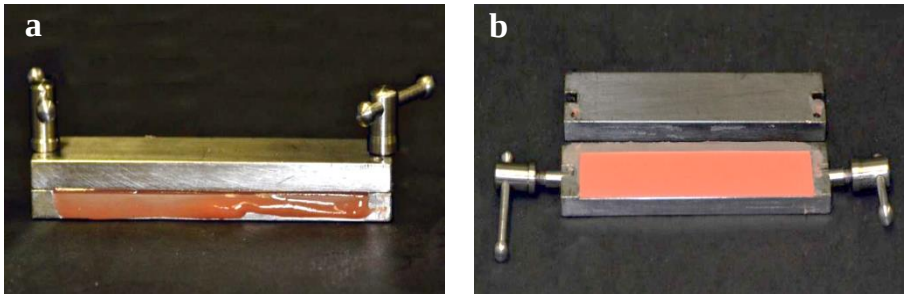

Figure 3.2.1 – Preparation of the specimens: a) Compression of the resin through metal mold compression; b) After the cure is complete.

Chemical ageing procedure

The procedure of chemical ageing consists of immerging each specimen in a 50 mL graduated falcon tube filled with artificial saliva, respecting a 1g/5mL ratio (Figure 3.2.2-a). To respect this proportion, specimens were weighted (A&D Company, Limited, Tokyo, Japan) and the calculation was obtained.

The solution used in the present study was artificial saliva at pH=7 and pH=3, prepared according to a Faculty of Pharmacy University of Lisbon formula, courtesy of Professor Joana Marto:

1) Determination of deionized water volume and PBS quantity (9,6g/1000mL) needed. Mixture both and boiling (F12-ED Refrigerated/Heating Circulator) half of the volume prepared at 60ºC (solution 1). Placing the magnet agitator inside the solution, turning the motor on at 700 rpm.

2) Sprinkling the quantity calculated of Xanthan gum (0,05g/100mL) into boiling buffer and stirring until total of xanthan gum was dissolved.

3) Dissolving of Calcium chloride dihydrate (0,04g/100mL) (EW-N/EG-N balance), Sodium chloride (0,08g/100mL) and Potassium chloride (0,08g/100mL) in solution 1 and stirring until total of materials were dissolved.

5) Dissolving the quantity calculated of Propylene glycol (15,0g/100mL) in solution 2 and stirring until total of Propylene glycol was dissolved.

7) Pouring the solution 3 into a graduated beaker and complete the solution with phosphate buffer pH=7.0 to the volume initially calculated. Removing the magnet agitator.

8) Adjusting the pH (Crison micro pH 2001) (Appendix 2, Figure 9) of artificial saliva to 3 with HCl 1N, since for pH=7 there is no need for adjustment.

9) Keep the solutions out of light, at room temperature.

Protocol of chemical ageing consisted in simulating oral conditions by placing the falcons into an incubator at 37ºC (Memmert, Schwabach, Germany) with constant gentle shaking (300 rpm) (Figure 3.2.2-b), following the sequence in Figure 3.2.3, until an ageing period of 28 days or 672 hours was achieved.

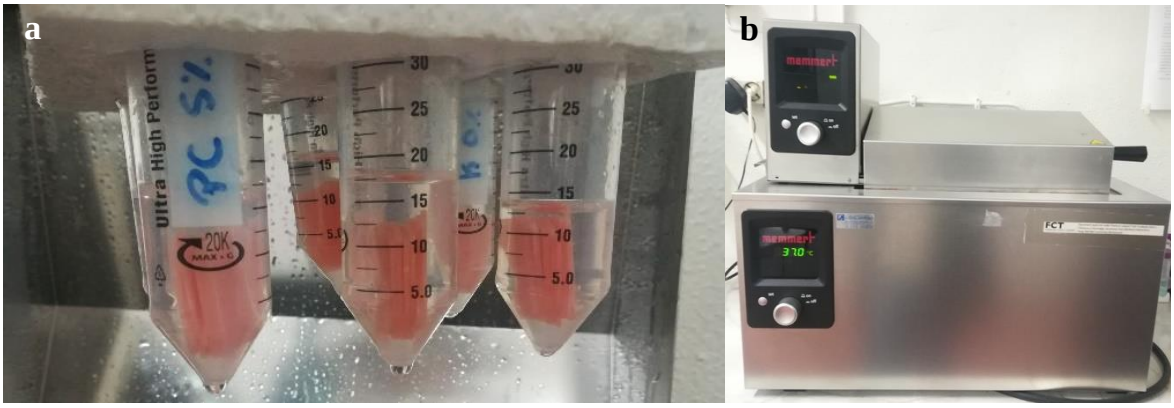

Figure 3.2.2 – Incubation of the specimens: a) in graduated falcon tubes with artificial saliva; b) in incubator at 37ºC.

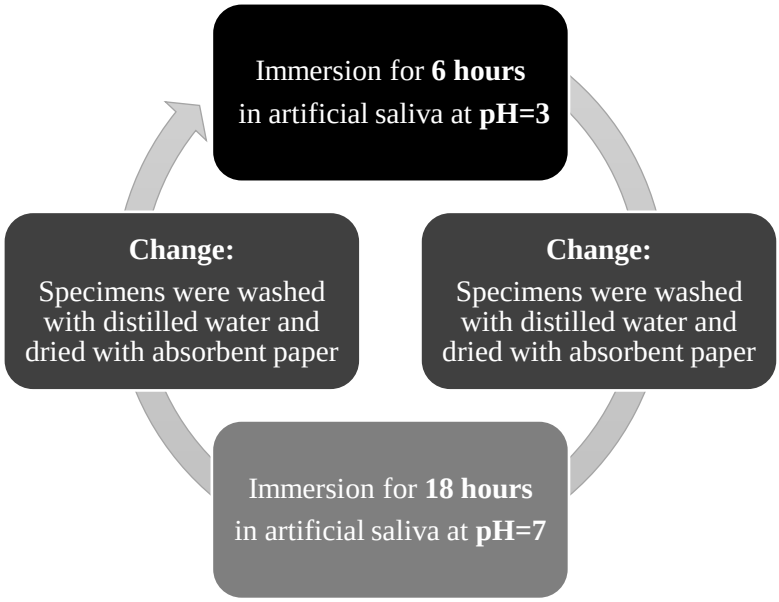

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Figure 3.2.3 – Sequence of a chemical ageing cycle

Surface free energy assessment

After the chemical ageing procedure was complete, the dimensions of each specimen (height, width and thickness) (Appendix 1, Tables 1.1, 1.2 and 1.3) were measured with digital micrometer (Mitutoyo Digimatic, MFG.Co., Ltd Tokyo, Japan) (Appendix 2 , Figure 10) with precision ± 0.01mm and introduced in the software of a computer connected with a Tensiometer K12 (Kruss, Hamburg, Germany) (Appendix 2, Figure 11).

Firstly, the specimen was suspended on the balance (sensitivity = 10^{-4} g) of the equipment, following the immersion of 4 mm in the liquid (water and 1,2-propanediol) at a speed of 20μms^{-1} (Appendix 2, Figure 12). In all the procedure, careful was taken not to handle the surfaces of the specimens to reduce the chance of contamination. The measurement of contact angles of distilled water and 1,2-propanediol of the specimens, at room temperature, were obtained applying the Wilhelmy plate technique.(55) Advancing contact angles were used to estimate total surface free energy (γ) of all specimens, as well as its dispersive (γ^d) and polar components (γ^p), based on the harmonic mean method proposed by Wu.(56)

The 1,2-propanediol used in this study had a total surface free energy (γ) of 38 mN/m, with a dispersive component (γ^d) of 28.6 mN/m and a polar component (γ^p) of 9.4 mN/m. The density was 1.04 kg/m^3 and the respective molar mass was 76.09 g/mol (1-2 Propanediol R.822324-1L; Merck, Germany) (Appendix 2, Figure 13). The water used was of Milli-RX quality (Merck Millipore, Germany).

3.3. Microtensile Bond Strenght

Preparation of denture base specimens

A total of thirty-six specimens of heat-polymerizing denture base acrylic resin Probase Hot (Ivoclar Vivadent AG, Liechtenstein) (Appendix 2, Figure 14) were produced. A conventional flasking technique was used, in which all the wax specimens obtained with a putty elastomer mold with a quadrangular shape (10 × 10 × 10 mm) were flasked and placed above a stratum of gypsum type II**.** Afterwards, a coat of vaseline above the primary stratum of gypsum was applied, placing another compound of gypsum type II and III mixture on the superior half, covering the specimens. Then the top of the flask was positioned, allowing the excess of gypsum to flow throw the holes. After the complete set of the gypsum was achieved, the flask was placed under boiling water between 4 to 6 minutes and, once removed from the boiling water, it was opened to clear the wax. A separating fluid was applied on the impressed gypsum (Ivoclar Vivadent AG, Liechtenstein), and then a heat-polymerizing resin (Probase Hot, Ivoclar Vivadent AG, Liechtenstein) was prepared and packed into the flask with a powder/liquid ratio of 22.5/10 g/mL. The set was subjected to polymerization through a hydraulic system which guaranteed the conditions indicated by the manufacturer (heat up to 100ºC and boil for 45 min). After being removed from the water, the set was let to cool at room temperature before removing the specimens.

In order to simulate a three month ageing process inside the oral cavity, all specimens were submitted to 2500 thermocycling cycles composed of alternating submersions of 20 seconds at 5ºC and 55ºC, with an interval of 5 seconds between each bath, on a thermocycling machine (Refri 200-E, Aralab, Cascais, Portugal) (Figure 3.3.1).

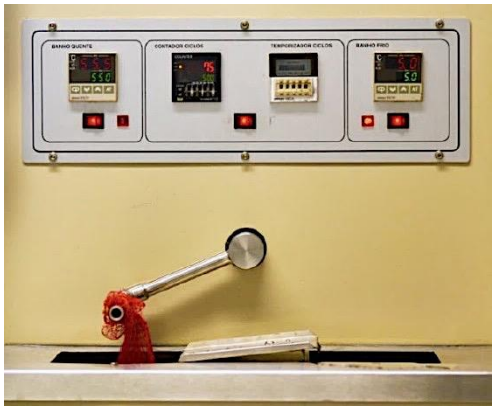

Figure 3.3.1 – Thermocycling machine.

Relining procedure

The measures of the denture base specimens were confirmed using a digital micrometer (Mitutoyo Digimatic, MFG.Co, Ltd. Tokyo, Japan) with a precision of ± 0.01mm and adjusted in a rotational polishing machine (DAP- U, Struers, Denmark) with a 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL).

A denture base specimen was placed in a putty elastomer mold (Figure 3.3.2) and, prior to relining with Kooliner or Probase Cold, correspondent monomer of these reline resins was soaked on the bonding area. In Ufi Gel Hard relining, a specific conditioner was applied and then dried in the air for about 30 seconds, as recommend by the manufacturer.

Two groups (control and experimental group with CHX) of six specimens (n=6) were prepared for each material, as presented in Table 3.2.

The relining procedure was carried out placing the mixed material above the denture base cube and with specific conditions, according to the manufacturer's instructions (Table 3.1). After polymerization, all the samples were removed from the molds and were polished manually with 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL) in order to remove irregularities. The face corresponding to the denture base was identified with nail varnish, applying a different color for each experimental group.

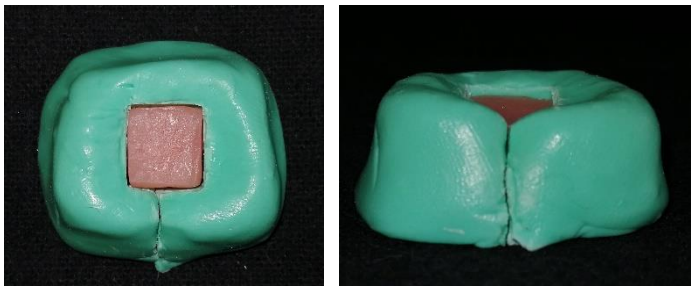

Figure 3.3.2 – Putty elastomer mold used for relining procedure.

Preparation of specimens for microtensile bond strength assessment

The relined cubes were assembled perpendicularly to the large axis of an acrylic resin cylinder, with the varnished base up, and fixed with sticky wax. Then the relined cubes were positioned on an Isomet cutting machine 1000 Precision Saw (Serial No. 666-IPS-03518; Buehler, Lake Bluff, IL, USA) (Figure 3.3.3-a) parallel to the diamond cutting blade (Lapcraft, OH, EUA; 4" x .012" x ½") and sectioned with 550 rpm and cooling, first on the X axis and

then on the Y axis to obtain sticks (parallelepiped specimens) (Figure 3.3.3-b,c) with a sectional area of 1mm^2.

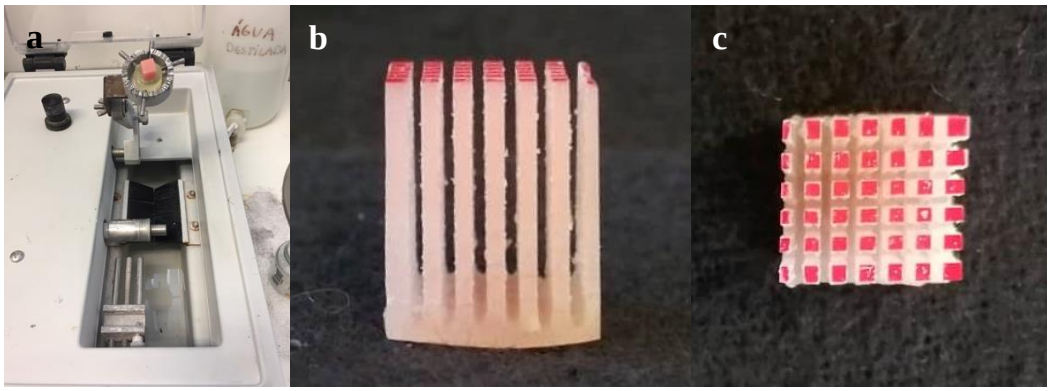

Figure 3.3.3 – Preparation of specimens: a) Position on Isomet cutting machine; b) and c) After section in X and Y axis to obtain sticks.

Measurements of each stick were taken with a digital micrometer (Mitutoyo Digimatic, MFG.Co, Ltd. Tokyo, Japan) with a precision of ± 0.01 and the five most uniform were selected.

Chemical ageing procedure

At this point, the five selected sticks of each relined cube (n=6) were allocated in a eppendorf falcon tubes of 1.5mL filled with artificial saliva (Figure 3.3.4), respecting a 1g/5mL ratio and submitted to the same chemical ageing procedure explained above in section 3.2 – Chemical Ageing Procedure and exemplified in Figure 3.2.3.

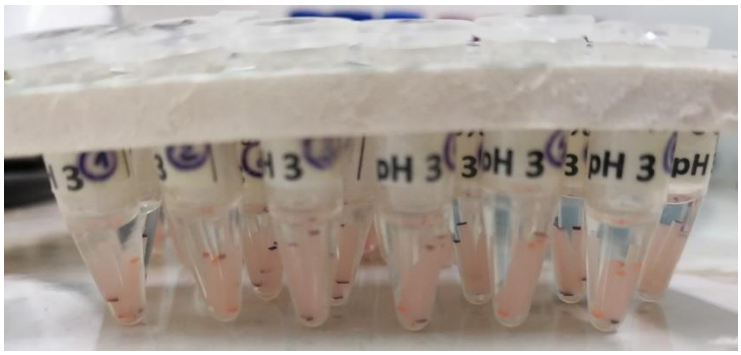

Figure 3.3.4 – specimens in graduated falcon tubes with artificial saliva.

Microtensile bond strength assessment

After the ageing process each stick was placed on a stainless-steel device, *Geraldeli's Jig*, in which the extremities were fixed with cyanoacrylate glue (PERMABOND, Permabond Adhesive, S. Paulo, Brazil) (Appendix 2, Figure 15). The placement of the sticks was performed

with the help of a stereomicroscope (EMZ-8TR, Meiji Techno Co, Saitama, Japan) (Appendix 2, Figure 16), in order to ensure that the interface was placed at the center of the device (Figure 3.3.5). Also, the side of the device correspondent to the denture base resin was identified with a permanent marker.

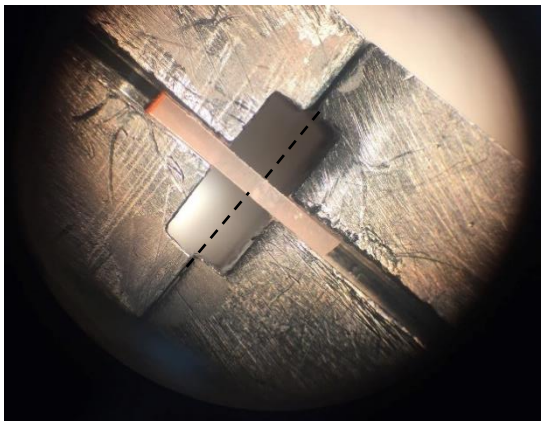

Figure 3.3.5 – Positioning the stick in the Geraldeli's Jig with the interface centered, using the stereomicroscope.

The device was installed in a universal testing machine model 4502 (Instron Ltd., Bucks, HP 12 3SY, England) (Figure 3.3.6) and the test was runned with 1kN load cell and crosshead speed of 1mm/min until fracture.

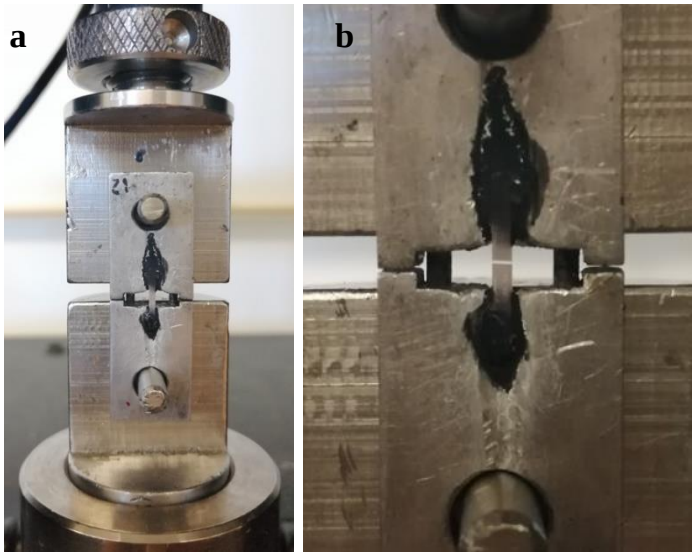

Figure 3.3.6 – Sticks fixed to *Geraldeli's Jig* with cyanoacrylate glue and placed at Instron universal testing machine. a) Before fracture; b) After fracture.

After fracture occurred, the measures of the bonding area were registered using a digital micrometer (Mitutoyo Digimatic, MFG.Co, Ltd. Tokyo, Japan) with a precision of ± 0.01mm (Figure 3.3.7). The microtensile bond strength (μTBS) value, expressed in MPa, was obtained by the Series IX program (Series IX, Automated materials test system, version 8.34.00, serial

number 21744H, Instron Corporation, Grove City, PA, EUA), through the relation between the load at the time of fracture and the stick interfacial area.

Figure 3.3.7 – Measurement of stick's bonding area with a digital micrometer.

Failure mode assessment

The failure mode on the separated surfaces was assessed by two observers with a stereomicroscope and classified as adhesive, cohesive or mixed (Figure 3.3.8). The failures were considered adhesive if occurred between the reline resin and the denture base resin and cohesive if the fracture occurred exclusively within one of the resins. If the fracture occurred in the interface of the two resins but included vestiges of reline resin, it was considered mixed.

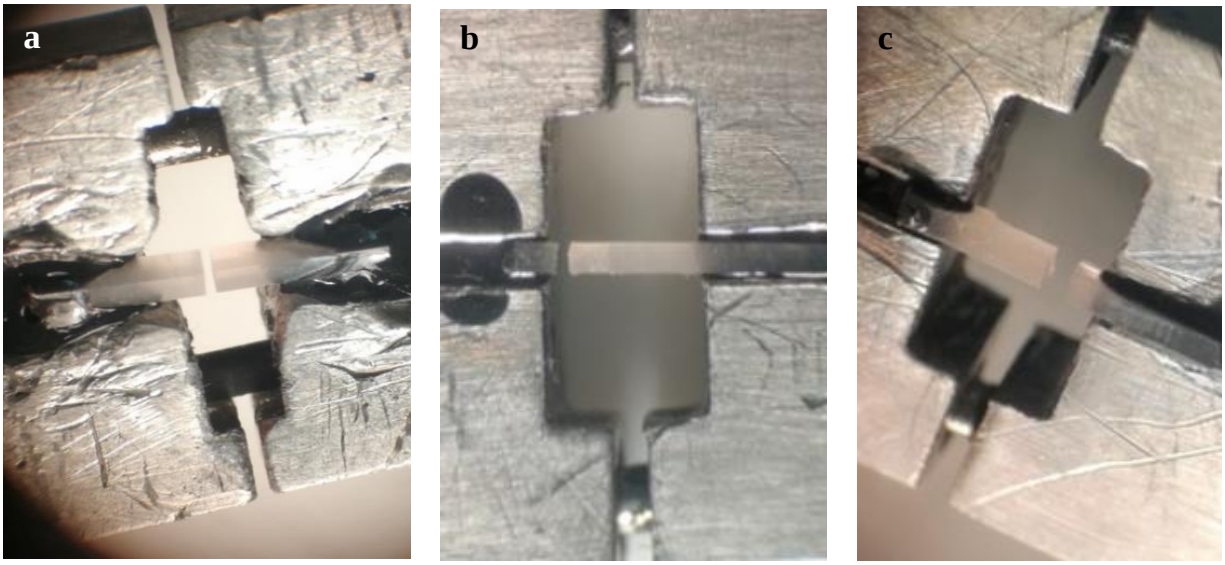

Figure 3.3.8 – Stereomicroscope's images of the three types of failures: a) Adhesive; b) Cohesive; c) Mixed.

3.4. Statistical Analysis

For microtensile bond strength test, each cube was considered as an experimental unit, assuming the mean of the values obtained from all the sticks of the same cube as an independent observation for the purpose of statistical analysis. In the case of failure mode assessment, each stick was considered as an experimental unit for the purpose of statistical analysis.

Descriptive statistics of surface free energy and microtensile bond strength values were carried out being determined the mean, median, standard deviation and interquartile range per group.

Data were statistically analyzed using SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA) and did not follow a normal distribution for the studied variables in the Shapiro-Wilk normality test. Therefore, the results were submitted to the nonparametric tests according to the Kruskal-Wallis method, followed by multiple corrections using Mann-Whitney tests. To determine the association between the failure mode and the incorporation of CHX, chi-square test and the Fisher's exact test were applied.

In all statistical tests, it was considered the 5% level of significance ($p<0.05$).

4. Results

4.1. Surface Free Energy

Descriptive analysis of the data was carried out for each material, including mean, median, standard deviation and minimum and maximum values for contact angle (Appendix 1, Table 1.4) and surface free energy (Appendix 1, Tables 1.5, 1.6 and 1.7).

The values of the total surface free energy (**γ**) and their components, the dispersive (γ^d) and polar (γ^p), are summarized in Table 4.1. Likewise, the mean, median, standard deviations and interquartile range of the groups by reline resin were registered.

Table 4.1 – Total surface free energy data, as well as the dispersive and polar components, by reline resin.

Material	% CHX loaded		Surface Free Energy (γ) (mN/m)		
			γ	γ^d	γ^p
Kooliner	**0%**	**M±SD**	32.1 ± 3.19[a]	15.8 ± 6.64[a]	16.3 ± 9.23[a]
		m (IR)	31.8 (4.20)	16.7 (4.30)	14.0 (7.60)
	2.5%	**M±SD**	34.1 ± 2.26[b]	16.2 ± 3.04[b]	17.9 ± 4.79[b]
		m (IR)	34.4 (2.30)	15.2 (5.00)	19.2 (4.20)
Ufi Gel Hard	**0%**	**M±SD**	39.89 ± 3.48[a]	19.04 ± 2.39[a]	20.81 ± 5.48[a]
		m (IR)	41.5 (4.40)	18.7 (2.40)	21.1 (6.70)
	5%	**M±SD**	41.9 ± 1.09[b]	18.1 ± 2.69[b]	23.8 ± 2.92[b]
		m (IR)	42.0 (1.80)	19.0 (4.70)	24.2 (3.80)
Probase Cold	**0%**	**M±SD**	36.7 ± 4.60[a]	12.3 ± 5.50[a]	24.4 ± 6.78[a]
		m (IR)	37.2 (4.40)	15.4 (9.50)	23.3 (10.4)
	5%	**M±SD**	37.2 ± 1.75[b]	19.1 ± 3.74[a]	18.1 ± 4.59[b]
		m (IR)	36.6 (2.80)	18.1 (2.90)	19.3 (3.50)

γ=Total surface free energy; γ^d=Dispersive surface free energy; γ^p=Polar surface free energy;

M=Mean; SD=Standard deviation; m=Median; IR=Interquartile range

Vertically identical superscripted letters denote significant differences among each group of the same material ($p<0.05$).

Considering Kooliner specimens (Table 4.1), significant differences have not occurred either in the total surface free energy or in its correspondent components, dispersive (γ^d) and polar (γ^p) ($p>0.05$).

For Ufi Gel Hard (Table 4.1), as well as in the previous acrylic reline resin, there were no statistical differences in total surface free energy and in the dispersive (γ^d) and polar (γ^p) components ($p>0.05$).

Regarding Probase Cold specimens (Table 4.1), a statistically significant difference ($p=0.025$) in the dispersive component (γ^d) was found, with specimens loaded with 5% of CHX exhibiting significant higher values than the control group. In the total surface free energy and in the polar component (γ^p), no significant differences were found ($p>0.05$) between groups.

4.2. Microtensile Bond Strenght

The mean (M) values of microtensile bond strength for each group are summarized in Table 4.2, as well as the standard deviation (SD), median (m) and interquartile range (IR).

Table 4.2 – Microtensile bond strength data by reline resin (*n*=6).

Material	% CHX loaded	Microtensile Bond Strenght (MPa)		
		M ± (SD)	m	IR
Kooliner	0%	13,0 ± 3,7	12,7	5,9
	2.5%	13,5 ± 3,6	13,4	6,0
Ufi Gel Hard	0%	22,6 ± 7,4	22,2	14,0
	5%	18,3 ± 5,6	16,8	9,6
Probase Cold	0%	45,0 ± 3,3	44,0	5,8
	5%	33,7 ± 1,9	33,1	3,3

M=Mean; SD=Standard deviation; m=Median; IR=Interquartile range.

Regarding Kooliner specimens (Figure 4.2.1), no statistically significant differences were found on microtensile bond strength between the control group and the 5% CHX loaded group ($p>0.05$).

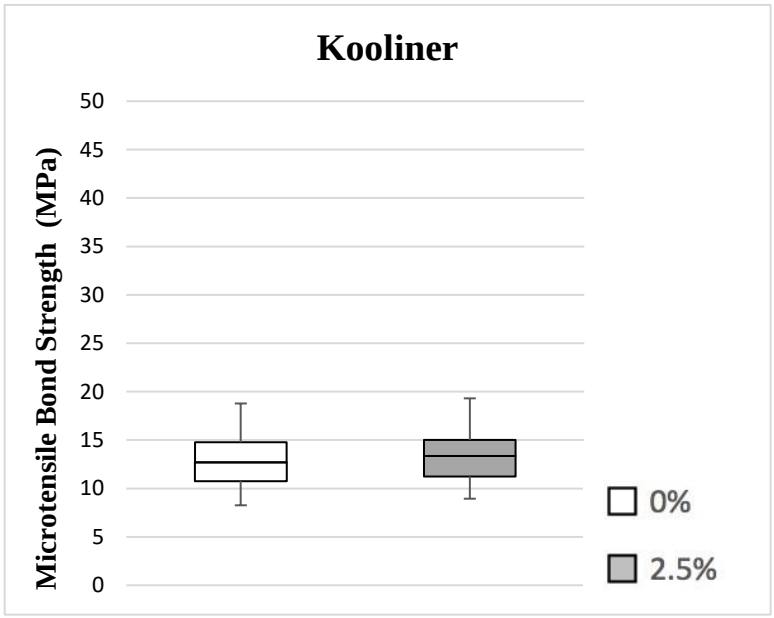

Figure 4.2.1 – Box plot of microtensile bond strength (MPa) of Kooliner. No statistically significant differences were found between groups ($p>0.05$).

Also, for Ufi Gel Hard (Figure 4.2.2) significant differences have not occurred on microtensile bond strength values between 5% CHX loaded group and control group (p>0.05).

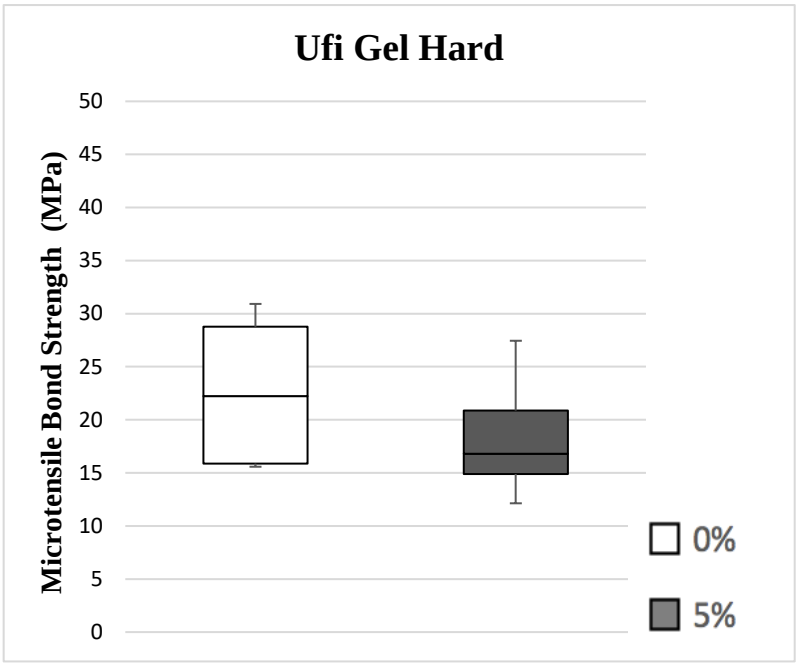

Figure 4.2.2 – Box plot of microtensile bond strength (MPa) of Ufi Gel Hard. No statistically significant differences were found between groups (p>0.05).

Considering Probase Cold (Figure 4.2.3), 5% CHX group had lower microtensile bond strength values compared to the control group (p=0.004). Horizontal line below the boxes denote significant differences among groups (p<0.05).

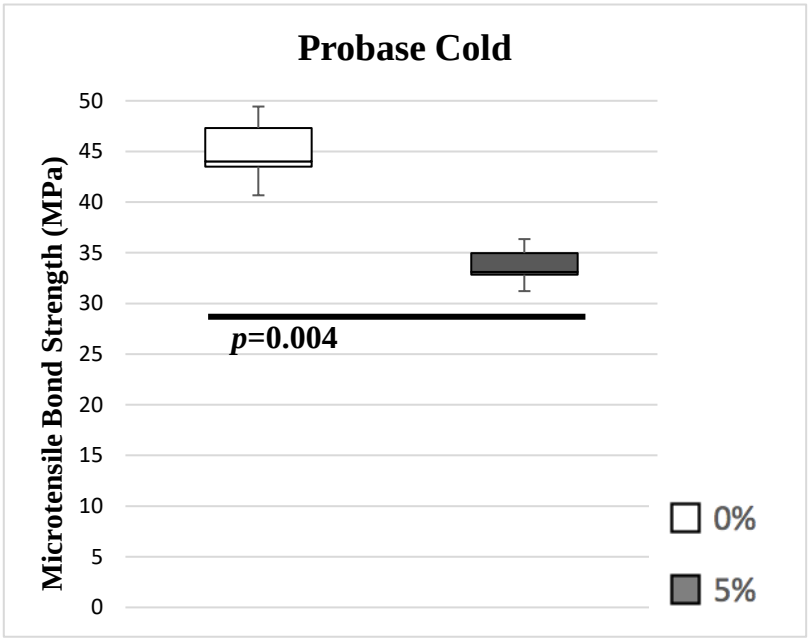

Figure 4.2.3 – Box plot of microtensile bond strength (MPa) of Probase Cold. Statistically significant differences were found between control group and 5% CHX group (p=0.004).

All specimens were observed in a stereomicroscope to assess the type of bonding failure, which percentages within each group are specified in Figure 4.2.4.

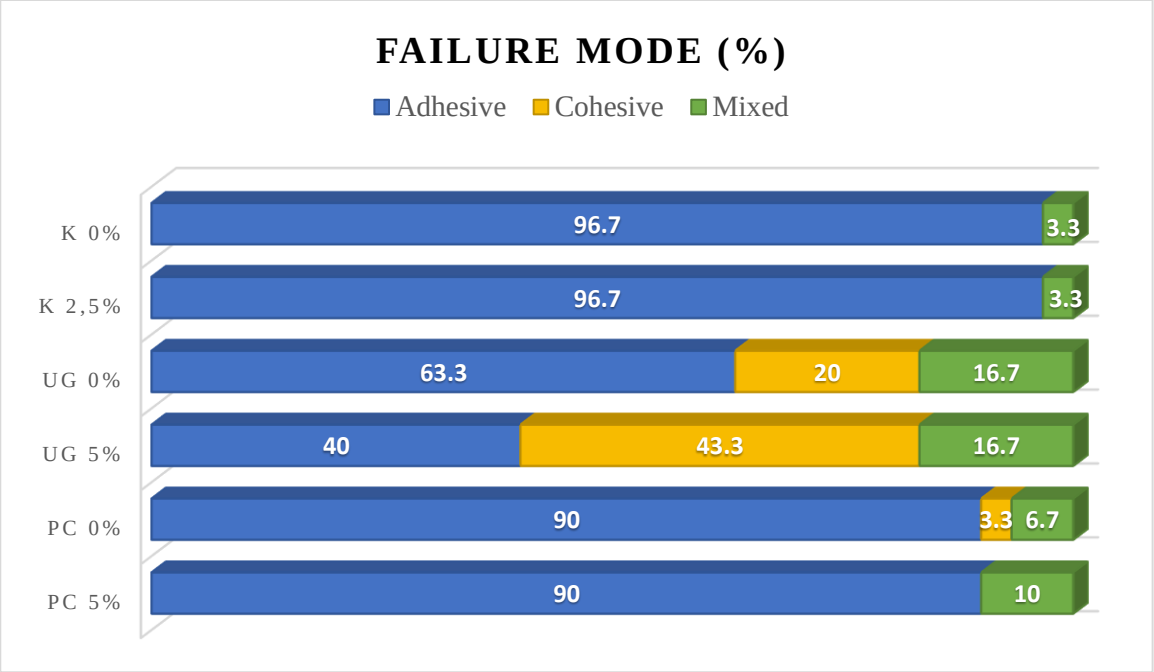

Figure 4.2.4 – Percentage of failure according to the acrylic reline resin and proportion of CHX loaded.

The predominant type of failure in the study was adhesive, with 79.4% of the sticks tested (N=180) showing this type of failure.

Considering Kooliner, in both experimental and control group 96.7% of the failures were adhesive and no cohesive failures occurred, having no statiscally significant differences between them ($p>0.05$).

In the case of Ufi Gel Hard all types of failures occurred in both groups, however, the predominant type of failure in the control group was adhesive (63.3%), while in the experimental group was cohesive (43.3%). It was observed a decrease in the adhesive failures and an increase of cohesive failures in the experimental group. However, these differences were not statistically significant when compared to the control group ($p>0.05$).

For both Probase Cold groups 90% of the failures where adhesive. Mixed failures were higher on the experimental group (10%) but this difference was not statistically significant ($p>0.05$).

5. Discussion

The present study evaluated the effect of CHX loading on surface properties of different acrylic reline resins, specifically the surface free energy, microtensile bond strength and the type of failure seen.

Incorporation of CHX acrylic resins is a therapeutic approach for denture stomatitis in which a slow and sustained-releasing device is created. It has been widely evidenced in microbiological and release studies due to its broad-spectrum antimicrobial activity, including against *C. albicans*.(19,21,22,33,43) The antifungal effect of CHX was found to be more effective than other drugs, such as fluconazole, both on releasing and microbiological tests.(22,27,31) However, the incorporation of antimicrobial agents such as CHX into polymeric materials may affect their mechanical properties, making their evaluation imperative.(21,23,57-60) CHX concentrations used in this study were selected based on the results of previous studies that evaluated the 10%, 7.5%, 5%, 2.5 % and 1% concentrations, excluding the ones that influenced negatively the mechanical and physical properties of each material. A recent preliminary microbiological study by Costa established a concentration of 2.5% for Kooliner and 5% for both Ufi Gel Hard and Probase Cold as the minimal concentration effective against *Candida albicans.*(36) Others authors found that these concentrations have no negative influence on the acrylic resins mechanical properties.(51,61,62) Thus, this concentrations were selected for this study.

The three reline resins studied were chosen for their differences in chemical composition and structural arrangement. Direct reline resins Kooliner and Ufi Gel Hard are both poly(methyl methacrylate) based materials and are known for an anomalous water uptake behaviour (21,63,64), with higher drug release characteristics.(64) Kooliner forms a simple non-crosslinking net when polymerization is complete, while Ufi Gel Hard forms a more complex crosslinking net. Indirect reline resin Probase Cold is a poly(methyl methacrylate) based material forming a net with a reduced percentage of uncured monomer methyl methacrylate.(65-68) Since these resins have different physical structure and chemical composition, CHX molecules when incorporated in the net can create different links to the polymeric chains and change their properties in distinct magnitudes. Also, CHX incorporation can increase the distance between polymer molecules, resulting in an expected weaker polymer net.(61)

These biomaterials are submitted to biodegradation processes that can change their physical and biomechanical properties due to the oral environment conditions (16,67,69), being important to simulate oral cavity conditions *in vitro*.(43) Some authors studied the effect of thermal ageing (51,61,62), but it is also importante to mimic the conditions of the oral cavity through a chemical ageing process, which was an objective of this study. Other studies have concluded that the release of CHX from acrylic resins showed a high initial rate of elution from the material followed by a slower and steadier diffusion throughout at least 28 days.(19,21,22,27,31,35,70) Also, another study concluded that maximum cumulative release of CHX was higher at pH=3 and pH=7 for the this three materials.(71) Taking these results into account, in this study a cyclic procedure of 6 hours at pH 3 interchanging with 18 hours at pH 7 was applied for 28 days, because it has also been suggested that an individual with a cariogenic diet is subject, daily, to approximately 6 hours of acid environment.(20,37,68)

The first objective of this investigation was to assess the influence of loading CHX on the surface free energy of reline resins, after a chemical ageing procedure.

The total surface free energy of a solid consists in the sum of components arising from dispersive (apolar) and polar contributions. The technique to determine the surface free energy in this study is an indirect method, in which the contact angles formed on the acrylic resins were measured by immersing each specimen 4mm into two distinct liquids (water and 1,2-propanediol). Then, the contact angles were used to calculate the surface free energy by the Wu method.(55,56) The method enable the calculation of the unknown solid surface energy components (polar and dispersive) from contact angle measurements with the two mentioned liquids.(72-75) Changes in the surface free energy of the acrylic resin will have an impact in its surface wettability and, consequently, in the denture retention to support mucosa and adherence of microorganisms to removable dentures.(30,39,74,76,77)

Considering Kooliner and Ufi Gel Hard, the results showed that there were no statistical differences in total surface free energy, dispersive and polar components between the control and the experimental groups of both materials, similarly to Costa results.(51) However, the total surface free energy values obtained by Costa relatively to the CHX concentrations applied in this study were lower, demonstrating a different effect of a chemical ageing as opposed to thermal ageing process.(51) This could be explained by the results of Alexandre, in which higher release of CHX was seen at a pH 3.(71)

Similarly to a study by Arima, in this study CHX incorporation in Kooliner seems to reveal lower total surface free energy values than the other two materials.(65) The fact that both

Ufi Gel Hard and Kooliner groups weren't affected by the CHX loading might be explained by their similar chemical constitution, being both composed of pre-polymerized poly(ethyl methacrylate) particles.(65) At this time, it may be concluded that the first and second null hypothesis cannot be rejected, meaning that surface free energy seems to not be affected by CHX loading in both Kooliner and Ufi Gel Hard groups.

On Probase Cold, inspite of the CHX group showed significant higher values of the dispersive component compared to the control group (meaning that it could become more apolar with CHX incorporation), there were no significant differences in the total surface free energy.(25) With this knowledge, it may be concluded that the third null hypothesis can not be rejected, since the loading of CHX does not seem to affect the total surface free energy of the acrylic reline resin Probase Cold.

The other objective of this study was to evaluate whether the loading of CHX would interfere or not with the microtensile bond strength between the reline resins and the denture base resin, after a chemical ageing procedure.

Adequate bonding between denture base resin and reline material is essential, since a failure can harbor bacteria, promote staining, decrease the strength of the denture and cause fractures.(23,40,41,43,78,79) In past studies, reline resin adhesion to denture base resin has been determined by test methods such as tensile and shear bond strength. However, according to the current literature, there isn't a consensus on the most reliable test for evaluating the bond strength between denture base and reline resins, because they are not truly testing the bonded interface and tend to induce cohesive fractures.(41,80-82)

Microtensile has been suggested as the first-choice method to determine the bond strength of interfaces between other dental materials because of the reduced testing area and more uniform distribution interfacial stresses, often leading to more adesive failures(45) No studies were found in the existing literature that evaluated the effect of CHX incorporation on microtensile bond strength of acrylic reline resins to denture base resins, although some authors presented it as a viable method.(80,82) Therefore, this study is innovating by applying this test method.

In the present study a crosshead speed of 1mm/min until the separation of the denture base resin and the reline resin was used, since it is considered to be the speed that distributes a more uniform force in the adhesive interface.(83) Is it know that with higher crosshead speed the microtensile bond strength values tend to increase.(8,78) However, there are no previous studies that indicates the most suitable velocity for testing μTBS between acrylic resins. In this

work each cube was treated as an experimental unit for μTBS, in which an average of the values obtained from all sticks of the same cube was used for statistical analysis.(48) Meanwhile for failure mode assessment, the stick was considered as an experimental unit for statistical analysis.(48) Though, the use of sticks as an experimental unit is controversial, as it is associated with pseudoreplication of the results and compromises the independence condition of the specimens.(84)

Regarding the Kooliner and Ufi Gel Hard groups no statistically significant differences were found between experimental and control groups mean values of μTBS. These results are similar than early studies that tested shear bond test strength after thermal ageing from other authors.(23,50,51) Also the mean values obtained in Kooliner for both control (13,0 ± 3,7 MPa) and 2.5% loaded CHX (13,5 ± 3,6 MPa) groups seems lower than the other resins, such as Costa obtained with shear bond test.(51) This may be due to the composition of its monomer isobutylmethacrylate, with a high molecular weight monomer that makes the dissolution of PMMA denture base resin surface difficult and leads to a less effective penetration of the reline resin into the denture base.(66,83) These findings sustained the theory that bond strength is dependent on the chemical composition of both materials (7,66,78,85-88), since bonding of autopolymerizing resins to denture base resin seems to be achieved by penetration and diffusion of monomer into the last one.

With these findings the fourth and fifth null hypothesis can not be rejected, since there were no differences between microtensile bond strength of experimental groups compared to the control in Kolliner and Ufi Gel Hard reline resins.

On the other hand, 5% CHX loaded Probase Cold group presented significantly lower mean microtensile bond strength values compared to the control group. This can be explained by the incorporation of CHX within the polymer matrix of the material, introducing more spaces, less homogeneity in the polymerized materials and weakening the bond strength.(23,31) Also the control group showed the highest mean microtensile bond values (45.0 ± 3.3 MPa) followed by the loaded CHX group with highest results (33.7 ± 1.9 MPa), once again accordingly to shear bond strength results of Costa.(51) This support Ahmad and colleagues hypotheses, since Probase Cold composition is identical to Probase Hot and a much easier diffusion and penetration of PMMA reline monomers of smaller molecular weight into denture base resin is achieved, forming an inter-penetrating polymer network.(44,66,78,85,86) At this point, the sixth null hypothesis can be rejected, since loading Probase Cold with 5% of CHX seem to affect the microtensile bond strength with the acrylic base resin.

In this study the predominant type of failure was adhesive, seen in 79.4% of the sticks when considering the entire sample (N=180). This is in accordance with the purpose of microtensile bond strength tests, where forces are directed towards the adhesive interface with a more uniform distribution, therefore validates the method chosen and applied in this study. An adhesive failure mode may indicate that the bond strength between the reline resin and the denture base is weaker than the reline material strength, which is an advantage if the objective is a temporary lining in practice.(7,31)

Contrary to what was done in this study, some authors defend to not include cohesive failure sticks in the statistical analysis, defending the non-overestimation of the results whose sticks did not fracture at the adhesive interface. However, according to Pashley and colleagues, the fact that the fractures did not occur at the adhesion interface does not mean that the adhesion is stronger than the intrinsic resistance of the substrate but rather that the test may not have been uniformly done and concentrated in a highly localized region.(89)

Regarding Kooliner, both 0% and 2.5% CHX groups, in 96.7% of the sticks an adhesive failure was observed and no cohesive failure. Also, a correlation between the type of failure and the microtensile bond strength values was detected, since a greater tendency for the occurrence of adhesive failures in sticks with lower μTBS values was observed both in 0% and 2.5% CHX Kooliner groups. This result is in agreement with the results of other authors.(48,90,91)

Concerning Ufi Gel Hard 5% CHX loaded specimens, the failure mode most obtained was cohesive (43.3%), higher than the 20% founded in the control group, followed by adhesive failures (40%). It can be stated that as the concentration of CHX incorporation increased a weakening of the internal structure of the UG occurred comparatively to the bond strength in the interface, leading to an increase of cohesive failures. Nevertheless, these differences were not statistically significant, meaning that more studies with a higher number of specimens are needed to confirm the conclusions of the present study, especially in the groups of Ufi Gel Hard were a high standard error was seen.

On the other hand, for the groups with higher μTBS values, such as Probase Cold, it would be expected to obtain more cohesive failures.(48,90,91) However, both experimental and control groups of this material showed a predominance of adhesive failures (90%). Also, a decrease in cohesive failures and an increase in mixed failures with the incorporation of 5% CHX was seen, possibly justified with the significant reduction of μTBS values occurred. A limitation of failure mode assessment in this material is the difficulty of observing the type of failures in the stereomicroscope, since the denture base resin and the reline resin have an

identical composition and an easier diffusion of the reline monomers into the denture base resin is obtained in the interface.(44,66,78,85,86) Although, the statistical analysis did not show significant differences between the type of failures obtained in the experimental and the control groups of all three reline resins. Thus, the seventh, eighth and ninth hypothesis can not be rejected, since no relation was found in the type of failure occurred between the denture base resin and the three reline resins with the respective CHX loaded.

This study led to important conclusions about the effect of CHX incorporation on reline resins followed by a chemical ageing procedure in their surface properties, namely surface free energy, microtensile bond strength and type of failure. So, it can be concluded that the concentration of 2.5% CHX for Kooliner and 5% CHX for Ufi Gel Hard may be valid because, since it is effective against Candida albicans and did not negatively affect the studied properties of these direct reline acrylic resins. On the other hand, loading Probase Cold with a concentration of 5% CHX may not be desirable.

However, more experimental studies under more closely simulated clinical conditions are needed, since it consists on a multifactorial process including exposure to saliva, chewing, breathing and chemical, thermal and dietary changes.(16,43,67) Also, for the microtensile bond strength test, applied for the first time to evaluate the adhesion interface between denture base resin and reline resin, it is essential to stablished a padronizated protocol to follow in further studies. The bond tests applied so far, including μTBS, does not allow to simulate correctly the forces existent in the oral cavity, since in this conditions the adhesion failure usually results from fatigue by repeated application of masticatory forces and not due a single force. So as to replicate these forces in vitro, an experimental method similar to that followed by Attia could be applied, submitting the relined specimens to several masticatory cycles in a simulator machine, analyzing the number of cycles required for its fracture.(92,93) Additionally, more studies are needed to assess how the release of CHX, in long term, is affected by this same conditions of the oral cavity. It would be advantageous to observe and characterize the adhesive interface with the use of scanning electron microscopy and analyze its influence on the microtensile bond strenght value.

6. Conclusions

Within the limitations of the study and considering the results obtained, the main conclusions are:

- Loading Kooliner with 2.5% of CHX and Ufi Gel Hard and Probase Cold with 5% of CHX does not demonstrate to affect the surface free energy.

- Loading Kooliner with 2.5% of CHX and Ufi Gel Hard with 5% of CHX doesn't seem to affect the microtensile bond strength to the denture base resin.

- Loading Probase Cold with 5% of CHX is suggested to affect the microtensile bond strength to the acrylic base resin, with this group presenting lower values than the control group.

- Loading Kooliner, Ufi Gel Hard and Probase Cold with a specific concentration of CHX does not demonstrate to influence the type of failure between them and the denture base resin. The predominant type of failure assessed in the study was the adhesive.

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Appendices

Appendix 1 – Tables

Table 1.1 - Measures of Kooliner specimens for surface free energy study.

% CHX loaded	Specimen Number	Measures (mm) Width	Height	Thickness	Weight (g)
Control 0%	1	24,91	15,87	1,08	0,501
	2	24,87	15,92	1,05	0,462
	3	24,92	16,1	1,16	0,511
	4	24,97	15,89	1,17	0,492
	5	25,06	16,08	1,24	0,464
	6	25,18	15,91	1,2	0,48
	7	24,64	15,95	1,19	0,495
2.5%	1	24,79	15,96	1,16	0,51
	2	24,93	15,85	1,18	0,495
	3	24,92	15,98	1,13	0,464
	4	24,72	15,82	1,27	0,449
	5	24,88	15,78	1,13	0,468
	6	24,8	16,04	1,06	0,512
	7	24,67	15,9	1,17	0,511

Table 1.2 - Measures of Ufi Gel Hard specimens for surface free energy study.

% CHX loaded	Specimen Number	Measures (mm) Width	Height	Thickness	Weight (g)
Control 0%	1	24,5	15,95	1,13	0,451
	2	24,36	15,88	1,04	0,501
	3	24,55	16	1,1	0,485
	4	25,03	15,94	1,01	0,473
	5	23,16	16,03	1,17	0,451
	6	25,13	15,95	1,06	0,462
	7	24,33	15,9	1,09	0,491
5%	1	25,04	15,88	1,02	0,455
	2	24,6	16,13	1,1	0,508
	3	24,4	16,09	1,07	0,509
	4	24,5	15,26	1,14	0,471
	5	24,77	15,88	1,15	0,488
	6	24,67	16,06	1,06	0,508
	7	24,79	15,89	1,16	0,469

Table 1.3 - Measures of Probase Cold specimens for surface free energy study.

% CHX Loaded	Specimen Number	Measures (mm) Width	Height	Thickness	Weight (g)
Control 0%	1	24,97	15,97	1,06	0,492
	2	24,5	15,76	1,12	0,506
	3	24,19	15,99	1,14	0,517
	4	24,59	15,86	1,08	0,507
	5	24,15	16,04	1,07	0,509
	6	24,33	15,89	1,09	0,513
	7	24,47	15,71	1,12	0,518
2.5%	1	24,34	15,85	1,14	0,492
	2	24,42	15,88	1,12	0,496
	3	24,62	15,92	1,01	0,492
	4	24,22	15,95	1,12	0,49
	5	24,52	15,86	1,12	0,501
	6	24,33	15,77	1,01	0,495
	7	24,48	15,91	1,13	0,497

Table 1.4 – Contact angle by reline resin.

Material	% CHX loaded	Contact Angle (°) Water M ± SD	Min	Max	1,2 - Propanediol M ± SD	Min	Max
Kooliner	0%	81.66 ± 5.20	75.63	88.93	50.26 ± 16.64	35.24	86.31
	2.5%	75.67 ± 5.66	68.52	86.98	43.86 ± 5.04	35.89	49.58
Ufi Gel Hard	0%	67.89 ± 8.07	61.35	84.64	28.96 ± 6.09	20.61	39.37
	5%	63.50 ± 1.67	61.83	66.23	28.20 ± 9.72	17.42	43.96
Probase Cold	0%	71.62 ± 6.44	66.02	84.69	52.80 ± 18.76	32.40	76.33
	5%	72.07 ± 5.55	67.31	84.01	32.49 ± 7.23	23.42	43.68

M=Mean; SD=Standard deviation; Min=Minimum; Max=Maximum.

Table 1.5 – Surface free energy data of Kooliner.

Material	% CHX loaded		Surface Free Energy (γ) (mN/m)		
			γ Total	γ Dispersive	γ Polar
Kooliner	0%	**M±SD**	32.10 ± 3.19	15.80 ± 6.64	16.30 ± 9.23
		m	31.80	16.70	14.00
		IR	4.20	4.30	7.60
	2.5%	**M±SD**	34.10 ± 2.26	16.21 ± 3.04	17.89 ± 4.79
		m	34.40	15.20	19.20
		IR	2.30	5.00	4.20

M=Mean; SD=Standard deviation; m=median; IR=Interquartile range.

Table 1.6 – Surface free energy data of Ufi Gel Hard.

Material	% CHX loaded		Surface Free Energy (γ) (mN/m)		
			γ Total	γ Dispersive	γ Polar
Ufi Gel Hard	0%	**M±SD**	39.89 ± 3.48	19.04 ± 2.39	20.81 ± 5.48
		m	41.50	18.70	21.10
		IR	4.40	2.40	6.70
	5%	**M±SD**	41.94 ± 1.09	18.11 ± 2.69	23.83 ± 2.92
		m	42.00	19.00	24.20
		IR	1.80	4.70	3.80

M=Mean; SD=Standard deviation; m=median; IR=Interquartile range.

Table 1.7 – Surface free energy data of Probase Cold.

Material	% CHX loaded		Surface Free Energy (γ) (mN/m)		
			γ Total	γ Dispersive	γ Polar
Probase Cold	0%	**M±SD**	36.71 ± 4.60	12.31 ± 5.50	24.40 ± 6.78
		m	37.20	15.40	23.30
		IR	4.40	9.50	10.40
	5%	**M±SD**	37.24 ± 1.75	19.14 ± 3.74	18.10 ± 4.59
		m	36.60	18.10	19.30
		IR	2.80	2.90	3.50

M=Mean; SD=Standard deviation; m=median; IR=Interquartile range.

Appendix 2 – Figures

Figure 1 - Kooliner

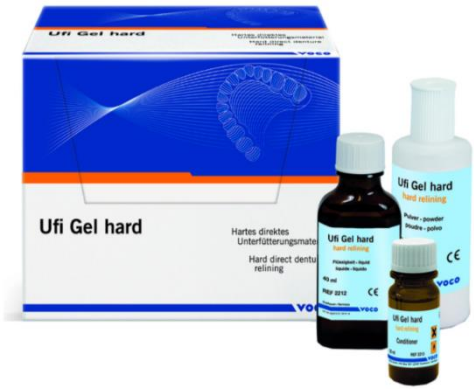


Figure 2 - Ufi Gel Hard

Figure 3 - Probase Cold

Figure 4 - Chlorhexidine Diacetate Monohydrate (CHX)

Figure 5 – Precision Balance

Figure 6 – Incubator

Figure 7 – Ivomat Pressure Device

Figure 8 – Rotational Polishing machine

Figure 9 - Ph meter – Crison micro pH 2001

Figure 10 - Digital micrometer – Mitutoyo

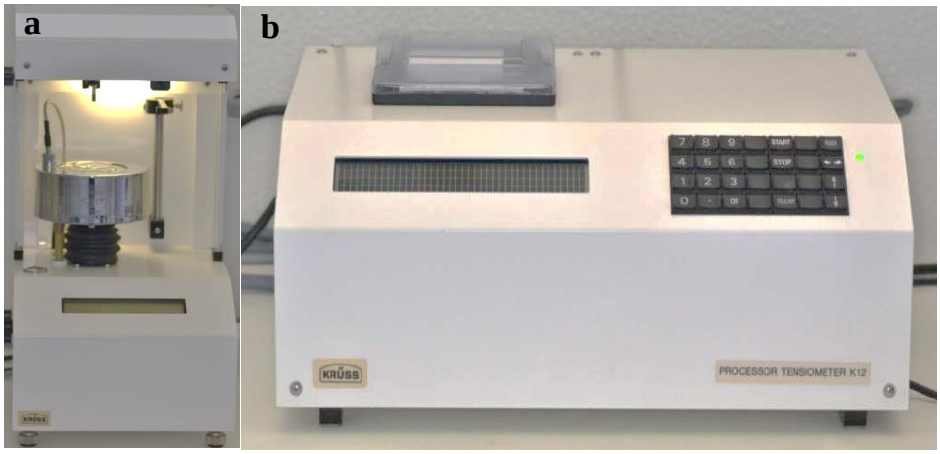


Figure 11 - Processor Tensiometer K12: Equipment used in Wilhelmy Plaque

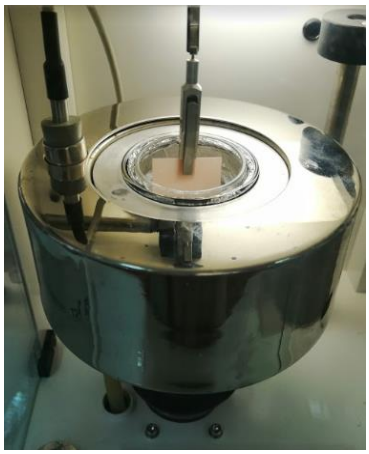


Figure 12 - Immersion of the specimen

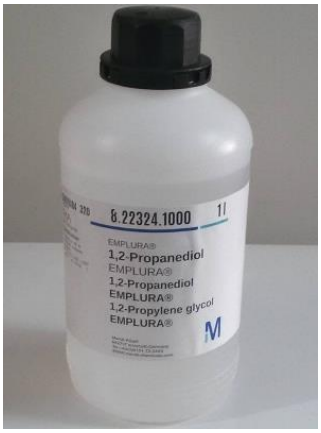


Figure 13 - 1,2-propanediol

Figure 14 - Probase Hot

Figure 15 – Cyanoacrylate glue

Figure 16 – Stereomicroscope: Equipment

Appendix 3 – Experimental Data

1. Surface free energy

1.1 – Kooliner

% CHX loaded	Specimen Number	Advance Contact Angle (°)		Surface Free Energy (γ) (mN/m)		
		Water	1,2-Propanediol	γ Total	γ Dispersive	γ Polar
0%	1	88.93	35.24	31.80	25.20	6.50
	2	84.51	44.56	29.70	18.10	11.60
	3	86.73	48.47	28.20	17.20	11.00
	4	77.70	50.33	32.40	13.80	18.60
	5	76.80	41.53	33.90	16.60	17.30
	6	75.63	86.31	38.00	3.00	35.00
	7	81.34	45.36	30.70	16.70	14.00
2.5%	1	73.38	43.04	34.90	15.70	19.20
	2	76.52	48.86	32.70	14.30	18.40
	3	86.98	38.97	30.50	21.90	8.60
	4	68.52	46.39	37.80	13.80	24.00
	5	75.31	49.58	33.40	13.80	19.60
	6	76.01	35.89	34.40	18.80	15.60
	7	73.00	44.28	35.00	15.20	19.80

1.2 - Ufi Gel Hard

% CHX loaded	Specimen Number	Advance Contact Angle (°)		Surface Free Energy (γ) (mN/m)		
		Water	1,2-Propanediol	γ Total	γ Dispersive	γ Polar
0%	1	61.59	39.37	42.60	15.00	27.60
	2	61.35	25.05	43.10	18.70	24.40
	3	64.76	20.61	41.50	20.30	21.10
	4	84.64	31.30	33.20	22.60	10.60
	5	64.54	28.14	41.80	18.30	23.50
	6	70.41	25.75	38.20	20.40	17.70
	7	67.92	32.48	38.80	18.00	20.80
5%	1	61.83	43.96	42.50	13.70	28.80
	2	64.56	39.32	40.50	15.20	25.30
	3	64.59	22.29	41.40	19.90	21.50
	4	61.95	26.64	42.70	18.50	24.20
	5	62.22	22.39	43.60	19.20	24.30
	6	63.15	25.41	42.00	19.00	23.10
	7	66.23	17.42	40.90	21.30	19.60

1.3 – Probase Cold

% CHX loaded	Specimen Number	Advance Contact Angle (°)		Surface Free Energy (γ) (mN/m)		
		Water	1,2-Propanediol	γ Total	γ Dispersive	γ Polar
0%	1	67.25	40.70	38.70	15.40	23.30
	2	70.86	32.40	37.20	18.60	18.60
	3	72.28	68.08	36.50	7.50	29.00
	4	66.02	76.33	42.30	5.00	37.30
	5	66.78	39.25	39.50	15.60	23.90
	6	84.69	73.04	27.70	7.30	20.40
	7	73.47	39.78	35.10	16.80	18.30
5%	1	67.31	23.42	39.90	20.30	19.60
	2	71.31	36.26	36.60	17.50	19.10
	3	84.01	23.83	34.90	26.80	8.10
	4	70.57	43.68	36.50	15.00	21.50
	5	69.05	34.32	38.70	17.40	21.30
	6	72.85	35.50	35.90	18.10	17.80
	7	69.41	30.45	38.20	18.90	19.30

2. Microtensile Bond strenght

2.1 – Kooliner

	n		Width (mm)	Lenght (mm)	Fracture Resistance (KN)	Adhesion Strength (MPa)	Failure
K 0%	1	1	0,93	0,94	0,0116	13,269	Adhesive
		2	0,87	0,98	0,0121	14,192	Adhesive
		3	0,99	0,93	0,0153	16,618	Mixed
		4	0,94	0,97	0,01	10,967	Adhesive
		5	0,98	0,93	0,018	19,750	Adhesive
	2	1	0,96	0,96	0,0107	11,610	Adhesive
		2	0,99	0,98	0,0099	10,204	Adhesive
		3	0,98	0,99	0,0083	8,555	Adhesive
		4	0,98	0,95	0,0178	19,119	Adhesive
		5	0,96	0,96	0,0058	6,293	Adhesive
	3	1	0,93	0,95	0,007	7,923	Adhesive
		2	0,99	0,91	0,0124	13,764	Adhesive
		3	0,96	0,98	0,0129	13,712	Adhesive
		4	0,99	0,97	0,0004	0,417	Adhesive
		5	0,95	0,95	0,005	5,540	Adhesive
	4	1	0,89	0,83	0,0124	16,786	Adhesive
		2	0,86	0,9	0,014	18,088	Adhesive
		3	0,87	0,89	0,0216	27,896	Adhesive
		4	0,86	0,99	0,0125	14,6823	Adhesive
		5	0,88	0,85	0,0123	16,444	Adhesive
	5	1	1	0,8	0,011	13,750	Adhesive
		2	1,02	0,86	0,0136	15,504	Adhesive
		3	0,98	0,78	0,0101	13,213	Adhesive
		4	0,97	0,84	0,0134	16,446	Adhesive
		5	0,98	0,82	0,0098	12,195	Adhesive
	6	1	0,99	0,76	0,0095	12,626	Adhesive
		2	1,04	1	0,011	10,577	Adhesive
		3	1,06	0,98	0,0079	7,605	Adhesive
		4	0,99	0,84	0,0099	11,905	Adhesive
		5	1	0,95	0,0099	10,421	Adhesive

	n		Width (mm)	Length (mm)	Fracture Resistance (KN)	Adhesion Strenght (MPa)	Failure
K 2.5%	1	1	1,03	0,84	0,0081	9,362	Adhesive
		2	0,94	0,87	0,0104	12,717	Adhesive
		3	0,92	0,87	0,0091	11,369	Adhesive
		4	0,9	0,87	0,0157	20,051	Adhesive
		5	0,91	0,89	0,0078	9,631	Adhesive
	2	1	0,97	0,88	0,0187	21,907	Adhesive
		2	0,98	0,9	0,0171	19,388	Adhesive
		3	0,97	0,98	0,0176	18,515	Adhesive
		4	0,91	0,83	0,0134	17,741	Adhesive
		5	0,92	0,79	0,0138	18,987	Mixed
	3	1	0,93	0,95	0,0083	9,394	Adhesive
		2	0,94	0,88	0,0069	8,341	Adhesive
		3	0,97	0,95	0,0049	5,317	Adhesive
		4	0,86	0,85	0,0087	11,902	Adhesive
		5	0,9	0,83	0,0073	9,772	Adhesive
	4	1	0,91	0,88	0,0149	18,606	Adhesive
		2	0,88	0,82	0,0101	13,997	Adhesive
		3	0,93	0,8	0,0097	13,038	Adhesive
		4	0,93	0,87	0,0067	8,281	Adhesive
		5	1,02	0,92	0,0155	16,517	Adhesive
	5	1	0,95	0,89	0,0093	10,999	Adhesive
		2	0,99	0,88	0,016	18,365	Adhesive
		3	0,91	0,87	0,0173	21,852	Adhesive
		4	0,91	0,86	0,01	12,778	Adhesive
		5	0,93	0,86	0,0101	12,628	Adhesive
	6	1	0,98	0,89	0,0135	15,478	Adhesive
		2	0,96	0,83	0,0088	11,044	Adhesive
		3	0,99	0,81	0,0079	9,852	Adhesive
		4	0,97	0,86	0,0063	7,552	Adhesive
		5	0,95	0,88	0,0083	9,928	Adhesive

2.2 – Ufi Gel Hard

	n		Width (mm)	Length (mm)	Fracture Resistance (KN)	Adhesion Strength (MPa)	Failure
UG 0%	1	1	1,03	0,89	0,0191	20,836	Mixed
		2	1,02	0,97	0,0463	46,796	Mixed
		3	1,03	0,93	0,0287	29,961	Mixed
		4	0,99	0,86	0,0249	29,246	Mixed
		5	1,01	0,86	0,0241	27,746	Adhesive
	2	1	0,95	0,93	0,0173	19,581	Adhesive
		2	1,03	0,89	0,0177	19,308	Cohesive
		3	0,93	0,89	0,0155	18,727	Adhesive
		4	0,9	0,88	0,009	11,364	Adhesive
		5	0,95	0,88	0,0121	14,474	Adhesive
	3	1	0,95	0,81	0,0139	18,064	Adhesive
		2	0,89	0,86	0,0107	13,980	Adhesive
		3	0,88	0,83	0,0117	16,019	Adhesive
		4	0,9	0,81	0,0098	13,443	Adhesive
		5	0,87	0,85	0,0122	16,498	Cohesive
	4	1	0,98	0,88	0,0233	27,018	Cohesive
		2	0,89	0,87	0,0265	34,224	Adhesive
		3	0,93	0,84	0,0251	32,130	Adhesive
		4	0,97	0,83	0,0184	22,854	Adhesive
		5	0,91	0,81	0,0167	22,656	Adhesive
	5	1	0,89	0,82	0,0231	31,653	Adhesive
		2	0,88	0,82	0,0104	14,412	Mixed
		3	0,88	0,84	0,0198	26,786	Cohesive
		4	0,89	0,83	0,0215	29,105	Cohesive
		5	0,91	0,85	0,0337	43,568	Adhesive
	6	1	0,95	0,89	0,0113	13,3656	Adhesive
		2	0,85	0,8	0,0179	26,324	Adhesive
		3	0,93	0,79	0,0113	15,380	Cohesive
		4	0,94	0,88	0,0132	15,957	Adhesive
		5	0,88	0,86	0,0052	6,871	Adhesive

	n		Width (mm)	Length (mm)	Fracture Resistance (KN)	Adhesion Strength (MPa)	Failure
UG 5%	1	1	0,98	0,85	0,0109	13,085	Adhesive
		2	1,01	0,89	0,0125	13,906	Mixed
		3	0,99	0,86	0,0097	11,393	Cohesive
		4	1,03	0,85	0,0083	9,480	Adhesive
		5	0,99	0,86	0,0109	12,802	Adhesive
	2	1	0,89	0,81	0,0125	17,339	Cohesive
		2	0,88	0,85	0,0333	44,519	Cohesive
		3	0,9	0,81	0,0132	18,107	Mixed
		4	0,94	0,79	0,0163	21,950	Mixed
		5	0,86	0,81	0,0246	35,314	Cohesive
	3	1	0,94	0,82	0,0167	21,666	Adhesive
		2	0,91	0,75	0,0141	20,659	Adhesive
		3	0,88	0,8	0,0051	7,244	Adhesive
		4	0,97	0,8	0,0095	12,242	Adhesive
		5	0,89	0,82	0,0075	10,277	Adhesive
	4	1	0,87	0,8	0,0133	19,109	Cohesive
		2	0,88	0,79	0,0101	14,528	Adhesive
		3	0,87	0,87	0,0142	18,761	Cohesive
		4	0,95	0,82	0,0118	15,148	Adhesive
		5	0,87	0,84	0,0102	13,957	Cohesive
	5	1	0,86	0,81	0,0087	12,489	C (UG)
		2	0,87	0,85	0,0132	17,850	M
		3	0,89	0,8	0,0147	20,646	C (UG)
		4	0,94	0,89	0,0121	14,463	M
		5	0,89	0,86	0,0161	21,035	C (UG)
	6	1	0,84	0,8	0,0197	29,315	C (UG)
		2	0,92	0,83	0,0148	19,382	Adhesive
		3	0,98	0,82	0,0246	30,612	C (UG)
		4	0,91	0,85	0,0115	14,867	Adhesive
		5	0,9	0,84	0,0122	16,138	Cohesive

2.3 – Probase Cold

	n		Width (mm)	Length (mm)	Fracture Resistance (KN)	Adhesion Strength (MPa)	Failure
PC 0%	1	1	0,87	0,62	0,0214	39,674	Adhesive
		2	0,92	0,81	0,0285	38,245	Cohesive
		3	0,61	0,69	0,0175	41,578	Adhesive
		4	0,88	0,86	0,0296	39,112	Adhesive
		5	1,03	0,82	0,0378	44,755	Adhesive
	2	1	1,07	1,02	0,0356	32,619	Adhesive
		2	0,98	0,96	0,0507	53,890	Adhesive
		3	0,96	0,9	0,0463	53,588	Adhesive
		4	0,95	0,87	0,042	50,817	Adhesive
		5	0,98	0,96	0,0476	50,595	Adhesive
	3	1	1,01	0,86	0,0425	48,929	Adhesive
		2	0,9	0,8	0,0299	41,528	Adhesive
		3	0,96	0,95	0,0401	43,970	Adhesive
		4	0,98	0,81	0,0355	44,722	Adhesive
		5	1,02	0,98	0,0425	42,517	Adhesive
	4	1	1,03	0,92	0,0476	50,232	Adhesive
		2	0,9	0,86	0,0264	34,109	Adhesive
		3	1,08	0,7	0,0329	43,519	Adhesive
		4	1,09	0,88	0,0429	44,725	Mixed
		5	1	0,88	0,0404	45,909	Adhesive
	5	1	1,01	0,89	0,0433	48,170	Adhesive
		2	1,09	0,92	0,0525	52,353	Adhesive
		3	0,94	0,92	0,0356	41,166	Mixed
		4	1	0,83	0,0462	55,663	Adhesive
		5	1,03	0,92	0,0472	49,810	Adhesive
	6	1	1,01	0,98	0,036	36,371	Adhesive
		2	1,02	0,82	0,0383	45,791	Adhesive
		3	0,94	0,91	0,0421	49,217	Adhesive
		4	0,88	0,8	0,036	51,136	Adhesive
		5	0,82	0,82	0,0233	34,652	Adhesive

	n		Width (mm)	Length (mm)	Fracture Resistance (KN)	Adhesion Strength (MPa)	Failure
PC 5%	1	1	0,9	0,87	0,0269	34,355	Adhesive
		2	0,92	0,81	0,0241	32,340	Adhesive
		3	1,01	0,95	0,0371	38,666	Mixed
		4	0,86	0,78	0,0224	33,393	Adhesive
		5	0,91	0,88	0,0312	38,961	Adhesive
	2	1	0,84	0,81	0,0242	35,567	Adhesive
		2	0,86	0,82	0,0226	32,048	Adhesive
		3	0,89	0,84	0,0256	34,243	Adhesive
		4	0,88	0,79	0,0209	30,063	Adhesive
		5	0,89	0,68	0,0207	34,203	Adhesive
	3	1	0,95	0,87	0,0228	27,586	Adhesive
		2	0,87	0,84	0,024	32,840	Adhesive
		3	0,84	0,79	0,0207	31,193	Adhesive
		4	0,89	0,8	0,0212	29,775	Adhesive
		5	0,9	0,88	0,0345	43,561	Mixed
	4	1	0,87	0,82	0,0232	32,520	Adhesive
		2	0,82	0,8	0,0199	30,335	Adhesive
		3	0,85	0,83	0,0228	32,318	Adhesive
		4	0,84	0,79	0,0214	32,248	Adhesive
		5	0,84	0,81	0,0195	28,660	Adhesive
	5	1	0,94	0,85	0,0275	34,418	Adhesive
		2	0,97	0,8	0,0304	39,175	Adhesive
		3	0,86	0,84	0,0278	38,483	Adhesive
		4	0,89	0,8	0,0246	34,551	Adhesive
		5	0,82	0,75	0,0216	35,122	Adhesive
	6	1	0,88	0,81	0,026	36,476	Adhesive
		2	0,9	0,89	0,0289	36,080	Mixed
		3	0,83	0,74	0,0256	41,680	Adhesive
		4	1	0,87	0,0175	20,115	Adhesive
		5	0,83	0,81	0,0199	29,600	Adhesive

Appendix 4 – Manufacturer's instructions

1. Kooliner

Prior to use, carefully read the instructions for use. EN

KOOLINER™

HARD CHAIRSIDE DENTURE RELINE

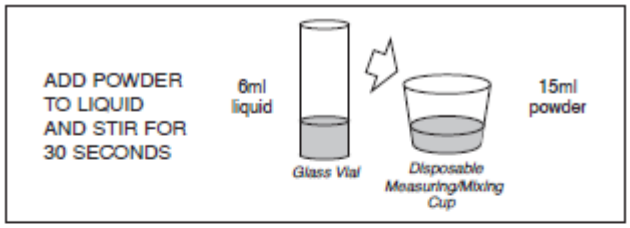
For use only by a dental professional in the recommended indications.

RECOMMENDED INDICATIONS
A temporary lining for acrylic dentures. For use in chairside procedures.

CONTRAINDICATIONS
Patients who have shown sensitivity to methacrylates. In case of allergy refer to a physician. Not intended for permanent lining.

DIRECTIONS FOR USE

1. **Preparation of the denture:**
   Relieve and roughen the area of the denture to be relined. Clean and dry the denture thoroughly. Coat labial and buccal surfaces of the denture with COE LUBRICANT. Do not apply coating within 3mm (1/8 inch) of the peripheral border. If the denture has plastic teeth also protect them with COE LUBRICANT.
2. **Preparation of KOOLINER:**
   Recommended powder / liquid ratio is 15ml powder to 6ml liquid. Pour liquid into the mixing cup and then add the powder slowly. Stir thoroughly for no more than 30 seconds and avoid the introduction of air bubbles.



3. **Application:**
   After approximately 1-2 minutes, spread the mixture of KOOLINER over the area to be relined. Seat the denture in the manner of taking an impression and instruct the patient to close lightly into occlusion. After 3 minutes, instruct the patient to move lips and cheeks so that a muscle trimmed periphery is obtained. Remove the denture and rinse under cold water. Trim away excess material. Re-seat the denture and instruct the patient to close FIRMLY into occlusion, and to hold this position for 5 minutes. Remove the denture and rinse again in cold water.
4. **Finishing:**
   Peak curing temperature: Approximately 43°C/110°F at 7 minutes when tested according to ADA/ANSI specification number 17. In thicker applications, peak temperature may exceed that stated above, possibly producing a hazardous condition in the mouth during curing. When curing is complete (10 minutes), trim away excess. For smoothing the edges, use a hot spatula.

STORAGE
Recommended for optimal performance, store at temperature of 4-25°C (39-77°F).

PACKAGES

345001 KOOLINER Professional Package
345002 KOOLINER Powder, 3 oz (80 g)
345091 KOOLINER Liquid, 55 mL

CAUTION

1. Patient should clean daily to remove food deposits and plaque. Recommend a commercially available denture cleaner and brush. Do not recommend toothpastes or hard bristle brushes as they may damage the denture liner or denture.
2. If patient notices damage to denture (e.g., chipping, delamination, distortion, etc.) or changes in tissue condition (e.g., inflammation, discomfort, allergic reaction, etc.) have patient discontinue use and return for evaluation and consultation.
3. Personal Protective Equipment (PPE) such as gloves, face masks and safety eyewear should always be worn.
4. Ensure good ventilation/exhaustion at the workplace. Keep ignition sources away.

CLEANING AND DISINFECTING RECOMMENDATION
MULTI-USE DELIVERY SYSTEMS: To avoid cross-contamination between patients the bottles and measuring devices require mid-level disinfection. Immediately after use inspect the bottles, measuring devices and label for deterioration. Discard if damaged.
DO NOT IMMERSE. Thoroughly clean bottles and measuring devices to prevent drying and accumulation of contaminants. Disinfect with a mid-level registered healthcare-grade infection control product according to regional/national guidelines.

Some products referenced in the present IFU may be classified as hazardous according to GHS. Always familiarize yourself with the Safety Data Sheets available at:
http://www.gceurope.com
or for the Americas:
http://www.gcamerica.com
They can also be obtained from your supplier.

Last Revised: 04/2018

CE 0086 Rx Only

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PRINTED IN THE U.S.A.

2. Ufi Gel Hard

VOCO

Ufi Gel hard

GB CE

Instructions for use

Ufi Gel hard is a cold-curing permanently hard relining material for dentures on polymethacrylate basis. It is simple and quick to use for direct as well as indirect relinings.
The **Ufi Gel hard** liquid contains no methyl-methacrylates, thus minimising the risk of allergies and irritations of the mucosa. The set additionally contains a conditioner to achieve a permanent and stable bond.

Fields of application:
- hard, permanent, total or partial relinings to restore the functions of partial and total dentures
- lengthening of denture margins

Application:

1. Preparation of the denture
Check occlusion and carry out corrections, if necessary. Clean denture thoroughly with a brush and dry. Roughen all areas to be relined incl. buccal and labial margins with a suitable bur. Then clean and dry. Insulate areas not to be relined (e. g. artificial teeth, labial and buccal area below roughened surfaces), with e. g. vaseline.
Clasps, anchors and attachments of partial dentures have to be blocked out with thin-flowing silicone or wax with low melting point.

2. Application of the conditioner
Apply conditioner with enclosed brush on all surfaces to be relined and let dry in the air (approx. 30 s). First coat the labial and buccal surfaces, then the base of the denture. The conditioner remains effective for about 10 min after application.
Attention: Close the bottle tightly immediately after use because of high volatility.
The brush can be cleaned with e. g. alcohol.

3. Dosing and mixing
Prior to first use, exchange the transportation cap against the dropper.
Ufi Gel hard is mixed in a proportion 1 ml liquid to 3 ml (≈ 1.8 g) powder. This corresponds to 2 graduation marks of the dropper to 1 graduation mark of the glass cylinder.

2 graduation marks liquid : 1 graduation mark powder

A thicker consistency, e. g. for lengthening the margins of the denture, is achieved by taking more powder.
Take out liquid with the dropper and put it into a PP-mixing cup. Shake the powder shortly and dispense it into the glass cylinder. For exact dosing make a smooth surface of the powder by slightly tapping on the side of the glass cylinder. Put powder into the liquid and mix carefully homogeneously with plastic spatula. Bubbles should be avoided by stirring slowly and along the side of the mixing cup. Let any bubbles rise to the top by tapping the mixing cup. Let the material soak until a workable consistency is achieved (approx. 1.5 min after the begin of mixing).

4. Application of Ufi Gel hard
Apply the material with a plastic spatula evenly onto the prepared margin and/or base of the denture, avoid excess and remove with a suitable instrument respectively.
Re-insert denture and have the patient exert slight occlusional pressure for 1 min. Then carry out functional, chewing and swallowing movements for 2 min. Make sure that no material flows into the throat when relining an upper denture, especially at the transition from hard to soft palate (A-line).

4.1 Partial and total denture <u>with</u> undercuts
Remove denture after 5 min after the begin of mixing and remove excess immediately with scissors or a scalpel. Insert again into the mouth for a final occlusion check and let cure for about 2 - 3 min.
Instead of re-inserting the denture, curing can also be completed in warm water, i.e. in a pressure pot at approx. 40°C. **Do not let material cure under contact to air** since oxygen will cause an uncured inhibition layer on the surface.

4.2 Total dentures <u>without</u> undercuts
Excess material can be removed intra- or extraorally before final polymerization.
Intraorally: remove excess material after 5 min (beginning of mixing) with a suitable instrument. Let the denture cure for further 2 - 3 min in the mouth until **Ufi Gel hard** is completely cured.
Extraorally: see position 4.1

5. Finishing the relining
The relined denture can be finished and polished with the usual instruments (tungsten carbide bur, silicone polisher, polishing disc).

beginning of mixing	2 min	mix and apply
insert denture into the mouth	1 min	exert pressure
	2 min	functional movements
remove denture if necessary	1.5 min	remove excess
insert denture into the mouth	2 min	curing
remove denture	1 min	finishing, polishing
	9.5 min	

☐ extraorally ☐ intraorally ☐ intra- or extraorally

Indications, precautionary measures:
- Store bottles of liquid and conditioner carefully closed and in upright position
- Avoid contact of liquid or conditioner to skin. Rinse contacted parts of the skin thoroughly with water and soap
- In case of contact with the eyes rinse thoroughly with water and consult an ophtalmologist
- **Ufi Gel hard** contains hydroxyethylmethacrylate, benzoylperoxid, acetone, do not use in case of allergies against these ingredients
- The stated time periods refer to a room temperature of 25°C as well as temperature in the mouth of 35°C.
  Polymerization will be slower at lower temperatures, higher temperatures will accelerate polymerization
- Too long or violent mixing might lead to air bubbles and unhomogeneous consistency which produces a rough surface
- Use the mixing cup several times
- Store at temperatures 4°C - 23°C

Cleaning indications:
Dentures relined with **Ufi Gel hard** can be cleaned with the usual cleansers and procedures. This refers to domestic as well as to professional cleaning.

Our preparations have been developed for use in dentistry. As far as the application of the products delivered by us is concerned, our verbal and/or written information has been given to the best of our knowledge and without obligation. Our information and/or advice do not relieve you from examining the materials delivered by us as to their suitability for the intended purposes of application. As the application of our preparations is beyond our control, the user is fully responsible for the application. Of course, we guarantee the quality of our preparations in accordance with the existing standards and corresponding to the conditions as stipulated in our general terms of sale and delivery.

3. Probase Cold

ProBase® Cold

Instructions for Use
Verarbeitungsanleitung
Mode d'emploi
Istruzioni d'uso
Instrucciones de uso
Instruções de Uso
Bruksanvisning
Brugsanvisning
Käyttöohjeet
Bruksanvisning
Productinformatie
Οδηγίες Χρήσεως
Kullanma Talimatı
Инструкция по применению
Instrukcja stosowania

BPS

CE 0123

Complies with / entspricht
ISO 20795-1; EN ISO 20795-1

For dental use only.
Rx only

Manufacturer:
Ivoclar Vivadent AG, FL-9494 Schaan/Liechtenstein
www.ivoclarvivadent.com

ivoclar vivadent: technical

english

Description
ProBase® Cold is a self-curing denture base material. It demonstrates excellent flow and modelling properties. It is easy and reliable to use with the pouring or packing technique, even when two or more saddles are present. The material is available in a variety of shades. As the powder and liquid can be dosed as desired within the usual limits, users can vary the consistency and working time of ProBase Cold.

Composition
Powder
Polymethyl methacrylate, softening agent, benzoyl peroxide, catalyst, pigments

Liquid
Methyl methacrylate, dimethacrylate, catalyst

Indication
- Partial dentures
- Combination dentures
- Relining
- Repairs
- Complete dentures

Contraindication
- Direct intraoral contact of unpolymerized material.
- If the patient is known to be allergic to any of the ingredients in ProBase Cold

Side effects
In individual cases, local allergic reactions to polymethyl methacrylate materials have been reported.

Application
Pouring technique
Preparation
Isolate boiled-out, well-wetted plaster surfaces with two layers of Ivoclar Vivadent Separating Fluid. Allow to dry, well roughen the teeth, apply mechanical retention, and wet with monomer to ensure an adequate bond with the denture base.

Dosage
- **Ideal mixing ratio**
  15 g polymer (powder) : 10 ml monomer (liquid)

Mixing
Thoroughly mix polymer and monomer with the spatula. Subsequently allow the mixture to rest for 15 seconds to permit any trapped air to rise.
Flow phase
At room temperature (23 °C / 73 °F), the flow phase is approx. 2.5 to 3 minutes. Pour the material into the saddle within this time span.

Modelling phase
The material is set after a transition period of approx. 4 minutes. It can be modelled during an additional 3 minutes.
High room temperatures shorten the flow and modelling phase.

Polymerization
Polymerization is carried out in a pressure device (e.g. Ivomat) at 40 °C / 104 °F and at 2 to 6 bar pressure for 15 minutes.

Finishing
Remove the precast, finish and polish in the usual manner.

Packing technique
Preparation
Isolate boiled-out, well-wetted plaster surfaces with two layers of Separating Fluid and allow to dry completely. Well roughen the teeth and wet with monomer to ensure an adequate bond with the denture base.

Dosage
Ideal mixing ratio for one denture: 20.5 g polymer
(= 1st graduation on measuring cylinder): 10 ml monomer

Integrated dosage system
This system ensures an ideal mixing ratio and, therefore, minimum polymerization shrinkage of ProBase Cold. The measuring cylinder for the polymer indicates the quantity of material required for one or two medium-sized dentures. The graduation on the measuring cylinder for the monomer is in millilitres.

Mixing
Thoroughly mix polymer and monomer in the given mixing ratio with a spatula. Mix thoroughly. Allow the dough to mature in a closed mixing cup for approx. 3 to 4 minutes. Subsequently, work the dough within approx. 2 minutes.
High room temperatures shorten the working time.

Pressing
Place a sufficient quantity of the resin dough in the hand-warm, wetted and isolated flask halves. Carefully close flask and load with 80 bar pressure. Fix with a clamp.

Polymerization
Polymerization is carried out by means of the clamp or a pressing device under constant pressure for 30 minutes (if the room temperture is 23 °C / 73 °F).
Deflasking and finishing
Open the flask and remove plaster. Check occlusion of the denture. Subsequently, finish and polish in the usual manner.

Repair and correction possibilities with ProBase Cold
- Corrections and repairs of ProBase Hot, ProBase Cold and SR Ivocap® may be carried out with ProBase Cold by using the pouring technique. Thoroughly roughen the corresponding surfaces and wet with monomer.
- The residual monomer content after polymerizing the material according to the method described is < 4,5%.

Warnings
- The monomer contains methyl methacrylate (MMA).
- Methyl methacrylate is easily flammable and irritating (flash point +10 °C / 50 °F).
- Irritating to eyes, skin, and respiratory system.
- May cause sensitization by skin contact.
- Avoid contact of the skin with monomer or uncured material. Commercial medical gloves do not provide protection against the sensitizing effect of methacrylates.
- Do not breathe vapour.
- Keep away from sources of ignition – no smoking.
- Do not empty into drains.
- Take precautionary measures against static discharges.

Storage
- Store material in a cool, dark, well-ventilated place. Storage temperature: 2–28 °C (36–82 °F).
- Do not use the materials after the indicated date of expiration.
- Keep out of the reach of children.

Date information prepared: 08/2012

4. Probase Hot

ProBase® Hot

Instructions for Use
Verarbeitungsanleitung
Mode d'emploi
Istruzioni d'uso
Instrucciones de uso
Instruções de Uso
Bruksanvisning
Brugsanvisning
Käyttöohjeet
Bruksanvisning
Productinformatie
Οδηγίες Χρήσεως
Kullanma Talimatı
Инструкция по применению
Instrukcja stosowania

BPS

CE 0123

Complies with / entspricht
ISO 20795-1; EN ISO 20795-1

For dental use only.
Rx only

Manufacturer:
Ivoclar Vivadent AG, FL-9494 Schaan/Liechtenstein
www.ivoclarvivadent.com

ivoclar vivadent: technical

english

Description
ProBase® Hot sets quality standards for heat-curing denture base materials with regard to working properties, shape and shade stability and comfort of fit. The material is available in a variety of shades. Various methods of polymerization render the material suitable for a number of application possibilities.

Composition
Powder
Polymethyl methacrylate, softening agent, benzoyl peroxide, pigments

Liquid
Methyl methacrylate, dimethacrylate (linking agent), catalyst

Indication
- Complete dentures
- Partial dentures
- Combination dentures
- Relining

Contraindication
- Direct intraoral contact of unpolymerized material.
- If the patient is known to be allergic to any of the ingredients in ProBase Hot

Side effects
In individual cases, local allergic reactions to polymethyl methacrylate materials have been reported.

Application
Preparation
Isolate boiled-out, well-wetted plaster surfaces with two layers of Ivoclar Separating Fluid and allow to dry. To ensure an adequate bond with the denture base, well roughen the teeth and wet with monomer.
- **Isolate plaster surfaces twice.**
- **Invest wax model with plaster in the flask.**

Dosage
- **Ideal mixing ratio for one denture**
  22.5 g polymer (powder) : 10 ml monomer (liquid)
- **With dosage system**
  1 graduation mark polymer : 10 ml monomer

Integrated dosage system
The integrated dosage system ensures an ideal mixing ratio and, therefore, minimum polymerization shrinkage of ProBase Hot. The measuring cylinder for the polymer indicates the quantity of material used for one or two medium-sized dentures. The graduation on the measuring cylinder for the monomer is in millilitres. Use the appropriate graduation mark.

Mixing
Thoroughly mix polymer and monomer in the given ratio by means of a spatula. Mix thoroughly. Subsequently, leave the material to mature in the closed mixing cup at room temperature (23 °C / 73 °F) for approx. 8 to 10 minutes.

Working time
When the material has matured sufficiently and is no longer sticky, it can be worked for approx. 20 minutes at 23 °C/73 °F.
- **Thoroughly mix powder and liquid.**
- **Dough time and working time depend on the temperature.**

Pressing
Place a sufficient quantity of the resin dough in the hand-warm (approx. 40 °C / 104 °F), isolated flask halves. Carefully close flask, load with 80 bar pressure and fix with a clamp. **Maintain pressure.**

Polymerization
Heat-polymerization can be carried out in different ways:

Standard procedure (recommended method)
Place closed flask in cold water. Heat up to 100 °C / 212 °F and let boil for 45 minutes.

Alternative methods
- Place flask in cold water, heat up to 70 °C / 158 °F and leave it for 30 minutes. Then heat up to 100 °C / 212 °F and let boil for 30 minutes.
- Place flask in water of 70 °C / 158 °F and leave it for 60 minutes. Subsequently, heat up to 100 °C / 212 °F and let boil for 30 minutes.
- Place flask in boiling water. Bring the water to the boil again and then let boil for 40 minutes. This procedure is only suitable for medium-sized dentures.
- Place flask in cold water, heat up to 80 °C / 176 °F and polymerize for 10 hours. Switch off heat source and leave the flask to cool in the same water bath over night.
- Polymerize the contents of the flask for 10 hours at 80 °C / 176 °F in the drying cabinet.

Residual monomer content can be reduced by increasing the polymerization temperature and by prolonging the polymerization length. We recommend using the standard procedure to keep the residual monomer content at minimum levels. The residual monomer content after polymerizing the material according to the standard procedure is <2.2%.

Cooling
Let the flask cool at room temperature for 30 minutes. Subsequently, completely cool the flask with cold water.

Deflasking and finishing
Open the completely cooled flask and remove plaster. Check occlusion of the denture. Subsequently, finish and polish in the usual manner.

Repair and correction possibilities of ProBase Hot
Corrections and repairs can be carried out with the cold-curing ProBase Cold material, using the pouring technique. Thoroughly roughen the corresponding surfaces and wet them with monomer.

Warnings
- The monomer contains methyl methacrylate (MMA).
- Methyl methacrylate is easily flammable and irritating (flash point + 10 °C / 50 °F).
- Irritating to eyes, skin, and respiratory system.
- May cause sensitization by skin contact.
- Avoid contact of the skin with monomer or uncured material. Commercial medical gloves do not provide protection against the sensitizing effect of methacrylates.
- Do not breathe vapour.
- Keep away from sources of ignition – no smoking.
- Do not empty into drains.
- Take precautionary measures against static discharges.

Storage
- Store material in a cool, dark, well-ventilated place. Storage temperature: 2–28 °C (36–82 °F).
- Do not use the materials after the indicated date of expiration.
- Keep out of the reach of children.

Date information prepared: 08/2012