Universidade de Lisboa
Faculdade de Medicina Dentária

A Chlorhexidine Delivery System Based on Reline Acrylic Resins

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Orientadores: Prof. Doutora Maria Cristina Bettencourt Neves
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Tese especialmente elaborada para obtenção do grau de Doutor em Medicina Dentária, especialidade de Biomateriais

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Prefácio

A presente dissertação tem como objetivo a avaliação *in vitro* de um novo sistema de libertação localizada e controlada de fármaco para ser utilizado no contexto do tratamento e controlo da estomatite protética associada à *Candida albicans*.

Com o objetivo de selecionar o fármaco, bem como o biomaterial mais adequado para este novo sistema, foram inicialmente realizadas uma revisão narrativa e uma revisão sistemática. A primeira descreve os sistemas de libertação de fármacos utilizados nas diversas áreas da Medicina Dentária (capítulo 1), e a segunda os possíveis fármacos, bem como a sua forma de adição, em resinas acrílicas, a partir de uma análise sistemática de toda a literatura existente até à data (capítulo 2).

O novo sistema proposto consiste na incorporação de clorexidina em resinas acrílicas de rebasamento duro, de polimerização direta e indireta, utilizadas em próteses dentárias removíveis (capítulo 3).

Para atingir o objetivo inicialmente descrito foram realizados estudos experimentais que permitiram entender o efeito da incorporação da clorexidina nas resinas acrílicas de rebasamento (capítulo 4), que incluíram: a) a caracterização da atividade microbiológica contra *Candida albicans* e *Streptococcus oralis* das resinas acrílicas de rebasamento com incorporação de clorexidina, b) a caracterização das propriedades mecânicas (microdureza e resistência à flexão), estruturais (morfologia, porosidade total e composição química) e de superfície (porosidade, energia livre, microscopia de superfície e resistência ao corte), c) a caracterização da quantidade de fármaco libertada durante 28 dias e d) a caracterização do efeito biológico em fibroblastos dos lixiviados das resinas acrílicas de rebasamento com incorporação do fármaco.

Este trabalho inclui também o estudo do efeito da incorporação de clorexidina após o envelhecimento térmico ou químico deste sistema (capítulo 5). Para atingir este objetivo foram realizados estudos experimentais com os seguintes objetivos específicos: a) a caracterização das propriedades mecânicas (microdureza e resistência à flexão) e de superfície (energia livre) das resinas acrílicas após envelhecimento e b) a determinação da estabilidade cromática das resinas acrílicas de rebasamento.

A caracterização da atividade microbiológica foi realizada através do método de difusão em ágar e da formação do biofilme, efetuados no laboratório do "Chemical

Biology and Toxicology" do Instituto de Investigação do Medicamento (iMed.ULisboa) da Faculdade de Farmácia da Universidade de Lisboa e no laboratório de Microbiologia da Faculdade de Medicina Dentária da Universidade de Granada, em Espanha. As observações em microscopia de varrimento foram efetuadas num microscópio SEM do Instituto Superior Técnico da Universidade de Lisboa.

A caracterização das propriedades mecânicas do sistema de libertação foi realizada através de estudos de microdureza e de resistência à flexão, efetuados no laboratório de Biomateriais (BioLAB) da Unidade de Investigação em Ciências Orais e Biomédicas (UICOB) da Faculdade de Medicina Dentária da Universidade de Lisboa, bem como os estudos de resistência ao corte.

A caracterização da morfologia e porosidade total foi realizada através de imagens tridimensionais de microtomografia computadorizada (micro-CT) no Centro para o Desenvolvimento Rápido e Sustentável do Produto (CDRSP) do Instituto Politécnico de Leiria. A variação da composição química foi analisada por espectroscopia de "Fourier transform infrared - attenuated total reflectance" (FTIR-ATR) no mesmo laboratório, bem como a análise morfológica da superfície por microscopia SEM.

A caracterização da porosidade aberta de superfície foi realizada pelo método de imersão, no Laboratório de Engenharia Mecânica do Centro de Desenvolvimento de Produto e Transferência de Tecnologia (CDP2T) na Escola Superior de Tecnologia de Setúbal do Instituto Politécnico de Setúbal.

A caracterização da energia livre de superfície foi efetuada no laboratório do "Chemical Biology and Toxicology" do iMed.ULisboa da Faculdade de Farmácia da Universidade de Lisboa, bem como a determinação da quantidade de fármaco libertada através de estudos de libertação e a avaliação do efeito biológico, através de estudos de citotoxicidade.

O processo de envelhecimento térmico foi realizado no BioLAB da UICOB da Faculdade de Medicina Dentária da Universidade de Lisboa, e o de envelhecimento químico, no laboratório do iMed.ULisboa da Faculdade de Farmácia da Universidade de Lisboa.

A determinação da estabilidade cromática foi realizada com recurso a dois espectrofotómetros no laboratório do Grupo de Investigação em Biologia e Bioquímica Oral (GIBBO) da UICOB.

Resumo

Os sistemas de libertação de fármacos são amplamente utilizados na área da Medicina Dentária e surgiram como uma modalidade de tratamento para diversas patologias. Estão descritas na literatura três técnicas para introduzir um fármaco no biomaterial: *coating*, imersão e incorporação. Diversos compostos como a prata, o miconazol, o óxido de zinco, o dióxido de titânio, a clorexidina, o fluconazol, a nistatina, *peptide mimetic compounds*, o zircónio, o etanol, bem como compostos de origem natural, têm sido introduzidos como agentes antimicrobianos em resinas acrílicas como forma de tratamento da estomatite protética.

A estomatite protética é uma condição patológica que afeta os indivíduos portadores de próteses dentárias removíveis. Localiza-se frequentemente na maxila e manifesta-se com o aparecimento de edema e eritema na mucosa de suporte, sendo geralmente assintomática. É caracterizada pela presença de um processo inflamatório na mucosa oral com etiologia multifatorial, sendo os fungos da espécie *Candida* apontados como principal fator etiológico. Outros fatores como os fatores traumáticos (existência de próteses mal adaptadas ou instáveis), bem como fatores predisponentes locais e/ou sistémicos também estão presentes na etiopatogenia desta patologia.

A terapêutica convencional com antifúngicos tópicos e/ou sistémicos tem-se demonstrado insuficiente, pelo que se tem estudado uma nova forma de tratamento a partir de sistemas de libertação localizada de fármacos. Este tratamento incide, em simultâneo, na base da prótese removível, no biofilme microbiano, bem como na mucosa de suporte. Estes sistemas com consequente libertação do fármaco para a cavidade oral, permitem inibir a adesão e o crescimento microbiano, induzindo um efeito terapêutico para esta condição. Essa libertação ocorre diretamente no local de ação de uma forma gradual e constante. Para além disso, minimiza a necessidade de *compliance* por parte do paciente e está associada a menos efeitos adversos quando comparada com a terapia sistémica.

No contexto da estomatite protética associada à *Candida albicans*, o novo sistema de libertação, localizada e controlada, de fármaco proposto consiste na incorporação de clorexidina em diferentes resinas acrílicas de rebasamento duro, de polimerização direta e indireta, utilizadas em próteses dentárias removíveis.

O objetivo do capítulo número quatro deste trabalho foi avaliar o efeito da incorporação de clorexidina na atividade microbiológica contra *Candida albicans* e *Streptococcus oralis*, bem como nas propriedades mecânicas, estruturais e de superfície de resinas acrílicas de rebasamento. Foi também caracterizada a quantidade de fármaco libertada, bem como o efeito citotóxico deste novo sistema.

Foram selecionadas três resinas acrílicas de rebasamento, Kooliner e Ufi Gel Hard (ambas de rebasamento direto) e Probase Cold (de rebasamento indireto). Os grupos experimentais incluíram Kooliner com 2,5% (m/m), Ufi Gel Hard e Probase Cold com 5% (m/m) de clorexidina. Para cada resina também foram produzidos espécimes com 0% clorexidina (grupo controlo). Estes grupos foram definidos pelo método de difusão em ágar com *Candida albicans* inicialmente realizado. Em cada grupo, a clorexidina foi misturada a seco com o pó da resina acrílica correspondente. Após a incorporação do líquido à mistura foram produzidos espécimes a partir de moldes de aço com formato e dimensões de acordo com o teste a realizar.

Foi calculada previamente a concentração mínima inibitória do fármaco para as estirpes selecionadas. Para determinar a atividade microbiológica através do método de difusão em ágar, os grupos de espécimes (n=3) foram colocados em placas inoculadas com *Candida albicans* ou *Streptococcus oralis*. Os espécimes com biofilme à superfície foram analisados através de microscopia SEM. Para avaliar as propriedades mecânicas, testes de microdureza *Knoop* e testes de resistência à flexão com três pontos de carga (n=8) foram realizados. Foram realizadas análises de microtomografia computadorizada para avaliar a morfologia 3D e de espetroscopia de infravermelho FTIR-ATR para avaliar a composição química dos espécimes. A caracterização da superfície incluiu a porosidade aberta à superfície calculada através do método de imersão (n=5); a energia de superfície calculada a partir da determinação do ângulo de contacto pela técnica da placa de *Wilhelmy* (n=5); a morfologia da superfície avaliada por microscopia SEM; e os testes de resistência ao corte entre as resinas de rebasamento e uma resina de base da prótese (n=10). A quantidade de clorexidina libertada foi determinada por espectroscopia (n=3) e a citotoxicidade dos extratos com culturas de fibroblastos foi determinada pelo ensaio de redução do brometo de tetrazólio (MTT) (n=2). Por não se ter verificado a normalidade da distribuição da amostra, os resultados obtidos foram analisados estatisticamente através de testes não paramétricos *Kruskal-Wallis* e *Mann-Whitney*. Em todos os testes estatísticos foi considerado o nível de significância de 5%.

A incorporação de clorexidina em Probase Cold demonstrou uma inibição da formação do biofilme e uma diminuição da resistência à flexão (p=0,005). Nas outras duas resinas verificou-se a formação de uma camada de biofilme em ambos os grupos. Não se verificaram diferenças significativas (p>0,05) nos valores das propriedades mecânicas dos espécimes incorporados com clorexidina, exceto para Ufi Gel Hard em que se verificou o aumentou (p=0,003) dos valores de microdureza. A adição de clorexidina em todas as resinas diminuiu a porosidade total e não provocou alterações na estrutura química. A incorporação de clorexidina em Ufi Gel Hard e Probase Cold apresentou valores superiores (p=0,008) de energia de superfície quando comparada com o grupo controlo. As imagens SEM demonstraram alterações na morfologia da superfície de Probase Cold aquando da incorporação de clorexidina. A incorporação de clorexidina em Kooliner e Ufi Gel Hard aumentou (p<0,05) a resistência ao corte, mas em Probase Cold diminuiu (p<0,001). A libertação de clorexidina ocorreu de forma gradual e constante após 1 a 2 dias em todas as resinas, sendo estatisticamente (p<0,001) superior na resina Ufi Gel Hard quando comparada com as restantes. A incorporação de clorexidina em Probase Cold foi considerada a menos citotóxica quando em contacto com a cultura de fibroblastos.

A incorporação de clorexidina em Probase Cold apresentou uma atividade antimicrobiana efetiva, sem afetar negativamente as propriedades mecânicas, estruturais e de superfície. Com uma difusão lenta e gradual do fármaco, foi a resina incorporada com clorexidina que apresentou menor citotoxidade.

Considerando o fato que um sistema de libertação de fármacos aplicado na área da medicina dentária está sujeito a processos de biodegradação oral, revela-se importante o seguinte estudo *in vitro*.

Após a caracterização do sistema de libertação de clorexidina em resinas acrílicas de rebasamento no imediato, no capítulo cinco deste trabalho avaliou-se o efeito da incorporação de clorexidina na microdureza, resistência à flexão, energia de superfície e estabilidade cromática, após processos de envelhecimento térmico ou químico.

Foram utilizados os mesmos grupos experimentais, definidos previamente no capítulo quatro (Kooliner: 0 e 2,5% clorexidina; Ufi Gel Hard: 0 e 5% clorexidina; Probase Cold: 0 e 5% clorexidina). Após um processo de envelhecimento térmico (1000 ciclos, 5ºC-55ºC) ou químico durante 4 semanas (ciclos de 6 horas em pH=3 e 18 horas

em pH=7 em saliva artificial), os espécimes (n=8) foram submetidos a testes de microdureza *Knoop* e, de seguida, a testes de resistência à flexão com três pontos de carga numa máquina de testes universal. A energia de superfície (n=5) foi calculada a partir da determinação do ângulo de contacto pela técnica da placa de *Wilhelmy* e a medição da cor (n=5) com recurso a dois espetrofotómetros, antes e após ambos os envelhecimentos. Os valores de L, C e h foram convertidos para o sistema CIELab e a diferença de cor foi calculada (ΔE) e transformado em unidades *NBS (National Bureau of Standards)*. Por não se ter verificado a normalidade da distribuição da amostra, todos os dados foram analisados estatisticamente com testes não paramétricos *Mann-Whitney* (α=0,05).

A incorporação de clorexidina não influenciou de forma significativa (p>0,05) os valores de microdureza, resistência à flexão e energia de superfície, em Kooliner e Ufi Gel Hard, após ambos os envelhecimentos. Verificou-se, no entanto, exceção para a resina Kooliner incorporada com clorexidina que atingiu valores de resistência à flexão superiores (p=0,050) ao grupo controlo. Após envelhecimento térmico, a incorporação de clorexidina em Probase Cold diminuiu os valores de microdureza (p=0,010) e resistência à flexão (p=0,038) e aumentou os de energia de superfície (p=0,008) comparado com o grupo controlo. Após envelhecimento químico, neste material, a incorporação do fármaco só diminuiu os valores da resistência à flexão (p=0,021). A incorporação de clorexidina aumentou o ΔE (p<0,05) de forma significativa na resina Kooliner após envelhecimento térmico e químico. No entanto, nas outras duas resinas, a incorporação da clorexidina só mostrou valores ΔE superiores (Ufi Gel Hard – p=0,008; Probase Cold – p=0,008) ao grupo controlo após envelhecimento químico, não se verificando diferenças (p>0,05) após o envelhecimento térmico.

A incorporação de clorexidina em Kooliner e Ufi Gel Hard não afetou negativamente a microdureza, resistência à flexão e energia de superfície após o envelhecimento térmico ou químico, afetando apenas a estabilidade cromática deste biomaterial. Contudo, a incorporação de clorexidina em Probase Cold diminuiu a microdureza e a resistência à flexão e aumentou a energia de superfície após o envelhecimento térmico, sem afetar a cor. Após o envelhecimento químico só a resistência à flexão e a estabilidade da cor da resina é que foram negativamente afetadas.

Dentro das limitações destes estudos *in vitro* pode-se concluir que os sistemas de libertação de clorexidina baseados em resinas acrílicas de rebasamento de próteses dentárias removíveis poderão vir a ser uma potencial alternativa no tratamento da estomatite protética. No entanto, mais estudos *in vivo* são necessários para avaliar a potencialidade da sua aplicação clínica.

Palavras-chave: Clorexidina, Resinas acrílicas, Estomatite protética, Sistemas de libertação de fármacos, Prostodontia.

Abstract

Drug delivery systems have been widely used in dentistry to prevent and treat several diseases. The methods of fabrication of a drug delivery system vary between coating, immersion, or incorporation. Compounds with antimicrobial activity like miconazole, silver, zinc oxide, titanium dioxide, chlorhexidine, fluconazole, nystatin, peptide mimetic compounds, zirconia, ethanol, and natural compounds have been added into acrylic resins to treat denture stomatitis.

Denture stomatitis is a pathological condition among people who wear removable dentures, characterized by erythema and edema of the oral mucosal areas. Usually, it is located in the upper jaw. Despite its multifactorial etiology, the infection by *Candida* species is considered the main etiologic factor. Trauma (caused by unstable dentures) and other local and systemic factors are also associated with denture stomatitis' etiopathogenesis.

Since conventional therapy with topical and/or systemic antifungals seems to be unsatisfying, drug delivery systems have been investigated. This treatment simultaneously targets the removable denture, the microbial biofilm, and the oral mucosa. The use of drug delivery systems in the oral cavity inhibits microbial growth and adhesion and are based on a continued, localized controlled release of a drug over a long period, promoting high therapeutic efficiency. It does not require patient compliance and presents minimal adverse risks when compared with systemic therapy.

In *Candida albicans*-associated denture stomatitis, the treatment with the proposed novel drug delivery system consists of incorporation chlorhexidine into three different reline acrylic resins by mixing the drug with the acrylic powder.

The study reported in Chapter 4 aimed to evaluate the effect of loading chlorhexidine on the antimicrobial activity against *Candida albicans* and *Streptococcus oralis* and the reline acrylic resins' mechanical, structural, and surface properties. The amount of drug release was also determined, as well as the cytotoxicity of this novel system.

Kooliner, Ufi Gel Hard, and Probase Cold were loaded with chlorhexidine diacetate. The results of an agar diffusion assay for *Candida albicans* defined the groups: control (with no addition of drug) and experimental (2.5% chlorhexidine (w/w)

to Kooliner and 5% to Ufi Gel Hard and Probase Cold). In each group, the reline resin powder and chlorhexidine were mixed and, after incorporating the liquid into the mixture, specimens were produced using steel molds with shapes and dimensions depending on the test to be performed.

The minimum inhibitory concentration of chlorhexidine for selected strains was previously determined. Specimens (n=3) were placed on agar plates inoculated with strains for antimicrobial activity study with the agar diffusion assay. Other specimens were submitted to the biofilm inhibition assay with SEM. The Knoop microhardness and the three-point bending flexural strength tests (n=8) were performed for mechanical characterization. A micro-CT device was used for morphometric and total porosity analysis and FTIR-ATR for chemical analysis. Surface characterization included porosity by the water immersion method (n=5), free energy using the Wilhelmy plate method (n=5), surface morphology with SEM, and shear bond strength testing between reline resins and the denture base resin (n=10). Chlorhexidine release was determined by spectrophotometry (n=3), and cytotoxicity was measured by the fibroblast viability endpoint MTT test (n=2). Since normality was not verified, quantitative data were submitted to the Kruskal-Wallis and Mann-Whitney tests (α=0.05).

Chlorhexidine-loaded Probase Cold showed a pronounced biofilm inhibition and decreased flexural strength (p=0.005). The other two reline resins showed biofilm formation in both control and experimental groups and no significantly (p>0.05) differences in mechanical properties, except for Ufi Gel Hard with significantly (p=0.003) higher microhardness values in the experimental group. The addition of chlorhexidine in all resins decreased total porosity, not changing their chemical structure. Chlorhexidine-loaded Ufi Gel Hard and Probase Cold showed significantly (p=0.008) higher surface free energy than the controls. SEM images showed a change in the surface morphology of chlorhexidine-loaded Probase Cold. Incorporating chlorhexidine increased (p<0.05) the Kooliner's and Ufi Gel Hard's bond strength but decreased (p<0.001) the Probase Cold's bond strength to the denture base resin. Chlorhexidine release followed a slow and steady pattern after 1-2 days in all resins and was higher (p<0.001) in Ufi Gel Hard. Chlorhexidine-loaded Probase Cold was considered slightly cytotoxic for the fibroblast cell line.

Loading Probase Cold with 5% chlorhexidine demonstrated effective antimicrobial activity and did not negatively affect its mechanical, structural, and

surface properties. Also, with a steadier long-lasting chlorhexidine diffusion, Probase Cold was the least cytotoxicity loaded resin under evaluation.

In Chapter 5, an *in-vitro* study was developed to investigate how these drug delivery systems were affected by biodegradation phenomena in the oral cavity. The main purpose was to evaluate the effect of chlorhexidine incorporation on the microhardness, flexural strength, surface energy, and color stability of the three reline acrylic resins after a thermal or a 28-day chemical aging process.

The same experimental groups previously defined in Chapter 4 were set: Kooliner with 0% and 2.5% chlorhexidine); Ufi Gel Hard with 0% and 5% chlorhexidine; and Probase Cold with 0% and 5% chlorhexidine. After thermal aging (1000 cycles, 5ºC-55ºC) and a 4-week chemical aging process (cycles of 6 hours at pH=3 and 18 hours at pH=7 in artificial saliva), the Knoop microhardness and the three-point flexural strength tests (n=8) were performed. Surface energy was estimated by determining contact angles using the Wilhelmy plaque technique (n=5) and color measurement with two spectrophotometers before and after both aging methods. The values were converted to the CIELab system, and the overall color change (ΔE) (n=5) was calculated and converted to National Bureau of Standards units. Since normality was not verified, data were submitted to the Mann-Whitney nonparametric statistical tests (α=0.05).

Chlorhexidine incorporation did not significantly (p>0.05) affect the microhardness, flexural strength, and surface free energy of the Kooliner and Ufi Gel Hard resins after either thermal or chemical aging, except for chlorhexidine-loaded Kooliner, which showed higher (p=0.050) microhardness values than the control group. Loading Probase Cold with chlorhexidine led to lower microhardness (p=0.010) and flexural strength (p=0.038), and higher surface free energy (p=0.008) values than the control group after thermal aging. After chemical aging, drug loading only decreased flexural strength (p=0.021). Loading Kooliner with chlorhexidine led to an increase (p<0.05) of ΔE values after either aging method. However, in the other two resins, chlorhexidine loading only caused higher ΔE values (Ufi Gel Hard – p=0.008; Probase Cold – p=0.008) than the control group after chemical aging, with no differences (p>0.05) after thermal aging.

Loading Kooliner and Ufi Gel Hard with chlorhexidine does not negatively affect their microhardness, flexural strength, and surface free energy after aging, only

affecting their color stability. However, loading Probase Cold with chlorhexidine decreases its microhardness and flexural strength and increases its surface free energy while not affecting the color stability after thermal aging. After chemical aging, Probase Cold led to decreased flexural strength and affected color stability.

Within the limitations of these *in-vitro* studies, chlorhexidine delivery systems based on reline acrylic resins of removable dentures may be used in the prevention or treatment of denture stomatitis. However, further *in-vivo* studies are necessary to recommend clinical use.

Keywords: Chlorhexidine, Acrylic resins, Denture stomatitis, Drug delivery systems, Prosthodontics.

List of Publications and Communications

Within the scope of this thesis, the following **full papers** were published or submitted in international and national peer-review journals:

1. Costa J, Bettencourt A, Portugal J, Neves CB. **Should local drug delivery systems be used in dentistry?**

To be submitted

2. Costa J, Neves CB, Ribeiro I, Arias Moliz MT, Santos CF, Dias JR, Franco M, Alves NM, Gonçalves L, Portugal J, Bettencourt A. **Engineering a novel chlorhexidine delivery system based on reline acrylic resins to treat denture stomatitis.**

To be submitted

3. Costa J, Bettencourt A, Portugal J, Neves CB. **Color and mechanical properties' stability in a novel chlorhexidine delivery system based on reline acrylic resins.**

To be submitted

4. Camilleri J, Arias Moliz T, Bettencourt A, Costa J, Martins F, Rabadijeva D, Rodriguez D, Visai L, Combes C, Farrugia C, Koidis P, Neves CB. **Standardization of antimicrobial testing of dental devices.** Dent Mater. 2020;36(3):e59-73. [https://doi.org/10.1016/j.dental.2019.12.006]

5. Costa J, Bettencourt A, Madeira A, Nepomuceno L, Portugal J, Neves CB. **Surface properties after chemical aging of chlorhexidine delivery systems based on acrylic resin.** Rev Port Estomatol Cir Maxilofac. 2019;60(4):155-62. [http://doi.org/10.24873/j.rpemd.2019.12.688]

6. Neves CB, Costa J, Nepomuceno L, Madeira A, Portugal J, Bettencourt A. **Microhardness and flexural strength after chemical aging of chlorhexidine**

delivery systems based on acrylic resin. Rev Port Estomatol Cir Maxilofac. 2019;60(3):104-10. [http://doi.org/10.24873/j.rpemd.2019.10.458]

7. Rijo I, Pedro D, Costa J, Bettencourt A, Portugal J, Neves CB. **Chlorhexidine loading of acrylic reline resins – microhardness and flexural strength after thermal aging.** Rev Port Estomatol Cir Maxilofac. 2018;59(3):154-61. [http://doi.org/10.24873/j.rpemd.2018.11.237]

Award "Prémio de investigação SPEMD" da Sociedade Portuguesa de Estomatologia e Medicina Dentária"

From the data of this thesis, the following **abstracts** were published in scientific journals:

1. Costa J, Ribeiro I, Gonçalves L, Arias Moliz MT, Bettencourt A, Portugal J, Neves CB. **Chlorhexidine-delivery-system based on acrylic resins – microbiological and biocompatibility studies.** Journal of Dental Research. 2019;98(Special Issue B): abstract nº 0518.

2. Nepomuceno LFG, Madeira ALA, Costa JV, Bettencourt A, Portugal J, Neves CB. **Sistema de libertação de clorexidina – propriedades mecânicas após envelhecimento químico.** Rev Port Estomatol Cir Maxilofac. 2019;60(S1):56, abstract nº 136. [http://doi.org/10.24873/j.rpemd.2019.12.597]

3. Costa JV, Bettencourt A, Portugal J, Neves CB. **Efeito da incorporação de clorexidina na estabilidade cromática de resinas acrílicas.** Rev Port Estomatol Cir Maxilofac. 2019;60(S1):56, abstract nº 137. [http://doi.org/10.24873/j.rpemd.2019.12.598]

4. Madeira ALA, Nepomuceno LFG, Costa JV, Bettencourt A, Portugal J, Neves CB. **Sistema de libertação de clorexidina – propriedades superfície após envelhecimento químico.** Rev Port Estomatol Cir Maxilofac. 2019;60(S1):56-7, abstract nº 138. [http://doi.org/10.24873/j.rpemd.2019.12.599]

5. Costa J, Rijo I, Pedro D, Bettencourt A, Portugal J, Neves CB. **Insights on chlorhexidine loaded acrylic resins after aging.** Journal of Dental Research. 2018;97(Special Issue B): abstract nº 3333.

6. Pedro DR, Rijo IC, Costa JV, Bettencourt A, Portugal J, Neves CB. **Incorporação de clorexidina em resinas acrílicas envelhecidas – efeito na microdureza.** Rev Port Estomatol Cir Maxilofac. 2018;59(S1):43, abstract nº 108. [http://doi.org/10.24873/j.rpemd.2018.11.436]

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9. Costa JV, Ribeiro I, Gonçalves L, Bettencourt A, Portugal J, Neves CB. **Veiculação de clorexidina em resinas acrílicas – atividade antibiofilme e citotoxicidade.** Rev Port Estomatol Cir Maxilofac. 2018;59(S1):48-9, abstract nº 121. [https://revista.spemd.pt/article/1200]

10. Costa J, Alexandre F, Marcelino N, Ribeiro I, Bettencourt A, Portugal J, Neves CB. **Drug incorporation of acrylic resins - microbiological and release studies.** Journal of Dental Research. 2017;96(Special Issue B): abstract nº 0048.

11. Costa JV, Alexandre F, Bettencourt A, Ribeiro I, Portugal J, Neves CB. **Incorporação de clorexidina em resinas acrílicas – estudos microbiológicos.** Rev Port Estomatol Cir Maxilofac. 2017;58(S1):51, abstract nº 133. [http://doi.org/10.24873/j.rpemd.2017.12.155]

12. Alexandre F, Costa J, Gonçalves L, Bettencourt AF, Neves CB. **Envelhecimento químico e libertação de clorexidina em resinas acrílicas de rebasamento.** Rev Port Estomatol Cir Maxilofac. 2017;58(S1):51-2, asbtract nº 134. [http://revista.spemd.pt/article/748]

13. Sousa C, Costa J, Matos A, Bettencourt A, Portugal J, Neves CB. **Efeito da incorporação de clorexidina nas propriedades de resinas acrílicas de rebasamento**. Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial 2014;55(S1):e23-e4, abstract nº 51. [http://dx.doi.org/10.1016/j.rpemd.2014.11.161]

Or in abstract books of congresses:

14. Costa J, Franco M, Santos C, Bettencourt A, Portugal J, Alves N, Neves CB. **Caracterização estrutural de resinas acrílicas de rebasamento após incorporação de clorexidina.** Abstract Book of the 9th Portuguese Congress of Biomechanics 2021, page 240, abstract nº 18106.

15. Neves CB, Costa J, Bettencourt A. **Suitability of aging procedures on mechanical and release studies of drug-incorporated oral biomaterials.** Abstract Book of the 7th Meeting of the Improved Protection of Medical Devices Against Infection (IPROMEDAI) – COST Action TD: 1305 2018, page 21, abstract nº 41 (1.9.2.).

16. Costa J, Barreiros M, Bettencourt A, Portugal J, Neves CB. **Effect of chlorhexidine loading on the surface free energy of acrylic reline resins.** Abstract Book of the 3rd Meeting of the College of Chemistry 2018, page 51, abstract nº Mat.P7.

17. Costa J, Barreiros M, Bettencourt A, Portugal J, Neves CB. **Loading of acrylic reline resins with chlorhexidine – evaluation of a physical property.** 10th iMed.ULisboa Postgraduate Students Meeting & 3rd i3DU Meeting 2018, page 109.

From the data of this thesis, the following **oral communications** were presented in national or international scientific meetings:

1. Costa J, Franco M, Santos C, Bettencourt A, Portugal J, Alves N, Neves CB. **Caracterização estrutural de resinas acrílicas de rebasamento após incorporação de clorexidina.** 9th Portuguese Congress of Biomechanics, Porto, Portugal, 2021.

2. Costa JV, Bettencourt A, Portugal J, Neves CB. **Efeito da incorporação de clorexidina na estabilidade cromática de resinas acrílicas.** XXXIX *Sociedade Portuguesa de Estomatologia e Medicina Dentária* (SPEMD) Congress, Porto, Portugal, 2019.

3. Costa J. **Insights on drug loading into acrylic resins – microbiological studies.** IPROMEDAI Training School, Amsterdam, Holland, 2018.

4. Costa JV, Ribeiro I, Gonçalves L, Bettencourt A, Portugal J, Neves CB. **Veiculação de clorexidina em resinas acrílicas – atividade antibiofilme e citotoxicidade.** XXXVIII SPEMD Congress, Lisboa, Portugal, 2018.

1st place "Prémio Congresso 2018 na categoria de investigação"

5. Costa J, Alexandre F, Marcelino N, Ribeiro I, Bettencourt A, Portugal J, Neves CB. **Drug incorporation of acrylic resins – microbiological and release studies.** Oral Health Research Congress hosted by the CED-IADR/NOF, Vienna, Austria, 2017.

Travel Award

6. Costa JV, Alexandre F, Bettencourt A, Ribeiro I, Portugal J, Neves CB. **Incorporação de clorexidina em resinas acrílicas – estudos microbiológicos** XXXVII SPEMD Congress, Coimbra, Portugal, 2017.

Data from the studies were also presented in scientific meetings in the following **poster communications**:

1. Costa J, Ribeiro I, Gonçalves L, Arias Moliz MT, Bettencourt A, Portugal J, Neves CB. **Chlorhexidine-delivery-system based on acrylic resins – microbiological and biocompatibility studies.** Oral Health Research Congress hosted by the Continental European & Scandinavian divisions of the International Association for Dental Research (CED-IADR/NOF), Madrid, Spain, 2019.

2. Nepomuceno LFG, Madeira ALA, Costa JV, Bettencourt A, Portugal J, Neves CB. **Sistema de libertação de clorexidina – propriedades mecânicas após envelhecimento químico.** XXXIX SPEMD Congress, Porto, Portugal, 2019.

3. Costa JV, Bettencourt A, Portugal J, Neves CB. **Efeito da incorporação de clorexidina na estabilidade cromática de resinas acrílicas.** XXXIX SPEMD Congress, Porto, Portugal, 2019.

4. Madeira ALA, Nepomuceno LFG, Costa JV, Bettencourt A, Portugal J, Neves CB. **Sistema de libertação de clorexidina – propriedades superfície após envelhecimento químico.** XXXIX SPEMD Congress, Porto, Portugal, 2019.

5. Neves CB, Costa J, Bettencourt A. **Suitability of aging procedures on mechanical and release studies of drug-incorporated oral biomaterials.** 7th Meeting of the Improved Protection of Medical Devices Against Infection (IPROMEDAI) – COST Action TD: 1305, Belgrade, Serbia, 2018.

6. Costa J, Barreiros M, Bettencourt A, Portugal J, Neves CB. **Effect of chlorhexidine loading on the surface free energy of acrylic reline resins.** 3rd Meeting of the College of Chemistry, Lisbon, Portugal, 2018.

7. Costa J, Rijo I, Pedro D, Bettencourt A, Portugal J, Neves CB. **Insights on chlorhexidine loaded acrylic resins after aging.** 96th General Session of IADR and IADR Pan European Regional (PER) Congress, London, United Kingdom, 2018.

8. Pedro DR, Rijo IC, Costa JV, Bettencourt A, Portugal J, Neves CB. **Incorporação de clorexidina em resinas acrílicas envelhecidas – efeito na microdureza.** XXXVIII SPEMD Congress, Lisbon, Portugal, 2018.

9. Rijo I, Pedro D, Costa JV, Bettencourt A, Portugal J, Neves CB. **Efeito da clorexidina na resistência à flexão de resinas acrílicas envelhecidas.** XXXVIII SPEMD Congress, Lisbon, Portugal, 2018.

10. Costa N, Costa J, Portugal J, Bettencourt A, Neves CB. **Resinas acrílicas com clorexidina envelhecidas – estudos de adesão e energia de superfície.** XXXVIII SPEMD Congress, Lisbon, Portugal, 2018.

11. Costa JV, Ribeiro I, Gonçalves L, Bettencourt A, Portugal J, Neves CB. **Veiculação de clorexidina em resinas acrílicas – atividade antibiofilme e citotoxicidade.** XXXVIII SPEMD Congress, Lisbon, Portugal, 2018.

12. Costa J, Barreiros M, Bettencourt A, Portugal J, Neves CB. **Loading of acrylic reline resins with chlorhexidine – evaluation of a physical property.** 10th iMed.ULisboa Postgraduate Students Meeting & 3rd i3DU Meeting, Lisbon, Portugal, 2018.

13. Costa J, Alexandre F, Marcelino N, Ribeiro I, Bettencourt A, Portugal J, Neves CB. **Drug incorporation of acrylic resins – microbiological and release studies.** Oral Health Research Congress hosted by the CED-IADR/NOF, Vienna, Austria, 2017.

14. Costa JV, Alexandre F, Bettencourt A, Ribeiro I, Portugal J, Neves CB. **Incorporação de clorexidina em resinas acrílicas – estudos microbiológicos.** XXXVII SPEMD Congress, Coimbra, Portugal, 2017.

15. Alexandre F, Costa J, Gonçalves L, Bettencourt AF, Neves CB. **Envelhecimento químico e libertação de clorexidina em resinas acrílicas de rebasamento.** XXXVII SPEMD Congress, Coimbra, Portugal, 2017.

16. Sousa C, Costa J, Matos A, Bettencourt A, Portugal J, Neves CB. **Efeito da incorporação de clorexidina nas propriedades de resinas acrílicas de rebasamento.** XXXIV SPEMD Congress, Coimbra, Portugal, 2014.

Objectives and Structure of the Thesis

The present work was elaborated to evaluate the effectiveness of an innovative drug delivery system in the context of *Candida albicans*-associated denture stomatitis control and treatment. The main global objective was to evaluate *in vitro* the effect of chlorhexidine loading on reline acrylic resins' properties at baseline and after being submitted to a biodegradation process. This thesis is organized in six chapters.

Chapter 1 introduces the theme of the thesis. The chapter starts with a review on drug delivery systems used in dentistry, based on a review article to be submitted to a journal in the scope of this thesis. Also, brief concepts about denture stomatitis are addressed, including discussions on its etiology, etiopathogenesis, prevention, and possible treatments reported in the literature until this date.

Chapter 2 consists of a systematic review to answer the PICO question: "Can the addition of an antimicrobial agent to hard acrylic resins be a treatment option for denture stomatitis, without changing their properties?". A search was done on the Pubmed database and a total of 57 articles were selected for this review.

Chapters 1 and 2 helped select the drug and the biomaterial for a novel drug delivery system to be used in removable prosthodontics. **Chapter 3** presents a brief description of chlorhexidine, including its advantages, applications, and side effects, and an overview of acrylic resins. In the end, the advantages of this innovative drug delivery system are summarized.

Chapter 4 evaluates the effect of chlorhexidine loading on the mechanical, structural, surface, and biological properties of reline acrylic resins. This *in-vitro* experimental study was written in a scientific paper format organized into five parts: Introduction, Materials and Methods, Results, Discussion, and Conclusions. This chapter is based on the following objectives:

1) Evaluate the microbiological profile of reline acrylic resins loaded with chlorhexidine against *Candida* and *Streptococcus* species;

2) Characterize the mechanical, structural, and surface properties of the novel drug delivery system;

3) Evaluate the release profile of chlorhexidine incorporated in reline acrylic resins;

4) Evaluate the biological potential of the loaded biomaterial in fibroblasts.

Chapter 5 presents the *in-vitro* behavior of this novel drug delivery system over time when submitted to biodegradation processes that mimic the oral cavity, based on thermal and chemical aging processes. This chapter was also written in a scientific paper format.

Chapter 6 presents the concluding remarks of the experimental studies and prospects in this field.

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List of Abbreviations

ΔE	Overall color change
1,6-HDMA	1,6-hexanedioldimethacrylate
ATCC	American Type Culture Collection
b	Specimen's width
C	Chroma
C. albicans	*Candida albicans*
CA	Chemical aging
CFU	Colony-forming unit
CH	Calcium hydroxide
CHX	Chlorhexidine
d	Specimen's thickness
DABCO	1,4-Diazabicyclo[2.2.2]octane
DDS	Drug delivery system
DMAE-CB	Methacryloxylethylcetyldimethylammonium chloride
DMSO	Dimethylsulfoxide
DS	Denture stomatitis
EDTA	Ethylenediaminetetraacetic acid
ES	VITA Easyshade
FS	Flexural strength
FTIR-ATR	Fourier transform infrared – attenuated total reflectance
GIC	Glass-ionomer cement
GPYA	Glucose-peptone-yeast-agar

h	Hue
HA	Hydroxyapatite
HCL	Hydrochloric acid
HEMA	2-Hydroxyethylmethacrylate
IBMA	Isobutylmethacrylate
IQR	Interquartile range
ISO	International Organization for Standardization
K	Kooliner
KHN	Knoop hardness number
L	Liquid
L	Lightness
m	Mass
M	Mean
Max	Maximum
MDPB	Methacryloyloxydodecylpyridinium bromide
MED	Median
MIC	Minimum inhibitory concentration
Micro-CT	3D Cone-beam Microtomography
Min	Minimum
MMA	Methylmethacrylate
MSs	Microspheres
MTA	Mineral trioxide aggregate
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
NBS	National Bureau of Standards

P	Powder
PC	Probase Cold
PEMA	Polyethylmethacrylate
PICO	Population, Intervention, Comparison, Outcome
PLGA	Poly(D-L lactide-co-glycolide
PMMA	Polymethylmethacrylate
PTBAEMA	Poly (2-tert-butylaminoethyl) methacrylate
RPMI	Roswell Park Memorial Institute
S. oralis	*Streptococcus oralis*
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SS	Spectroshade Micro
TA	Thermal aging
TAT	1,3,5-triacryloylhexahydro-1,3,5-triazine
TBAEMA	2-Tert-butylaminoethyl methacrylate
UGH	Ufi Gel Hard
UV	Ultraviolet
V	Volume
W	Maximum load before fracture
$\mathbf{W_{dry}}$	Weight of reline acrylic resin specimen saturated with water
$\mathbf{W_{sat}}$	Dry weight of reline acrylic resin specimen
$\mathbf{W_{sus}}$	Weight of reline acrylic resin specimen suspended in water
γ	Surface free energy

γ^d	Dispersive component of surface free energy
γ^p	Polar component of surface free energy

List of Units

ºC	Degrees Celsius
%	Percent
cm	Centimeter
g	Grams
h	Hours
Kg	Kilogram
kV	Kilovolt
mA	Milliampere
min	Minutes
mL	Milliliter
mm	Millimeter
mM	Millimolar
MPa	Megapascal
ms	Millisecond
N	Newton
nm	Nanometer
pH	Potential of Hydrogen
rpm	Rotations per minute
ua	Arbitrary unit
µg	Microgram
µm	Micrometer

Chapter 1

General Introduction

1. General Introduction

This first chapter includes a narrative review about drug delivery systems (DDSs) used in dentistry and a theoretical framework with denture stomatitis' key concepts. It details a discussion of the etiology, etiopathogenesis, prevention, and possible treatments presented in the literature until this date.

1.1. Drug Delivery Systems

Biomaterial or biomedical material is defined as any substance or combination of substances of natural or synthetic origin which for an indefinite period may be used as a medical device to treat, increase, or replace any tissue, organ, or function of the body.[1,2] Materials of lipid[3,4], inorganic[5], and polymeric nature[6,7] have been used. DDSs can be achieved by combining drugs with biomaterials to provide a more effective way to deliver the compounds into the target tissues.[6,8,9]

Before 1950, all drugs for the oral cavity were provided as formulations that released the loaded drug immediately upon contact with water, without any ability to control the kinetics of the drug release.[10] Through this system, only a small fraction of the dose reaches the target tissue since most of the dose is distributed into other tissues and metabolized or excreted not reaching the site of action (Figure 1.1).

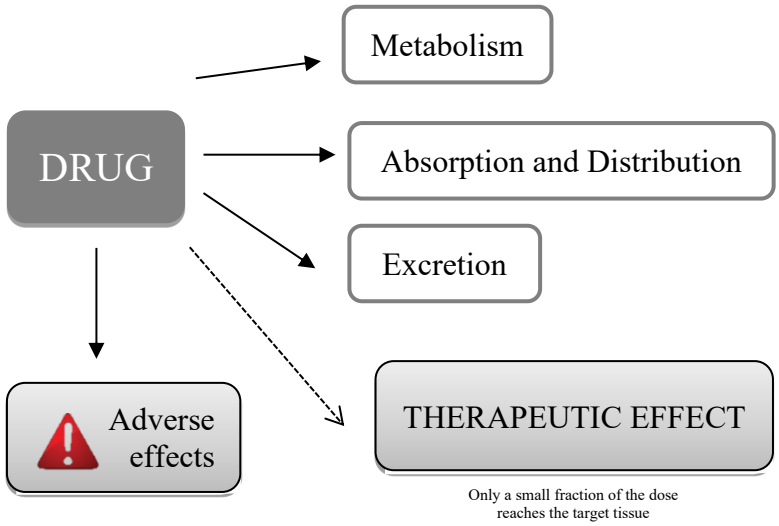

Figure 1.1 – Illustration of the disadvantages of the conventional drug formulations for targeting the oral cavity.

With a DDS, the effectiveness of the drug at the target site is increased.[11] In 1952, Smith Klein Beecham introduced the first sustained-release formulation: stimulant Dexedrine (dextroamphetamine sulfate) for narcolepsy. It was manufactured as a DDS that gradually released the drug over some time and achieved 12-hour efficacy.[12]

In the oral cavity, sites of bacterial infections, such as periodontal pockets and proximal or marginal areas of composite restorations, can be largely inaccessible to antimicrobial agents. This inaccessibility can lead to the establishment of bacterial or fungal biofilms, causing secondary caries, periodontal system inflammation, and biomaterials' degradation.[13] If sufficient drug is not available at the infection site, the drugs cannot penetrate the biofilm and the microorganisms may become resistant.[14] Therefore, efficient delivery and penetration of the antimicrobial agent to the exact infection site are highly desirable, especially in dentistry.

The maintenance of the drug at the site of action is another of the numerous advantages of DDS, overcoming the difficulty to maintain an effective drug concentration on the infected surfaces and tissues due to the salivary flow, tongue, and swallowing movements, which rapidly dissolve and eliminate the drug.[15] Furthermore, DDS presents a controlled-release technology to prolong drug actions, decrease drug metabolism, and minimize its overall distribution in the body.[16] Finally, it minimizes patient compliance during treatment by reducing the drug application frequency and presents minimal adverse risks, such as systemic toxicity.[17,18]

An antimicrobial agent can be included in a DDS if the drug reaches the intended site of action, maintains an adequate concentration for a sufficient duration, and presents substantivity.[19] However, there are some possible disadvantages, such as the risk of developing microbial tolerance or drug resistance due to a prolonged release.[20] Another problem related to DDS can be the detrimental of biomaterial's general properties when the drug is incorporated (e.g., what happens with the addition of antifungal drugs into soft liners).[21-24] Since this problem may restrict the use of the material as a DDS, *in-vitro* studies have to be conducted (Figure 1.2).[25]

In dentistry, a range of microbiological infections is still the major reason for recurrent and persistent infections despite the use of antibiotics. In the past, drugs and medical devices were developed separately until it was realized that their combined use potentiated synergistic therapeutic results. Nowadays, there has been an increasing interest and development of biomaterials loaded with drugs to treat or prevent

diseases.[1,26] In dentistry, there are many clinical situations where a DDS with a continued and local release would be desirable.

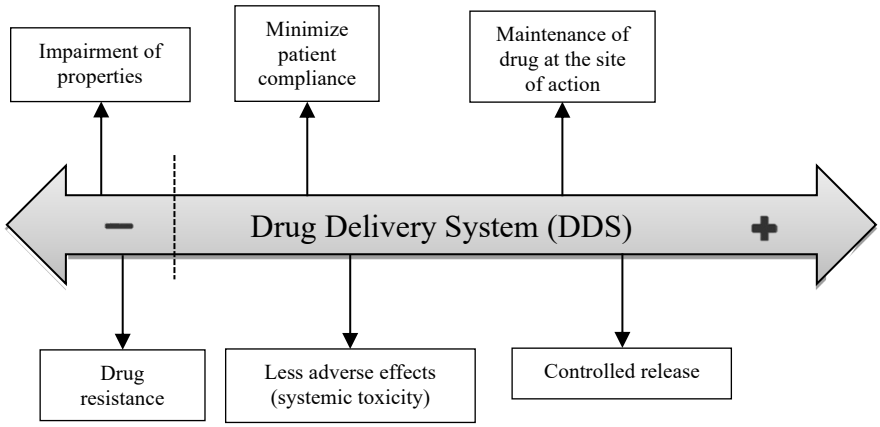

Figure 1.2 – Summary of the disadvantages and advantages of drug delivery systems.

1.1.1. Methods of Drug Inclusion in DDSs

The DDS production methods currently used in dentistry vary between immersion, coating and incorporation. The direct soaking (immersion) of medical devices into drug solutions usually does not result in efficient loading/controlled release. Thus, specific strategies are required to incorporate drugs and other bioactive substances. The approaches for preparing drug-eluting medical devices can be categorized into two broad groups: i) those that incorporate the drug in the outer layers of the device by coating procedures, covalent binding, or weak chemical interactions; and ii) those that incorporate the drug in the bulk of the material that constitutes the medical device during its fabrication (compounding) or in a later step (presoaking) (Figure 1.3).[26]

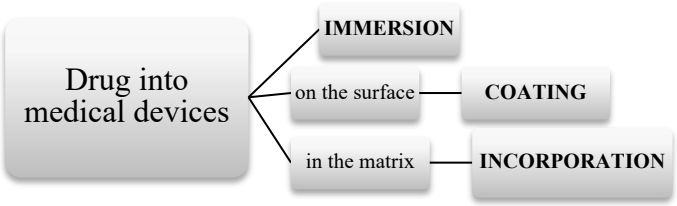

Figure 1.3 – Illustration of methods of drug inclusion into medical devices: immersion, coating, or incorporation.

The drug tends to be released into the surrounding environment in large quantities for a few days, followed by a decrease of its concentration in the material. Thus, the method of dispersing the drug in the resin matrix (coating or incorporation) does not allow controlling the kinetics of drug release.[27,28] The drug release profile is determined by several factors, including the characteristics of the release medium, the physicochemical properties of the drug and the polymer, and the interactions established between them.[29] The method of drug incorporation, the degree of crosslinking, the biomaterial's morphology (namely the presence of pores and their size and distribution), and the water sorption capacity are other important factors.[28] Therefore, the most suitable controlled-release systems should be chosen based on the specific application.[29]

1.1.2. Areas of Dentistry with DDSs

The present review provides an insight into DDSs regarding their potential application in various areas of dentistry, such as anesthesiology, oral diseases, cariology, restorative dentistry, periodontics, endodontic, implantology, fixed and removable prosthodontics, and orthodontics. The main interest for using a DDS will be to manage microbiological infections, which explain why most DDSs focus on antimicrobial drug delivery.

1.1.2.1. Anesthesiology

Lidocaine hydrochloride administered either topically or parenterally has been a widely used local anesthetic in dentistry since 1948. The parenteral route of administration is currently the route of choice for the induction of local dental anesthesia worldwide with the advantages of better penetration and rapid onset of action of the drug. This mode of delivery can be painful, especially for pediatric patients, and can be associated with the risk of transmitting infectious diseases. For these reasons, the search for alternatives has intensified, and the strategy for local DDS has been tested. A study performed by Taware *et al.* (1997)[16] reported the use of a bioadhesive as a DDS (film laminate composed of a nonmedicated backing membrane and a medicated layer) in alternative to infiltration anesthesia in dentistry. More recently, a study found that a lidocaine-containing bioadhesive system delivering topical anesthesia was highly effective in alleviating pain/discomfort arising from needle sticks, with and without

concomitant injection. However, that randomized controlled trial has the limitation of being associated only with periodontal treatment.[30,31]

A recent review[32] has been published focusing on DDSs used for topical anesthesia on the oral mucosa. With chemical methods, it is possible to create a DDS to increase topical anesthetic efficacy and achieve a better anesthetic effect. Liposomes, lipid nanoparticles, bioadhesive and mucoadhesive films, patches, and hydrogels have been successfully developed. Their possible clinical applications are scaling and root planning, rubber dam clamp placement, post-operative pain control, treatment and/or analgesia of painful conditions at the mucosa (oral ulcers and mucositis), soft-tissue biopsy, and needle-free dental treatment. Most of these promising formulations have shown interesting properties *in vitro*, with only a few clinical trials.[16,30,31,33-36]

1.1.2.2. Oral Diseases

DDSs' sustained-release capacity would be beneficial in the treatment of many oral diseases because it could reduce side effects and improve treatment outcomes. In the literature, different examples of DDSs are presented as potential methods for managing oral cancer and malignant oral disease, oral mucositis, immune-mediated diseases (oral lichen planus, pemphigus), infections (herpes simplex, candidiasis, and recurrent aphthous stomatitis), and neuropathic pain (Table 1.1).[37-47] However, further research is needed to improve treatment outcomes.

Table 1.1 – Drug delivery systems in oral diseases.

ORAL DISEASE	*MATRIX*	*DRUG*
Oral cancer and potentially malignant oral disease Squamous cell carcinomas, leukoplakia, and erythroplakias	Mucoadhesive disc[38]	Acitretin
	Gel[39]	Isotretinoin
Oral mucositis	Chitosan gel[40]	TGF-β3
Immunology mediated diseases oral lichen planus, pemphigus, mucous membrane pemphigoid, recurrent aphthous stomatitis	Spray, cream, gel or adhesive paste[41]	Fluticasone
	Cream, aqueous solution, or adhesive paste[41,42]	Clobetasol propionate
Infections herpes simplex, candidiasis, and recurrent aphthous stomatitis	Mucoadhesive[43]	Miconazole
	Mucoadhesive[44]	Acyclovir
	Cream[45]	Acyclovir Penciclovir Docosanol Foscarnet
	Chewing gum[46]	Probiotics
Neuropathic pain	Cream[47]	Capsaicin
	Patch[47]	Lidocaine

1.1.2.3. Cariology

Since 1999, chewing gums are increasingly viewed as DDSs for active agents, including pharmaceutical drugs.[48] The labeling of sugar-substituted chewing gum as "tooth-safe" or "tooth-friendly" is due to fluoride's inclusion to provide oral health benefits.[48,49] Although it has been hard to achieve in real-world scenarios, the reason for the superior efficacy of remineralizing agents from the chewing gum may be explained by saliva stimulation alone, coupled with the kinetics of release of the gum's active ingredients that causes them to be highly diluted in a relatively large volume of stimulated saliva that is rapidly swallowed.[49]

Another form of reduction of dental caries in children is the incorporation of probiotics in chewing gums. Probiotic is the term currently used to name ingested

microorganisms associated with beneficial effects to humans and other animals. The key event is the occupation by harmless microorganisms, such as *Lactobacilli* or *Bifidobacteria* strains, of a space in a biofilm that would otherwise be colonized by a pathogen.[50] These non-pathogenic microorganisms represent a valid alternative treatment in several oral affections, such as caries, periodontitis, halitosis, and fungal infection. A clinical study concluded that two gum-based systems containing *Lactobacillus spp.* and the *Lactobacillus reuteri* strain significantly reduced the counting number of *Streptococcus mutans* in saliva.[51] Another study was conducted with a similar conclusion but with a DDS of gums containing the probiotic *Latobacillus reuteri* strains and xylitol.[52]

1.1.2.4. Restorative Dentistry

In the dentistry area, restorative dental materials such as composites and glass-ionomers can be used as DDSs. In recent years, composite resin has been the most used restorative material in direct restorations of anterior and posterior teeth, as an alternative to amalgam.[53] However, unlike amalgam, which has been shown to possess antibacterial properties due to the release of metallic ions (silver, copper, and tin)[25], composite resin allows rapid bacterial colonization.[54-56] Furthermore, one of the major disadvantages of using this restorative material is its shrinkage after polymerization. The micro-gaps between the tooth and the restoration result in the micro-infiltration of bacteria into the dental cavity, potentially leading to the appearance of secondary caries.[57] Thus, it is easy to understand the importance and advantages of adding drugs, mainly antibacterial ones, to dental resins. The reduction and inactivation of cariogenic bacteria is a direct strategy to eliminate the cause of dental caries, increasing the longevity of the restorations.[58]

Chlorhexidine (CHX)[59-62], cetylpyridinium chloride[63], benzalkonium chloride[64-66], and triclosan[67-70] have been added as soluble antibacterial drugs to the dental composite resin matrix. CHX, commonly used as an antiseptic mouthwash in dentistry, is a bis-biguanide with bacterial and bacteriostatic action against a wide range of gram-positive and gram-negative bacteria.[71] The addition of small concentrations of CHX into dental resins increased the antibacterial activity[59,60], without compromising the mechanical properties (compressive, tensile, and restorative material-enamel adhesive shear strength tests).[59] The CHX release was highly correlated with the degree of water uptake/sorption, which, in turn, depends on the hydrophilic

characteristics of the resin's compositions. However, the solubility of the resin was probably affected by both the amount of CHX and unreached residual monomers present in the polymer matrix.[60] Last year, the combination of CHX crystals with magnetic nanoparticles was investigated to improve this system's efficacy. The purpose was to control the release of CHX from a resin using a magnetic field.[62]

Triclosan is an antibacterial and antifungal agent found in some consumer products. Recently, the effects of a novel triclosan methacrylate monomer incorporated into an experimental resin composite were tested *in vitro* against *S. mutans* biofilms. The results showed that the triclosan methacrylate monomer's presence causes harmful effects at molecular and cellular levels in *S. mutans*, implying a reduction in those microorganisms' virulence.[70] Other molecules with antibacterial properties also began to be polymerized directly to the resin matrix through covalent bonds. Half of these molecules are responsible for the antibacterial effect, and the other half binds to the resin. Once immobilized, this molecule is not released from the resin, and its antibacterial effect occurs by direct contact with the bacteria. Thus, the antibacterial effects are maintained longer, and the resin's mechanical properties do not deteriorate.[72] Since then, several monomers with antibacterial properties have been proposed, such as 12-methacryloyloxydodecylpyridinium bromide (MDPB).[73]

Since silver, gold, and zinc act as bactericidal and bacteriostatic agents, the addition of metal (oxide) particles/ions into restorative materials has been proposed.[74,75] The addition of zinc oxide nanoparticles into flowable resin composite significantly inhibited the growth of *S. mutans*. However, incorporating 1 wt. % was not enough to negatively affect the composite's mechanical properties (flexural and compressive strength and modulus, depth of cure, degree of conversion, and micro-shear bond strength).[76]

Similarly, it was possible to demonstrate that incorporating antibacterial monomers into adhesives systems could be beneficial to reduce biofilm accumulation.[73] CHX, benzalkonium chloride, MDPB, and methacryloxylethyl cetyl dimethyl ammonium chloride (DMEA-CB) have already been tested.[77-81] However, these materials' clinical effectiveness needs to be further studied since no clinical study has yet been conducted to confirm their antibacterial effect.[73]

Atraumatic restorative treatment is a minimally invasive technique in which demineralized tooth tissues are removed using manual instruments, and the cavity is restored using a filling material, usually a glass-ionomer cement (GIC).[82] This material

can release fluoride, which means that it has antibacterial activity.[58] GIC's antibacterial activity increases after introducing antibiotics, like ciprofloxacin and metronidazole, due to it increasing the fluoride release, as already proven with success rates in clinical trials.[83-86] However, it should be taken into account that higher concentrations of antibiotics exhibited lower material compressive strength and reduced bond to dentin.[84]

1.1.2.5. Periodontics

Periodontal diseases are bacterial infections characterized by inflammation and destruction of the attachment apparatus, often leading to tooth loss if not properly treated.[87,88] Mechanical debridement of the root surface by scaling and root planning associated with oral hygiene orientations have been one form of treatment.[19] However, the combination of that protocol with antibiotics and antiseptics is also reported in the literature.[89]

Because of low periodontal clearance, local delivery of antimicrobials has been investigated as a possible method for controlling and treating periodontal disease. The drugs (metronidazole, CHX, minocycline, doxycycline, and tetracycline) are inserted in periodontal pockets to inhibit or eliminate the pathogenic microorganisms and modulate the tissues' inflammatory response.[19,88,90]

Fibers, films, gels, strips, vesicles, microparticles, and nanoparticles loaded with the drugs are some of the DDSs available.[19,90] In periodontics, the DDS can be used in combination or not with mechanical debridement.[9] The use of controlled delivery systems as an adjunct for recurrent and refractory periodontitis is of particular interest.[88]

1.1.2.6. Endodontics

Various local DDSs with irrigants and medicaments for root canal system disinfection and resin materials used as sealers for root obturations are described below.

Recently, intracanal DDSs with antibiotics and non-steroidal anti-inflammatory drugs have been developed to eliminate bacterial infection and reduce or eliminate post-operative pain. Endodontic DDSs can be classified into microparticulated and nanoparticulated systems.[19] The development of polymeric biodegradable microspheres (MSs) of poly(D-L lactide-co-glycolide) (PLGA) and zein (a class of prolamine protein) allowed local delivery and preservation of amoxicillin's antimicrobial activity. These amoxicillin-loaded MSs are efficient against *Enterococcus*

faecalis and can be used for root canal disinfection.[91] The use of photoactive drugs encapsulated in PLGA nanoparticles may be a promising adjunct in antimicrobial endodontic treatment. The synergism of light and photosensitizer methylene blue-loaded nanoparticles led to reduced colony-forming units (CFUs) of *E. faecalis*. The encapsulation of methylene blue within PLGA nanoparticles may offer a novel nanoplatform design for an enhanced DDS for the root canal system and photodestruction of root canal biofilms.[92] Chitosan nanoparticles were also found to display a high antibacterial activity against *E. faecalis* and have good compatibility with the endodontic sealer.[93]

There are several irrigants and medicaments in endodontics: sodium hypochlorite, CHX, tetraclean, ethylenediaminetetraacetic acid (EDTA), calcium hydroxide, and mineral trioxide aggregate (MTA).[94] The antimicrobial activity of CHX alone is reduced, so its combination with cements, such as calcium hydroxide, has been developed as sustained-release systems, whose effectivity as intracanal medication against *E. faecalis* has been proven.[95-102] A cylindrical, needle-shaped device consisting of a core and a polymer coating loaded with 30-45% CHX showed positive results in *in-vitro* antimicrobial tests; however, these were carried out on a bovine tooth model.[103]

Another type of DDS developed in endodontics is absorbent papers with CHX. A study proposed coatings with chitosan, polylactic glycolic acid (PLGA), or polymethyl methacrylate (PMMA) to control this system's release of CHX digluconate. CHX's release rate was the greatest in the noncoated group, followed by the chitosan-coated group, the PLGA-coated group, and the PMMA-coated group. It was concluded that polymer coating could control CHX's release rate.[104]

Many authors considered the epoxy resin-based root canal sealer as the gold standard due to its good properties.[105-108] Numerous studies demonstrated that the incorporation of CHX, cetrimide, and benzalkonium chloride adds some degree of antimicrobial property to this sealer without significantly affecting its physical properties.[109-111]

The treatment of pulpal necrosis in immature permanent teeth is challenging due to its uncertain prognosis.[112-114] Apexification is one of the treatment options and can be done with calcium hydroxide (CH) or MTA. Calcium hydroxide microspheres (CHMSs) have been developed and studied to eliminate the requirement for multiple visits for this procedure. The CHMS is prepared by an emulsion technique that

encapsulates the CH in a carrier with an outer coating of alginate gel. It is a promising delivery vehicle for the sustained slow release of calcium and hydroxide ions in the root canal.[115]

Recently, regenerative endodontics has become an alternative to classical root apexification. One of the most critical steps is the complete eradication of residual infection, and this is achieved by applying CH or double or triple antibiotic paste as a second option.[116,117] The double antibiotic paste contains ciprofloxacin and metronidazole, and the triple adds minocycline.[116,118] The use of either product has demonstrated several side effects on dental pulp cells, as well as periodontal ligament fibroblasts, and tooth discoloration.[119] Since these products showed negative clinical consequences, drug delivery-based strategies based on antibiotic-containing nanofibers that can reduce the local drug concentration have recently been proposed[117,120-122] in the form of antibiotic-containing scaffolds or clindamycin-modified triple antibiotic (metronidazole, ciprofloxacin and clindamycin) polymers. These scaffolds could be a biologically safe antimicrobial DDSs for regenerative endodontics, because they inhibit the growth of *Enterococcus faecalis, Porphyromonas gingivalis* and *Fusobacterium nucleatum.*[119,123]

Local DDSs applied to endodontic and periodontal therapy have shown positive clinical and microbiological effects but increased the cost of therapy. However, the benefits outweigh the costs. For example, a site-specific administration of simple injectable formulations of micro- or nanoparticles in the periodontal pocket or the root canal cavity would reduce the number of treatment sessions for patients and may serve as an adjuvant to surgical protocols due to offering a means of saving teeth, which is the ultimate goal of any dental treatment.[19]

1.1.2.7. Implantology

Tooth loss is a significant problem affecting the elderly population[124], and the current aging population increases the number of edentulous and partially dentate patients.[125] The treatment options for these patients range from conventional complete dentures to fixed implant-supported restorations. In this area, various drugs have been combined with implants or synthetic grafts.

The microbiological component plays an important role in encouraging and facilitating infection during implant placement and later when the implant is in function in the mouth, representing a septic medium.[126] The initial bacterial adhesion to and

colonization of an implant surface play key roles in the pathogenesis of biomaterial-related infections.[127] Two strategies to prevent this are: (1) coating implants with antibiotics like amoxicillin or biphosphonates[8,128]; and (2) covalently attaching antimicrobial molecules (e.g., Ag, ZnO, CuO, nanoparticles, quercitrin, and CHX) onto the implant surface.[129,130]

Before implantation, we often need to replace missing bone using bone grafts. Growth factors, platelet lysate, hormones and phytohormones, antibiotics, alendronate, simvastatin, and raloxifene are included on synthetic grafts, constituting a local delivery system.[131] DDSs to the bone could be used to promote regeneration, prevent infection, or treat post-surgical pain.[8] Hydroxyapatite (HA), well known as a bone substitute material for its excellent biocompatibility and reasonable mechanical behavior, has been used as a bone graft to fill a cavity created after curettage of benign lytic lesions of the bone.[132,133] HA with gentamicin, vancomycin, and ciprofloxacin showed significantly better clinical results than the conventional therapies.[132,134-136] CHX digluconate uptake from an aqueous solution by HA has also been investigated in a potential adjuvant antimicrobial system for clinical use.[137-139] CHX solutions were prepared by dilution, and adsorption experiments were carried out. The results from *in-vitro* bioactivity, cytotoxicity, and microbiological assessments indicate that the HA/CHX association might be a potential adjuvant system for prophylaxis and treatment of oral infections.[137]

1.1.2.8. Removable Prosthodontics

Agents that transmit diseases such as HIV/AIDS, herpes, tuberculosis, and hepatitis can survive outside of the human body for a long period, so it is important to prevent the occurrence of crossed infection between the dental staff and/or the prosthesis laboratory, transmitted by contaminated dental impressions.[140,141] To promote the disinfection of impression material, the addition of CHX to alginate leads to a considerable reduction of the amounts of microorganisms, as proven by microbiological tests.[142,143]

With the aim of reducing the development of adherent biofilms into denture materials[144], several examples of DDSs have been proposed to treat and/or prevent denture stomatitis. A novel thin-film coating with the addition of various drugs (CHX diacetate, nystatin, and amphotericin) effectively inhibited *C. albicans* biofilm formation and should be evaluated as a potential preventive therapy for denture

stomatitis.[145] A pre-coating acrylic with histatins 5 and CHX for reducing *C. albicans*'s biofilm development in a denture stomatitis model has also been proposed.[146]

Another alternative proposed was immersing the denture in drugs: nystatin, amphotericin B, ketoconazole, fluconazole, and CHX gluconate. A brief exposure to these antifungal drugs suppressed fungal oral *Candida* strains' adhesion to the surface of acrylic dentures.[147,148] In an attempt to reinforce the drug's daily action, antifungal drugs were incorporated into denture adhesives. A new denture adhesive containing 2% of miconazole nitrate loaded in microparticles produced effective antifungal activity, good adhesive force, and no toxicity effect, being a promising therapeutic approach for removable denture wearers affected by denture stomatitis.[149]

The incorporation of antifungal and antimicrobial agents (nystatin, miconazole, ketoconazole, itraconazole, fluconazole, and CHX diacetate) into denture acrylic resins[17,150-156] or denture soft lining materials[18,21,157-161] offers a therapy based on the sustained release of antifungal drugs. The first studies proposed loading of reline resins with nystatin, miconazole, and ketoconazole and proven their antifungal activity against *C. albicans*.[157,161] Later studies were developed with the incorporation of fluconazole and CHX.[17,21,150-153,162]

Compared to other antifungals, CHX's *Candida* inhibition lasts longer than amphotericin B's and nystatin's.[148] CHX has shown better results than fluconazole on both releasing and microbiological tests.[145,150,153,163-165] The CHX concentration most frequently used in earlier studies[17,150,151,153,164] that evaluate the efficacy of a release delivery system against *C. albicans* is 10% (w/w). This concentration has shown to be effective and feasible. Moreover, it was also important to study the behavior of drug loading into acrylic resins. The degree of conversion and the color stability were influenced by the addition of CHX or fluconazole. However, the final values were comparable to other commonly used acrylic liners and within acceptable ranges.[154] Although hardness increased over time, CHX diacetate's incorporation into PMMA and polyethylmethacrylate (PEMA) denture liners probably does not lead to a clinically relevant change.[21] However, recent preliminary investigations tried to decrease the 10% concentration of CHX and tested its effects with microbiology studies, drug release studies, and mechanical and superficial tests.[156]

Other studies indicated that antimicrobial activity could be incorporated into denture base resin with nanoparticles.[166-169] Nano-silver showed antifungal activity and

inhibitory effects on *C. albicans* adhesion and biofilm formation in the denture base resin, especially at a higher concentration.[166,168] However, the reduction in impact strength can increase the risk of fracture, so new methodologies are needed to ensure better dispersion of the nanoparticles in the polymer matrix.[168]

1.1.2.9. Fixed Prosthodontics

Agents for cementing fixed dental prostheses, namely cements, have been developed as DDSs. *In-vitro* studies have proven that GICs release suitable amounts of fluoride, preventing secondary caries.[170-172] Thus, GICs presented higher antibacterial activity than the conventional material (zinc phosphate cement).[173,174] CHX-loaded zeolite nanoparticles have been incorporated into GIC and showed activity against *S. mutans* with an early CHX release burst.[175] These findings suggest that a range of antimicrobial drugs that inhibit the growth of oral bacteria can be incorporated efficiently into dental GICs.

Self-adhesive resin cements with benzalkonium chloride are another example of DDSs in fixed prosthodontics. This drug was included in concentrations of up to 1% and seemed acceptable considering the tested properties: degree of conversion, Vickers hardness, setting time, and biaxial flexural strength. It may also be a good option to improve the durability of the adhesive interface.[176]

1.1.2.10. Orthodontics

Orthodontic adhesives, brackets, and cements for band cementation have been loaded with drugs to create DDSs.

A concern about orthodontic treatment in young patients is the formation of white spot lesions around bonded orthodontic brackets. The placement of fixed appliances increases plaque accumulation and colonization by cariogenic bacteria, resulting in tooth enamel's demineralization.[177-179]

A few decades ago, one of the first recommendations for patients with orthodontic treatment was rinsing brackets with antimicrobial agents (e.g., 0.12% CHX digluconate or fluoride mouthwash).[180-184] The antimicrobial effect of mouthwashes against periodontal pathogenic microorganisms like *Aggregatibacter actinomycetemcomitans*, *S. mutans*, and *Lactobacilli*, known as the bacteria most closely associated with dental caries, has been proven.[181,182] To avoid the need for patient compliance, these antimicrobial agents, fluoride, CHX, and triclosan, have been

incorporated directly into orthodontic adhesives[81,185,186], which allowed the short-term release of the antimicrobial agent without influencing the mechanical properties.[187]

On the other hand, clinicians have been using GICs with CHX digluconate for band cementation. The addition of CHX to the liquid does not significantly influence the diametral tensile, compressive, or shear bond strength and increases the tested GIC's microhardness.[188]

Recently, newer technologies have been developed to prevent enamel demineralization and the formation of white spot lesions. Nanoparticles have been added into orthodontic adhesives and, in general, exhibit antibacterial activity with no adverse effects on mechanical properties. The incorporation of silver nanoparticle (AgNP) solutions into the Transbond™ XT adhesive primer showed *S. mutans* growth inhibition. The shear bond strength decreased after incorporating an AgNP solution of up to 0.33%, without compromising the adhesive primer's chemical and physical properties.[189] Another orthodontic adhesive containing 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT) is a promising antibacterial material, especially in concentrations of 15% and 20%. This antibacterial agent promotes a bacterial growth reduction, followed by a higher degree of conversion, less degradation, and greater bond strength.[190]

To prevent the accumulation of dental plaque and the development of dental caries during orthodontic treatment, some authors have performed studies using Ag[191-193], Ag-Pt[194], TiO_2[191,192,195,196], CuO, and ZnO[197] as brackets coatings. However, the longevity of these coatings is questionable, and more *in-vivo* studies are needed.[198] On the other hand, resin-based materials containing nanoparticles of amorphous calcium phosphate presented great biofilm growth reduction compared to a commercial orthodontic adhesive and did not adversely affect the shear bond strength.[199]

A promising approach to prevent *in-vivo* demineralization and therapeutic enamel remineralization consisted of incorporation triclosan into nano-compounds based on mesoporous aluminosilicate nanotubes as nano-carriers for antibacterial agents. This innovative therapeutic adhesive may promote long-term antimicrobial activity and mineral deposition, with an acceptable degree of conversion and shear bond strength.[186]

Despite this great evolution in dentistry to prevent the appearance of white lesions, sometimes they are still found after the removal of the orthodontic brackets and bans. One of the treatment options for demineralized lesions is using the resin-based

material ICON®.[200] It consists of a resin component based mainly on triethlyene glycol dimethacrylate (TEGMA), which can penetrate the pores of the affected enamel and prevent caries lesion progression.[201,202] A DDS incorporating CHX diacetate into this resin has been proposed. Infiltrants with CHX could arrest and reinforce initial caries lesions, and their antimicrobial effect could prevent new lesions in sound enamel adjacent to the infiltrated area.[81]

1.1.3. Immediate Effect or Long-Term Effect?

Some problems have been described with DDSs, such as deterioration of the matrix's properties or the possibility of adjacent tissue toxicity in the case of excessive drug release.[27] Given the different clinical situations, it is also important to study the behavior of the DDS under the biodegradation processes of the oral medium since it should maintain adequate properties over time in the oral environment.

Biodegradation can be defined as the changes in the chemical, physical, and mechanical properties of a biomaterial caused by the physiological environment.[11] It is important to conduct studies with aging, especially in dentistry, where the biomaterials are exposed to a harsh oral environment, subject to temperature and pH changes, food, and cleaning methods adopted by the patient.[137,138]

The aging of a species *in vitro* can be simulated by thermal, chemical, and/or physical (by occlusal mastication) processes. Thermocycling is a method widely used where the specimens are immersed in almost extreme-temperatures baths: 5 and 55±2ºC with a dwell time of 20 seconds.[124,203] It consists of an *in-vitro* test mimicking what happens in the oral cavity because, in clinical use, the biomaterial may experience considerably varying temperatures with the intake of hot and cold food and drinks or the use of warm or hot water in cleaning.[204] The literature described that 10 000 cycles of cyclic thermal stressing correspond to 1 year of intraoral conditions.[205] A novel method using a polymerase chain reaction (PCR) thermal cycler was recently developed.[206,207]

Chemical aging is an alternative method where the specimens are stored in saliva, an important physiological fluid in the oral cavity. *In-vitro* experiments can use natural saliva (e.g., when collected from a volunteer) or artificial saliva.[208,209] In the context of scientific research, many artificial saliva models have been developed.[210,211] A study by Bettencourt *et al.* (2016)[212] described a potential easy, reproductible formulation of artificial saliva from the mixing of several components, like phosphate

buffer, xanthan gum, calcium, sodium and potassium chloride, and propylene glycol. Several cycles at different pH values must be carried out in order to simulate oral cavity environments, where the pH changes with food and drinks ingestion.[213-215] Previous studies indicate that an individual with a cariogenic diet is subjected to approximately 6 daily hours of an acidic environment. Accordingly, a chemical aging protocol was described in the literature: specimens were immersed in artificial saliva at pH=3 or pH=5 in cycles of 6h interchanging with 18h at pH=7.[214,216-218]

Other forms of aging have been described in the literature, including enzymatic processes and even food pigments or staining solutions or UV (ultraviolet aging).[219] The artificial physical aging submits the specimens to a series of thermo-mechanical fatigue cycles in a masticatory simulator under a specific load depending on the biomaterial, simulating occlusal forces.[220]

The incorporation of CHX or ursolic acid into a commercial primer has been tested with and without thermocycling to analyze the effects of water aging on the antimicrobial activities (growth and biofilm assays) of these primers.[221] In restorative dentistry, the effects of adding the hydrophobic monomer 1,12 dodecanediol dimethacrylate (DDDMA) to experimental sealants were studied regarding the various mechanical and superficial properties with and without thermocycling.[222] Microtensile bonding strengths to enamel of sealants containing a synthesized antibacterial fluoride-releasing monomer (DDS) were tested after 24-hour storage in water at 37°C and after 1000 thermal cycles.[223] In the same area of dentistry, the antibacterial effect and the physical-mechanical properties of temporary restorative material containing antibacterial agents as a DDS was tested after being submitted to thermocycling.[224]

The design of controlled released systems depends on various factors, such as the physical-chemical properties of drugs and material carriers, expected release profiles, diseases being treated, the patient's condition, treatment duration, and challenges to translate the systems from the laboratory to the clinic. Thus, a more thorough understanding of the above factors will ultimately allow for the design of an optimal controlled-release system for a specific application.[29]

1.2. Denture Stomatitis

In developed countries, global average life expectancy increased by 5.5 years between 2000 and 2016, the fastest increase since the 1960s, and this tendency is expected to continue.[225] The number of edentulous and removable prosthesis users is also expected to grow, especially in low-income populations.[226-228] In Portugal, according to the 2019 National Oral Health Barometer (*Barómetro da Saúde Oral*)[229], 31.1% of the population was missing six or more natural teeth. Tooth loss is associated with decreased integrity of the masticatory system and negative aesthetic, functional, and psychological consequences.[230] So, the need for prosthodontic treatment increases. Moreover, 41.4% of the population used a removable denture, and only 12% used fixed rehabilitation to replace missing teeth.

The most common chronic mucosal lesion associated with removable prostheses is denture stomatitis (DS)[231], also referred to in the literature as erythematous candidiasis, denture-induced stomatitis, and chronic atrophic candidiasis.[232] In 2015, a study by the Portuguese Directorate-General of Health (*Direção Geral de Saúde*, DGS)[233] reported a prevalence of rehabilitation with removable dentures in 51.1% of people aged 65-74 years. In the population under study, between 15% and 70% of denture wearers had DS, with higher values in the elderly and women.[144,234] According to a study performed by Figueiral *et al.* (2007) in Portugal[235], 45.3% of patients with removable dentures showed DS, and it was more prevalent in females (59.8%).

DS is characterized by the presence of erythema and edema of the oral mucosal areas underlying a removable (partial or total) denture or appliance.[144,232,236-238] The lesion shows signs of moderate acanthosis with inflammatory cells and prominent blood vessels, as confirmed by histological findings.[239,240] However, it is almost invariably an asymptomatic lesion diagnosed in routine oral exams by the dentist.[238] Even so, signs and symptoms like bleeding, pain, discomfort, halitosis, unviable use of muco-supported dentures[241,242], and altered taste sensation, with consequent poor nutritional intake, were reported.[145,155,243] An association with angular cheilitis and/or median rhomboid glossitis has also been detected.[227,242]

The lesion is usually located in the upper jaw, especially on the palatal mucosa, confined to a discrete area of pinpoint inflammation related to the glands' ducts or an intense erythematous area covered by a denture.[227] It rarely appears in association with

mandibular dentures due to the action of the high salivary flow and the smaller surface of contact between the mucosa and the prosthesis.[244]

Diagnosis is based on clinical examination and patient medical history. Sometimes diagnostic tests (exfoliative cytology or microbial culture) are conducted for microbiologic identification and quantification of *Candida* spp.[245,246]

Different classifications of DS have been proposed. Newton, in 1962[247], classified DS into three subtypes (Figure 1.4), according to its clinical appearance based on the severity, distribution, and extent of the lesion:

- Type I may represent an early stage of this condition and is frequently the most common lesion. Its clinical appearance consists of red spots, usually around the minor palatal salivary glands (pinpoint hyperemic lesions with petechial hemorrhage), or a localized simple inflammation in a limited area of the palate mucosa.[248,249]
- Type II is uncommon and consists of a generalized simple inflammation characterized by more diffuse erythema (redness) and edema (swollen) confined to a part or all of the oral mucosal areas under the denture.[250,251]
- Type III is often observed in association with the other two types. Also described as papillary hyperplasia of the palate, its clinical appearance consists of hyperemia of the oral mucosa with the appearance of nodules or plaques, usually localized in the central hard palate and the alveolar ridge. Histopathologically, the lesion has been described as papillary projections covered by stratified squamous epithelium with chronic inflammation. Normally, the lesion results from chronic injury by an unstable denture and may not be associated with candidiasis. Thus, papillary hyperplasia of the palate can be considered an entity independent of DS, and some authors have described it as a premalignant lesion.[227,252]

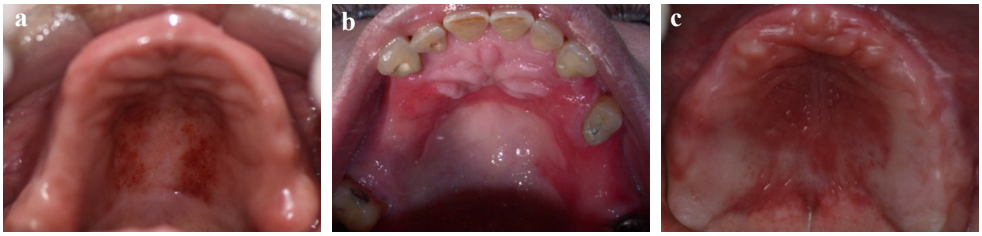

Figure 1.4 – Denture stomatitis clinical photographs based on Newton's classification.
a) Type I; b) Type II; c) Type III.

A study by Figueiral *et al.* (2007)[235] in a population of 140 patients from the Faculty of Dentistry of the University of Porto who wore removable maxillary acrylic dentures reported the following prevalences: DS type I – 41.4%, DS type II – 34.3%, and DS type III – 24.3%.

Budtz-Jorgensen and Bertram in 1970[253], Bergendal and Isacsson in 1983[239], and Barbeau *et al.* in 2003[254] proposed other classifications derived from Newton's original method where scoring is done according to the severity of the erythema.

1.2.1. Etiology

At present, there is still no consensus on a single etiologic factor of DS, given that no study has demonstrated a clear cause-and-effect relationship. The current trend is based on a multifactorial etiology of DS[144,254,255] that includes *Candida*'s capability to adhere and proliferate in the host epithelial tissues (infectious factors), trauma of the oral mucosa, and other host-related local and systemic factors (Figure 1.5).[256]

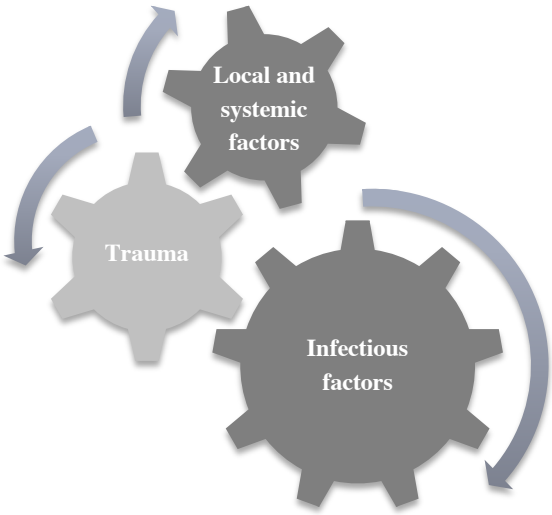

Figure 1.5 – Multifactorial etiology of denture stomatitis.

1.2.1.1. Infectious Factors

The infection by *Candida spp.* is considered the main etiological factor of DS.[18,22,145,159,236,237,257,258] *Candida albicans* (*C. albicans*) is frequently isolated in affected areas, but other fungi from the *Candida* group, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, have also been observed. These species can form biofilms on virtually any biomaterial implanted in a human host, such as a denture base.[254,259-262]

C. albicans is a commensal yeast localized in the oral cavity, gastrointestinal tract, vagina, and skin, normally present in reduced percentages on the oral microflora

in a large proportion of healthy people. It is part of the normal flora, maintaining a dynamic equilibrium with the other resident microorganisms.[263] When associated with the predisposing factors (trauma and/or local and systemic factors), it becomes a pathological microorganism with the ability to colonize the denture and oral mucosal surfaces.[264-267] The non-pathogenic yeast forms are transformed into pathogenic invasive forms (pseudohyphae and true hyphae) capable of evading or escaping phagocytic cells. Thus, *C. albicans*' morphology contributes as a major factor for the survival of the yeast at various sites or conditions.[268,269]

Candida biofilm formation starts with the initial adherence of yeast cells to the surface of medical devices, followed by the formation of microcolonies and the development of a hyphal/pseudohyphal layer that extends outward. This process is accompanied by the formation of an extracellular matrix surrounding both the hyphal and yeast layers. Like the others, the *Candida* biofilms display a complex and organized structure that consists of a dense network of microorganisms deeply embedded in an extracellular matrix composed of polysaccharides.[270-272] These biofilms show *Candida* fungi together with gram-positive bacteria like *Streptococcus and Staphylococcus spp*. Thus, they are different from teeth-associated biofilm, which consists predominantly of gram-positive bacteria with a reduced number of fungi.[273,274]

A study with *C. albicans* and *Streptococcus spp*. demonstrated that the interaction between these two species increases the presence of fungi. This finding suggests that antibacterial approaches should be adopted in addition to antifungal strategies to control stomatitis more effectively.[275] Furthermore, in addition to fungi, *Staphylococcus* and *Streptococcus* species can penetrate up to 1 mm in the resin and still survive.[276] These biofilms stay deeply embedded into cracks and imperfections of the biomaterials, protected from any action.[145]

These biofilm communities have unique characteristics that confer them survival and pathogenicity, allowing defense against harmful physical and chemical factors, metabolic activity reduction, and protection against host defenses. Moreover, they hinder the diffusion of antibiotics, increasing the resistance of microorganisms to their action.[145,270,277,278]

1.2.1.2. Trauma

Although denture trauma alone cannot induce DS, when combined with other factors, it may contribute to the appearance of the disease.[279] Patients with unstable

dentures by resorption of the alveolar ridge may present traumatic ulcers that can increase the epithelium's permeability to toxins.[280] This factor is more related to DS type I (localized simple inflammation).[144,279,281] When denture stability is provided, for example, by association with implants and formation of an overdenture, oral mucosal trauma reduces, and DS can be controlled.[280]

1.2.1.3. Local and Systemic Factors

Both these factors are associated with DS because they can potentiate the other two previously mentioned.

Local factors are those related to the saliva, the prosthesis (prosthetic factors), including decreased occlusal vertical dimension (DVO), lack of oral and denture hygiene, continuous and nocturnal denture wear, denture base materials, among others.[244]

- Saliva

Saliva has a physical cleaning effect due to the presence of defense molecules that act as a potent inhibitor of *Candida*'s acid protease synthesis.[242,282] However, saliva can also have the opposite effect due to the presence of other components in its constitution that facilitate the adherence of *C. albicans* to saliva-coated acrylic resins.[242] In conditions of trauma and poor oral hygiene, the adhesion of *C. albicans* to a continuously worn denture base may cause DS if the human saliva's oral antimicrobial peptides (with antifungal defense) are not present at a higher magnification.[254]

Xerostomia and altered salivary pH represent potential risk factors for DS development. Patients with Sjögren's syndrome, subjected to head and neck radiotherapy and/or xerostomizing drugs, may be more prone to the establishment of DS due to the decreased salivary flow.[283-287] In the oral cavity, the pH is approximately 7.0, although temporary environmental pH changes can be observed. In an acidic environment where pH decreases (e.g., by carbohydrate intake), *C. albicans* can colonize the denture surface.[284,288-290] Monroy *et al.* (2005)[291] reported atrophic DS in 50 patients with an average saliva pH of 5.2. Nevertheless, a decreased amount of saliva and an acidic pH were directly associated with the presence of yeasts, but not with the existence of DS.[234]

- Prosthetic factors

1. Occlusal vertical dimension (DVO)

A reduced DVO was associated with the appearance of angular cheilitis and, as previously mentioned, can be a sign of the presence of DS. Angular cheilitis is characterized by a lesion infected by either *C. albicans*, *Staphylococcus aureus* or both.(250) A decreased DVO may result in oral mucosa trauma due to an uneven load distribution.(280)

2. Poor oral and denture hygiene

A significant correlation between poor denture hygiene and the prevalence of *Candida* has been reported. Only 16.7% of dentures worn by the elderly are properly cleaned, and it tends to be worse with older dentures.(292) Correct oral hygiene is important for controlling the bacterial biofilm on the denture and the oral mucosa and is the fundamental base for preventing DS.(275)

3. Continuous and nocturnal denture wear

Wearing dentures continuously for 24 hours is associated with an increased frequency and density of *C. albicans* on the denture's surface. In some situations, it can even cause oral mucosa trauma.(242) Removing the dentures at night can be an efficient procedure to reduce *Candida* counts.(293)

The continued use of dentures does not allow elastic recovery of oral tissues and promotes the rapid growth of microorganisms due to the humidity and temperature conditions. Soft tissues have been reported to need about 3 hours to completely recover from a 10-minute strength.(294)

4. Denture base materials

The development of DS is also influenced by the denture base material.(23,145,262) Materials for dentures, such as acrylic resins, offer perfect support for biofilm formation. The chemical and physical characteristics of these materials' surface (e.g., surface free energy and surface roughness) support biofilm formation through reversible and then irreversible adhesion to the surface.(258,295,296) The literature on *C. albicans* adherence is still controversial regarding surface free energy.(263) In addition, other factors should also be considered, such as cell surface factors, diet, salivary composition and secretion rates, and antibody titers, which are all controlling factors in plaque formation and could therefore influence yeast attachment.(297)

Due to the intrinsic porous nature of acrylic resins and the surface deterioration, promoted by oral hygiene procedures and the oral biodegradation process, roughness is

promoted and influences the tendency for pathogenic microorganisms adhesion.[298-301] It is known that fungal adhesion may be greater in materials presenting higher surface roughness.[263]

- Diet

Diets with high sugar intake increase the risk of DS due to glucose being able to grow *Candida* species and potentiate its adhesion to oral mucosa and dentures. Sometimes sugar consumption is associated with poor denture hygiene, another risk factor for DS.[302-304]

- Tobacco and alcohol

Smoking has been associated with the presence of yeasts in the oral mucosa.[305,306] Moreover, alcohol consumption and other lifestyle factors, such as lower physical activity and bad dietary habits, enhance the effect of smoking on DS development.[307]

- Drug therapy (antibiotics and topical corticosteroids)

Antibiotics and topical corticosteroid administration can cause instability in the oral microflora. Drugs eliminate bacteria that compete with fungi, allowing them to grow and become phatogenic.[285,308]

- Oral cancer and leukoplakia

Patients with oral cancer and leukoplakia may have their immunity compromised, leading to candidal infection.[283,285,309]

Systemic Factors

- Diabetes Mellitus

Diabetic patients suffer from oral candidiasis more frequently than healthy individuals, and the risk increases if they are denture wearers. This higher frequency is probably due to the saliva of diabetic patients favoring *C. albicans* growth, increasing its frequency of colonization.[310,311] It is also known that in patients with type 2 diabetes, the severity of DS's signs and symptoms is higher.[243]

Diabetes mellitus can also predispose to infection because of reduced immunity. This predisposition is related to glycemic control, as an increase blood glucose concentration can cause a decrease in immunity.[312-314]

Other factors that allow a commensal microorganism to change into a pathogenic one are:[315]

- Heart diseases
- Rheumatic diseases
- Hypoparathyroidism
- Hypothyroidism
- Pregnancy
- Neoplasms (acute leukemias, agranulocytosis)
- Nutritional deficiencies (iron, folate, vitamin B12)
- Immune disorders (HIV infection)
- Immunosuppressive drugs (chemotherapy)
- Drug therapy (systemic corticosteroids)

For example, elderly patients with a reduced general condition and more likely to present the mentioned diseases have a higher risk of developing DS.[316]

Although all these risk factors contribute to DS's development, it is important to note that the presence of *Candida* spp. biofilm on the prosthesis is considered the most important factor for the establishment of DS.[144] The mentioned factors promote denture and mucosa colonization by *C. albicans*, which acts as an opportunistic pathogen, especially when the host's resistance is overcome by the virulence of the microorganisms.[317]

1.2.2. Etiopathogenesis

The appearance and development of DS depend on a pathophysiological mechanism involving multiple mechanisms and pathways.[318] According to the current literature, the adherence of *C. albicans* to the denture material's surface may be necessary to initiate DS.[24,319] Adhesins are proteins present on this fungus's surface that mediate its binding to abiotic surfaces, such as a denture base.[320-322] This biofilm adherence also depends on dental material properties like surface roughness, surface energy, and composition.[323,324] With this connection, the planktonic *Candida* cells overgrow and promote biofilm formation.

Candida spp. can also produce hydrolytic enzymes such as proteinases and phospholipases to play an important role in the adherence to and penetration in the oral

mucosal surface, invasion and destruction of host tissues, and inducing inflammatory reactions. Several studies have demonstrated a relationship between increased hydrolytic enzymes and the clinical signs of candidiasis.[325] Proteinases facilitate the colonization and invasion of host tissues by degrading physiological substrates and inhibiting phagocytosis-inducing inflammatory reactions. Phospholipases damage the host cell membranes.[326-329]

The attachment of *C. albicans* to salivary macromolecules is also a critical event in the development of DS. A lower pH, normally associated with denture wears, promotes salivary immunoglobulin A (IgA) inactivation and favors the growth and multiplication of *Candida* and bacterial biofilm. Furthermore, an acid environment provides conditions for both the production and activity of proteinases and lipases. On the other hand, saliva has defensive molecules, such as lysozyme, lactoferrin, calprotectin, and IgA, that decrease *Candida*'s adhesion to the oral surface.[248,320,330,331]

Candida spp., mainly *C. albicans*, have a wide range of virulence factors such as the expression of adhesins, the secretion of hydrolytic enzymes (proteinases and phospholipases), dimorphism (morphological transformation between yeast and hyphal forms), the formation of biofilm, and phenotypic variations (switching). Additionally, they can grow at 37ºC and adapt to temperature and pH changes, have molecules homologous to the human CR3 integrin, favoring adherence to epithelial cells, survive within phagocytes, and evade the immune cells.[266,332,333] Thus, this fungus can attach and adhere to denture surfaces and oral tissues, causing the infection onset. Then, it can invade tissues and escape the host defenses mechanisms, developing fungal diseases.[332,334] The candidiasis infection can evolve to generalized and severe conditions such as candidemia (affecting the blood), meningitis (brain), and endocarditis (heart).[335]

The interaction mediated by the microorganism's virulence factors and the host's predisposing factors may promote DS. This infectious and inflammatory process results from the transition of commensal to pathogenic organisms.[266,332] Understanding the etiopathogenesis (Figure 1.6) is crucial for developing new diagnostics strategies and therapies for DS.

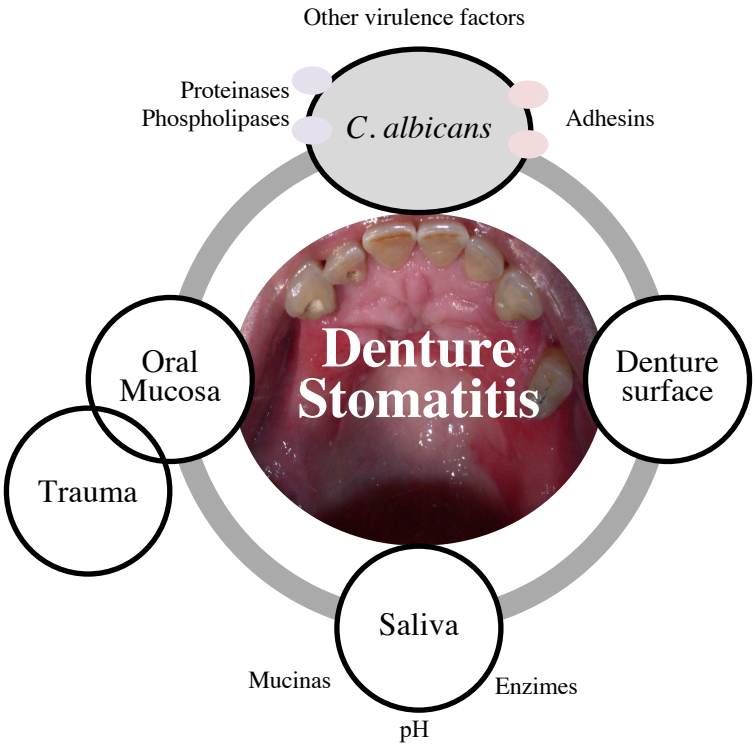

Figure 1.6 – Simplified diagram illustrating the etiopathogenesis of denture stomatitis.

1.2.3. Prevention

Given the diversity of predisposing clinical conditions, there are no simple preventive measures to prevent DS's onset and development. All etiological factors involved directly or indirectly should be taken into account.(317)

Since local factors are predisposing factors for this condition and may increase biofilms, it is fundamental that the patient follows correct prevention measures as instructed by the health professional. Recommendations for DS include correct denture and oral hygiene, removing the denture some time per day (normally at night), and regular dental visits.(336)

1.2.4. Treatment

Clinical diagnosis of this asymptomatic disease is imperative since DS can be a reservoir for microbes that promote infection in other sites of the body. Especially in the elderly, continual swallowing of microorganisms from denture plaque exposes patients to a risk of unexpected infections such as gastrointestinal infection, aspiration pneumonia, and chronic obstructive lung disease.(337,338)

The treatment of *Candida*-associated DS usually consists of one or more procedures already cited in the literature. The conventional treatment of oral fungal infections with topical and/or systemic antifungal therapy should be associated with reduced denture wearing time and the elimination of possible risk factors, such as tobacco habits[339], prolonged use of corticosteroids and antibiotics[246], and poor oral and denture hygiene[292] (Figure 1.7).

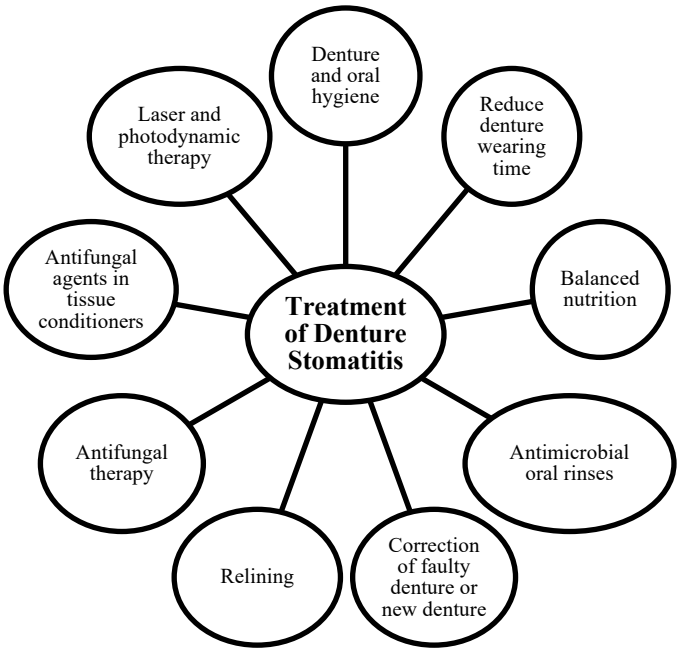

Figure 1.7 – A schematic presentation of treatment of denture-induced stomatitis. Graph illustration adapted from Iqbal *et al.* (2016) and Davoudi *et al.* (2018).[340,341]

Denture and oral hygiene

Proper hygienic care of removable dentures is an important way to maintain a healthy oral mucosa in denture wearers without pathogenic microorganisms. Mechanical denture cleansing is an effective measure for biofilm control; however, some denture wearers may have difficulty keeping their dentures clean. In geriatric patients and those with limited motor capacity, it is best to choose a combination of mechanical and chemical cleansing to reduce microbial biofilm formation in dentures.[342-345] Chemical products like sodium hypochlorite and peroxides, CHX, chlorine dioxide, and cetylpyridinium, marketed as effervescent cleansing tablets, have been used to disinfect these materials and prevent DS.[24,269] Since no relationship was found between hygiene and the growth rate of fungal microorganisms[346], this therapy

should not be considered alone. The literature also recommends that edentulous individuals with DS use palatal brushing to massage the tissues and reduce possible symptoms.[347]

Reduce denture wearing time

Patients with removable prostheses should also reduce denture wearing time to promote tissue rest.[348] Also, continuous denture wear may reduce the protective effect of saliva, decrease the cleaning effect of the tongue, and impair oral mucosa's oxygenation, thus contributing to DS.[254,349,350] A case report proposed a treatment protocol for DS that included the patient sleeping without dentures at night. This treatment protocol proved to be effective because remission of the inflammatory process occurred in all three cases.[241,351]

Balanced nutrition

Considering that nutritional deficiencies are a systemic predisposing factor for the development of this pathology, DS treatment includes balanced nutrition to decrease the susceptibility to infections.[304]

Antimicrobial oral rinses

Some antimicrobial oral rinses containing ethanol or essential oils have been used as disinfectants and cleansers for the treatment of DS.[163,283,352-354] Authors also suggested that the denture may be a reservoir for microorganisms and recommended daily antimicrobial therapy for the denture. Since 1972, CHX has been considered a denture disinfectant in the treatment of DS.[355] However, this treatment option has been associated with the recurrence of DS.[144,356]

Relining

The adherence of microorganisms over the surface of denture materials is necessary to initiate the process of DS. Eliminating the contact between the denture biofilm and infected tissues through denture base's relining has been recommended to enhance DS's treatment.[357] In case the stability and retention of the denture changes (frequently associated with tissue trauma), the denture should be relined, thus improving its fit and decreasing the porosities of acrylic and the adhered *Candida*.

Relining allows removing the top layer of contaminated old dentures, diminishing the recurrence of the disease.[241,358] The microorganisms' adhesion process can be influenced by the chemical/physical properties of microbial cell surfaces and the structure and composition of the biomaterial's surface.[359,360] Since tissue conditioners or soft liners are porous biomaterials susceptible to microbial colonization and biodegradation, this DS treatment option may present disadvantages.[361] Although it would be preferable to fabricate a new denture, it has been reported in the literature as an expensive treatment option for this pathology.[348]

Antifungal therapy

Usually, clinicians prescribe topical and/or systemic antifungal therapy along with oral hygiene instructions and denture adjustments.[18,362,363] Despite some antimycotics currently available in the market, the ideal antimicrobial for treating DS is not yet available. Three main groups of antifungal drugs are used to reduce the acute overgrowth of *Candida* species: the polyenes (amphotericin B and nystatin), the azoles (miconazole, ketoconazole, fluconazole, and itraconazole), and the antiseptics, such as CHX gluconate.[364]

Topical antifungal therapy

All topical antifungals are effective in minimizing potential signs and symptoms.[283,365]

- Nystatin

It can be used in the form of oral suspension, cream, and pastille. The commonly recommended therapy is a suspension of 100 000 units per mL for 1 or 2 weeks.[248] It should be rinsed and then swallowed due to its low absorption in the gastrointestinal tract.[366]

- Amphotericin B

This drug is usually presented as an oral suspension and is effective in signs remission. However, it has shown a partial reduction in *C. albicans* biofilm compared with nystatin.[277,367]

Both nystatin and amphotericin B have an unpleasant taste and sometimes their oral use may lead to gastrointestinal side effects.[368]

- Miconazole

It is available in a gel (Daktarin®) or cream form and can be applied three times daily for 1 or 2 weeks. This topical antifungal showed an inhibitory effect on *C. albicans* growth *in vitro*.[159,248] It should be taken into account that miconazole oral gel can potentiate warfarin anticoagulant activity.[369]

- Clotrimazole

These azole drugs used in the form of cream are broad-spectrum agents and affect the permeability of *Candida*'s membrane by interfering with the synthesis of ergosterol.

- CHX

CHX has antifungal and antibacterial activity because it significantly reduces the number of organisms both on the mucous membrane and denture. It also has an important antibiofilm effect because it is capable of inhibiting candidal adhesion to surfaces.[370]

In a double-blind study conducted in 53 wearers of complete dentures, the application of 2% CHX gluconate gel to the maxillary denture's fitting surface for 2 weeks produced a significant reduction of inflamed tissues beneath the denture.[371] Other studies suggested 1% CHX gel for DS treatment and reported a reduction in fungi cultures and clinical signs of the disease.[372]

In patients with systemic diseases, it is frequently used as an antiseptic in the form of CHX gluconate 0.2%. This drug cannot always be recommended for routine therapy because it stains the denture, and the yeast has relative resistance to the drug's action.[373,374]

Disadvantages of using this topical therapy have been reported. Saliva's diluent effect and the oral musculature's cleansing action tend to decrease the concentration of topical drugs to subtherapeutic levels, not reaching a therapeutic antifungal concentration on the denture surfaces.[150,278] Other problems are the mucosal reinfection after treatment completion and the necessity of compliance by patients.[365]

Systemic antifungal therapy

This therapy has been used in cases that do not respond well to topical antifungals, patients intolerant to topical treatment, and patients at high risk of developing systemic infections.[248,368] Systemic antifungal agents are similarly effective

in relieving DS's signs and symptoms but do not have an active role in eradicating the microorganisms from the denture surface.[15,375-377] The drug's poor penetration into the biofilm and the low patient compliance to this therapy remain the main causes of recurrence of DS.[277,378] Side effects such as gastrointestinal disturbances, hypersensitivity, and renal and liver toxicity can be disadvantages of this therapy.[352,365,379,380]

- Fluconazole

Fluconazole is a systemic antifungal commonly used because it is well-tolerated and has low toxicity, mild side effects, and low costs. However, it has poor efficacy on biofilms and, in elderly patients with reduced saliva production, there is a potential risk of low drug concentrations and the emergence of microbiological and clinical resistance. In addition, some *C. albicans* strains and other *Candida* species associated with DS are azole-resistant.[17] Although this therapy is commonly used for immunosuppressed patients, it has been associated with drug interactions.[249,362,381]

- Ketoconazole

This drug is used in a single dose of 200 mg for two weeks. However, it has shown hepatotoxic effects and cardiac arrhythmias when used in combination with other drugs.[248]

- Itraconazole

Itraconazole has shown minor side effects compared with other systemic antifungal drugs.[382]

All the above-mentioned systemic drugs have the same mechanism of action, interfering with the synthesis of ergosterol, a constituent of fungal cell membranes.[317]

DDS

Despite the various treatments available, DS is invasive and recurrent. It is difficult to eradicate because the biofilm cells show high resistance to antifungal treatments and the host's defense mechanisms and exhibit an excellent ability to adhere to biomaterials.[383] Thus, new developments related to denture materials are focusing on means to reduce the development of adherent biofilms.[144] An advanced strategy recently suggested is to impregnate denture materials with drugs for localized drug delivery near the infection site. This method prolongs *in-vivo* drug actions, decreases

drug metabolism, and minimizes its overall body distribution.[16] Moreover, this system has the advantages of being low-cost and successful, not requiring patient cooperation, and presenting minimal adverse risks, such as systemic toxicity.[17,18,24]

Because temporary soft denture liners are easily degradable and highly susceptible to microbial colonization, the first incorporation of antifungal agents as DDS was on these biomaterials.[21,158,159,384] A systematic review by Iqbal and Zafar (2016)[340] concluded that the incorporation of different antifungal medicaments in commercially available tissue conditioners could be recommended for managing denture-induced stomatitis; however, the optimal concentration remains uncertain.[24]

As previously mentioned, there is a concern that the drug inside the biomaterials may compromise its properties. However, adding nystatin, CHX, and ketoconazole to MICs to prevent *C. albicans* biofilms resulted in no harmful effects on the tensile strength and elongation percentage of the temporary soft denture liner materials up to 14 days.[385]

Unfortunately, because of the heterogeneous data, a meta-analysis could not be performed. Up to today, no definitive protocol (with the type of drug and its optimal concentration) has been established to prevent denture-related stomatitis using tissue conditioners or denture liners.[24]

New technologies and techniques

Considering the side effects of antifungal therapy, new technologies and techniques have been developed. New safe, efficient, user-friendly, and cost-effective treatment methods, like laser or photodynamic therapy, have been investigated for the treatment of DS.[341,386] As most DS lesions are caused by superficial candidiasis, they are typically accessible by laser photons, reducing the CFU/mL of *C. albicans* and improving soft-tissue inflammation without adverse effects.[387] However, more well-designed randomized clinical trials are needed.

Chapter 2

Drug Delivery Systems Based on Acrylic Resins

– A Systematic Review –

2. Drug Delivery Systems Based on Acrylic Resins – A Systematic Review

2.1. Introduction

Rehabilitation with partial and total removable dentures is one of the most common treatment options to restore the masticatory, aesthetic, and phonetic functions in edentulous patients.[388,389] According to the current literature, polymethylmethacrylate (PMMA) resin is still the ideal biomaterial for denture base fabrication since it is easy to manipulate, has a low cost, and offers adequate biocompatibility properties, color stability, and esthetics.[356,390] The mechanical properties of this polymeric material allow an adequate resistance to the masticatory forces.[391] However, this material is susceptible to microbial colonization in the oral environment. The absence of ionic charge in the PMMA denture base prevents the adsorption of salivary defense molecules, contributing to biofilm formation.[356] Other factors that have been associated with microbial colonization include local factors, such as surface roughness, porosity, poor denture hygiene methods, and continuous and nocturnal denture wearing[144,356,392], and systemic factors, such as xerostomia, immunodeficiency, malnutrition, and uncontrolled diabetes mellitus, normally in elderly patients.[262,275,356,392,393]

The microbial biofilm of removable dentures presents a wide range of bacterial species and more prevalent fungal *Candida* species such as *Candida albicans* (*C. albicans*), *Candida dubliniensis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*.[262,275,356,392,393] Changes in the equilibrium of the oral microflora and consequent biofilm formation can lead to the development of denture stomatitis, a pathological condition characterized by edema and erythema in the oral mucosal areas underlying a removable denture or appliance.[144,262] Palatal mucosa is the most affected location, and the most frequent form is oral candidiasis.[275,381,393]

The predominant microorganisms isolated from the affected mucosa include *C. albicans*, the main etiological factor for denture stomatitis.[262,356,392] This fungus can act as an opportunistic pathogen that can form a complex and heterogeneous biofilm on the oral cavity's hard and soft tissues.[144,275,394]

Denture stomatitis is commonly found among denture wearers despite being almost invariably asymptomatic and usually found in professional routine oral exams. Only a minority of individuals have symptoms such as pain and discomfort.[144,262,395] Even though microbial colonization is considered the main etiological factor, this pathology can also be related to denture trauma.[144,392]

The treatment options for denture stomatitis reported in the literature include improving the denture wearing habits, focusing on oral hygiene care and denture cleaning, and discontinuous use of the denture.[275,362,392] Moreover, disinfectant agents such as chlorhexidine have been used to clean the denture base to eliminate the bacteria and fungi and reduce biofilm.[249,275,356,392] Nonetheless, this treatment option has been associated with an incomplete denture disinfection, leading to a recurrence of the infection.[144,356]

The treatment with topical and/or systemic antifungal drugs is the conventional therapy for this pathology. Altough systemic therapy with fluconazole is commonly used for immunosuppressed patients, it has been associated with adverse systemic effects (nephrotoxic and hepatotoxic effects), antimicrobial resistance, and drug interactions.[249,275,362,381] Topical antifungal agents such as nystatin, miconazole, amphotericin B, and clotrimazole have proven to be effective in relieving the signs and symptoms of denture stomatitis associated with *C. albicans*.[249,356,365,392,395] Nevertheless, it is difficult to maintain an effective antifungal concentration on the infected surfaces and tissues due to the constant salivary flow, tongue, and swallowing movements. Other disadvantages of this therapy include low patient compliance, high cost, unpleasant taste, and requiring continuous use of dentures for local treatment, which contributes to reinfection and can cause additional trauma to previously injured and infected tissues.[381]

Drug delivery systems are an alternative to the above therapies and emerged based on a continued, localized controlled release of a drug over a long period.[396] Furthermore, it promotes high therapeutic efficiency and presents minimal adverse risks[150,397], having been widely used in several areas of Medicine.[398-400]

The treatment of denture stomatitis should reduce *C. albicans* levels in both the soft tissues and the denture base.[381,394] This reduction depends on antimicrobial substances achieving adequate therapeutic concentrations in both the soft tissues and the denture base.[393] Therefore, an alternative strategy for removable denture wearers affected by denture stomatitis is to impregnate acrylic resins with antimicrobial drugs

for localized delivery of drugs to soft tissues and, at the same time, act on the microorganisms in the polymeric matrix.[144,381] Additionally, unlike the conventional treatment, these systems are not time-dependent and allow for agent's diffusion through the polymeric matrix.[397] Besides their importance for microbial colonization, drug delivery systems also help prevent the actual appearance of denture stomatitis.[362,393,394] Moreover, these systems have the advantage of not depending on the patient compliance with a drug regimen since the patient is only required to use the denture.[381,397]

The addition of antimicrobial drugs to the denture base can be done through different processes: directly in the denture material, by imbibition through immersion of the denture base in a drug solution or by incorporation of the drug in the polymeric matrix during denture fabrication[166,401]; and indirectly by using a different material as a coating that will cover the denture base.[397]

Antimicrobial agents have been incorporated in denture bases or reline acrylic resins to prevent microbial infection. The relining procedure fills the existing gap between the original denture contour and the altered tissue contour with new material, thus replacing the denture base.[392,402] This procedure can be done with either a hard reline resin or a soft lining material.[403,404]

A soft reline resin allows incorporating the antimicrobial drugs and prevents direct contact between the biofilm on the denture base and the infected mucosa, thus reducing denture stomatitis's recurrence.[362,365,381] However, this is a short-term solution[403], since it is not recommended for longer than two weeks, because the soft resins are easily degradable, more prone to discoloration and microbial colonization, and their mechanical properties can suffer time-dependent changes.[405-407] On the other hand, hard resins are more appropriate for a long-term relining as they allow for substantial therapeutic action and drug release throughout time.[402,406,408] This procedure consists of readapting an old denture to the underneath support tissues and, thus, eliminate the need for new dentures, being a low-cost alternative.[405]

Acrylic resins used as delivery systems can be modified with other polymers to increase the drug release rate[150,397], for example, by adding macromolecules to the PMMA denture base.[356,362,409] Incorporating antimicrobial agents can completely prevent or eliminate *Candida* colonization and biofilm formation but can also affect the resins's properties.[150,381] For instance, Nam *et al.* (2012)[410] demonstrated poor color stability when the denture base was modified with silver nanoparticles.

Even though many scientific papers related to drug delivery systems based on acrylic resins have been published, there are no systematic reviews about hard acrylic resins.

2.2. Purpose

The present study aims to answer the following PICO research question by conducting a systematic review of the available literature: “Can the addition of an antimicrobial agent to hard acrylic resins be a treatment option for denture stomatitis, without changing their properties?”, where the population (P) is hard acrylic resins, the intervention (I) is a drug delivery system with an antimicrobial agent, the comparison (C) is the absence of therapy or placebo, and the outcome (O) is the control of denture stomatitis without changing their properties.

2.3. Materials and Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[411] The literature search was carried out using the online database PubMed/MEDLINE with the following keywords: "prosthodontics," "acrylic resins," "denture," "antimicrobial," and "antibacterial," associated with the "AND" and "OR" Boolean operators. A second search was carried out on the same database with the keywords described above and "stomatitis." All searches were conducted by two independent researchers from 2010 to the end of June 2020.

The studies were selected according to the eligibility criteria based on the PICOS strategy. A total of 195 articles were identified in the initial research. After duplicates exclusion, 155 articles were analyzed based on their title and abstract. The inclusion criteria for the articles were being written in English, Portuguese, or Spanish, having the full text available, and including *in-vitro* and *in-vivo* experimental studies. The exclusion criteria were articles with other types of studies, such as systematic reviews, guidelines, and case reports, and articles about soft acrylic resins and disinfectant agents used in prosthetic appliances (Appendix 1, Table 1). After the inclusion and exclusion criteria were applied, 105 articles were excluded, and a total of 50 articles were considered.

After the previous selection, seven additional articles of interest referred in the included studies' bibliography were added and respected the inclusion criteria. Thus, a total of 57 articles were considered for this systematic review (Figure 2.1) (Appendix 1, Table 2).

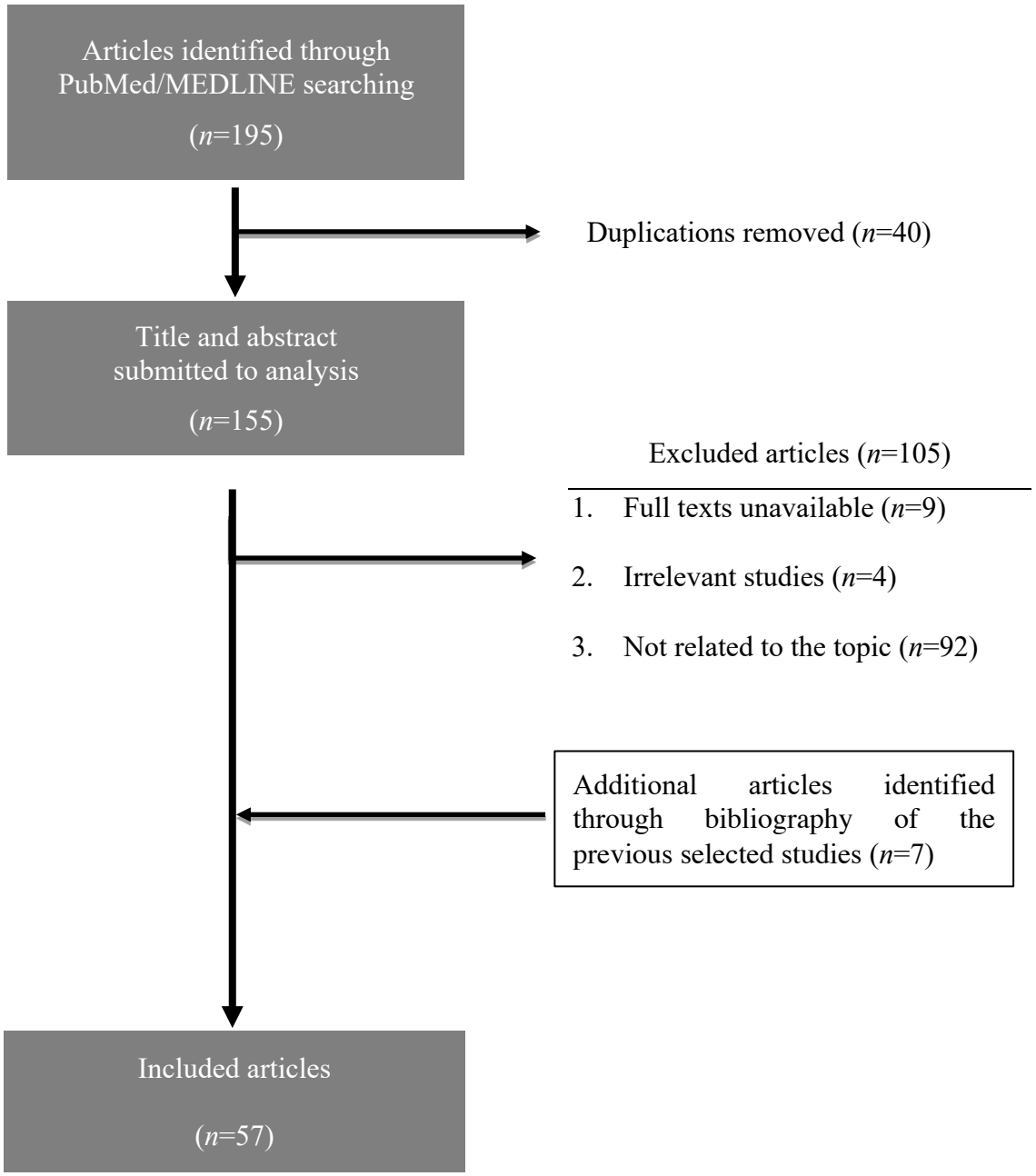

Figure 2.1 – Flow diagram of the screening and selection process.

2.4. Results

This review included 57 articles on antimicrobial drug delivery systems based on hard acrylic resins. Of these articles, nine refer to antimicrobial drug addition through coating, 11 through immersion, and 37 through incorporation in acrylic resins. All the studies included were made *in vitro*. The data obtained from each article included information about the microbiological studies, the properties of resins, drug release, and cytotoxicity studies (Table 2.1).

The compounds with antimicrobial activity mentioned were miconazole, silver, zinc oxide, titanium dioxide, chlorhexidine (CHX), fluconazole, nystatin, peptide mimetic compounds, zirconia, ethanol and natural compounds (*equisetum giganteum*, *punica granatum*, cellulose, thymoquinone, henna, and tea tree oil). Seven of these compounds were added to acrylic resins by more than one addition method (miconazole, silver, CHX, fluconazole, zinc oxide, titanium dioxide, and natural compounds). For example, CHX was directly added to the acrylic resins through immersion and incorporation.

Among the 37 papers related to the incorporation of antimicrobial agents in acrylic resins, ten reported the addition of monomers or copolymers like sodium fluoride, mesoporous silica, zirconia dioxide, pre-reacted glass ionomer fillers, quaternized ammonium, 1,4-diazabicyclo[2.2.2]octane (DABCO), methacryloyloxydodecylpyridinium bromide (MDPB), poly (2-tert-butylaminoethyl) methacrylate (PTBAEMA), and 2-tert-butylaminoethyl methacrylate (TBAEMA). The antimicrobial activity of each combination of resin with an antimicrobial agent was tested, except for ethanol, thymoquinone, and TBAEMA.[412-414] Thirty-one of the articles tested antimicrobial activity only against *C. albicans*, not including other species of *Candida* or bacterial or polymicrobial cultures.

Table 2.1 – Articles included in the systematic review on acrylic resins delivery systems.

SYSTEM	*DRUG*	*ANTIMICROBIAL ACTIVITY*			*PROPERTIES*	*RELEASE STUDIES*	*CYTOTOXICITY STUDIES*
		C. albicans	*Other species*	*Both*			
COATING (9 articles)	Miconazole	1			1		1
	Silver		1	1	2		
	Zinc oxide	1					
	Titanium dioxide		1				
	Natural compounds	1		1			
IMMERSION (11 articles)	Miconazole	2		2		3	
	CHX	3	1			2	
	Fluconazole	2	1		1		
	Nystatin	2	1		1		
	Peptide mimetic compounds	1					
	Natural compounds	3	1		1		1
INCORPORATION (37 articles)	Silver	7	2	1	8	3	4
	CHX	2	1		1	1	
	Fluconazole	2			1	1	
	Zinc oxide	1		1	2		1
	Titanium dioxide	1	1		1		
	Zirconia	1					
	Ethanol				1		
	Natural compounds	1		1	2		
	Sodium fluoride			1		1	
	Mesoporous silica			1	1		1
	Zirconia dioxide			1	1		1
	Pre-reacted glass ionomer filler particles	1			1		
	Quaternized ammonium			1	1	1	
	DABCO			1			1
	MDPB			1	1		1
	PTBAEMA			1			
	TBAEMA				1		

DABCO = 1,4-Diazabicyclo[2.2.2]octane, MDPB = Methacryloyloxyundecylpyridinium bromide, PTBAEMA = Poly (2-tert-butylaminoethyl) methacrylate, TBAEMA = 2-tert-butylaminoethyl methacrylate.

The properties of drug delivery systems based on hard acrylic resins were studied in 28 papers. In most of these papers, antimicrobial studies were also conducted. However, two of the selected papers exclusively evaluated the properties of the acrylic resins. One paper evaluated the effects of incorporating TBAEMA on the resins' flexural strength, and another paper evaluated the effects of incorporating ethanol on the resins' color and mechanical properties. Besides the acrylic resins' matrix properties, such as flexural, tensile and impact strength, fracture resistance, hardness, and elastic modulus, their surface properties, like roughness and contact angle measurement, and color were also studied.

Studies on drug release and cytotoxicity with cellular testing were conducted in nine and 11 articles, respectively. No article studied simultaneously all the properties and parameters of interest for this review, namely, microbiological studies, evaluation of the resins' properties, drug release, and cytotoxicity.

The results are described and discussed below according to the method of antimicrobial agent addition.

2.4.1. Addition of Antimicrobial Drugs through Coating

The addition of antimicrobial drugs can be done through denture base coating using denture adhesives. The new adhesives containing miconazole showed antifungal activity[149,415] with slight cytotoxicity associated with higher concentrations of the drug added (20%).[149] This study also reported results about the adhesiveness of the different denture adhesives loaded with drugs: when the drug was loaded to adhesives in microparticles, the drug maintained an adequate adhesive force[149], while other types such as cream or gel caused a change in adhesiveness.[415]

Silver nanoparticles added to denture adhesives for antibacterial coating influenced the denture base's surface properties by reducing roughness, monomer release, stress concentration, and fracture.[416] The antimicrobial activity was proven by the reduced adhesion of pathogenic species and the morphological changes in the resin surface.[417] Acrylic resin modification with zinc oxide or titanium dioxide coating also showed antimicrobial activity by preventing the acrylic resins' colonization.[418,419]

The natural compounds added through coating were herbal medicines such as *Equisetum giganteum* and *punica granatum*, and chitosan, a biopolymer abundant in crustacean shells. All these drug delivery systems presented antifungal activity, with

chitosan showing the best effectiveness with the highest concentration tested (40 mg/mL).[420,421]

2.4.2. Addition of Antimicrobial Drugs through Immersion

Studies on the addition of drugs through immersion were mainly based on the efficacy of the loaded resins' antimicrobial activity.[147,151,297,401,422,423] Antimicrobial studies that used CHX, miconazole, tea tree oil, and fluconazole solutions concluded that the first three drugs were more effective with *C. albicans* in the first 14 days, whereas the antifungal effect of fluconazole was not significant after 7 days.[147,401] The drug delivery system that used nystatin and fluconazole changed acrylic resins' surface properties, such as the contact angle and roughness.[297] CHX digluconate and miconazole presented similar results regarding antimicrobial activity and drug release, even though miconazole showed a longer antifungal effect, which is attributed to the higher water solubility of CHX.[151,236,265,422]

Another publication proposed immersing the denture in peptide mimetic compounds, a synthetic material that mimics a natural peptide. The results obtained with this compound revealed a potent, selective antimicrobial activity effective in eliminating or preventing the adhesion of *C. albicans* because these compounds demonstrated rapid fungicidal activity even in the presence of saliva.[424]

The natural compounds used were *Equisetum giganteum*[401,425], tea tree oil[423], and cellulose[426], which were obtained from plants and microorganisms. The first two compounds proved to have antimicrobial activity.[401,423,425] Even though cellulose did not show significant antimicrobial activity, it improved the acrylic resin's surface properties by reducing the contact angle and showed non-cytotoxic effects.[426]

2.4.3. Addition of Antimicrobial Drugs through Incorporation

Incorporation was the method of antimicrobial drug addition most cited in the papers reviewed. This method consists of mixing the drug with a powder, liquid, or even powder-liquid mix when preparing the material for polymerization.

The most studied drug was silver, and its incorporation was done through microparticle or nanoparticle processes. There were no significant changes in silver-modified acrylic resins' mechanical properties, namely, flexural strength and elastic

modulus.[427-431] Nine articles proved its antimicrobial effect against different species, especially at higher concentrations, and its cytotoxicity was low.[166-168,410,429,430,432-434]

The incorporation of CHX and fluconazole demonstrated an antifungal effect and a sustained drug release of up to 28 test days.[152,153] In the papers where these drugs were both studied, CHX showed higher inhibition of microbial colonization[435], even though it led to a decreased resistance to fracture of the resin.[436]

The acrylic resins incorporated with zinc oxide demonstrated inhibition of microbial colonization. On the other hand, this drug delivery system influenced the surface properties, particularity with 2.5% of nanoparticles.[437,438] Similarly, incorporating titanium dioxide into acrylic resins offered antifungal activity[439,440], but had an adverse effect on flexural strength directly proportional to the drug concentration.[441] As a new approach for denture stomatitis prevention, the acrylic resin modified with zirconia nanoparticles exhibited antifungal effects on *C. albicans*.[442]

The article that studied ethanol incorporation into acrylic resins did not mention antimicrobial testing and only evaluated the resin's properties. Namely, ethanol incorporation caused changes in the resins's color and elastic module.[413]

The natural compounds added through incorporation were plant extracts (thymoquinone and henna*)* and chitosan, a biopolymer abundant in crustacean shells.[443-446] Chitosan nanoparticles promoted antimicrobial activity.[443] Concentrations of thymoquinone greater than 1.5% caused a decrease in hardness, flexural strength, and modulus of elasticity and an increase in the resin's surface roughness.[445] Henna incorporation was mentioned in one paper that proved its effectiveness as an antifungal agent[444]; however, it also showed an adverse effect on the resin, as it changed surface roughness and hardness.[446]

Sodium fluoride as a copolymer incorporated into acrylic resins inhibited microbial colonization. Moreover, it showed a controlled release of the drug during 6 days, with fluoride release still detectable up to 6 months after, although with a very low concentration.[447]

The publication about mesoporous silica demonstrated its potential as an antimicrobial agent and as a possible vehicle for the release of other drugs. Additionally, it increased the resins' hardness in all tested concentrations (0.5, 1, 2.5, and 5%), decreased flexural strength, and increased surface roughness at the highest concentrations. It did not show any cytotoxicity.[448]

The addition of zirconium dioxide into acrylic resins demonstrated antimicrobial activity and no cytotoxicity. It also improved two resin properties: flexural strength and hardness.[449] The incorporation of charged particles based on glass ionomer reduced the colonization by *C. albicans* but increased the resin's surface roughness.[450]

Incorporating quaternary ammonia promoted a potent antimicrobial effect with a prolonged release but led to a decrease in the resin's flexural strength.[451]

Both incorporations of the compounds DABCO and MDPB promoted significant inhibition of microbial colonization and diminished cytotoxicity.[452,453] One the other hand, low concentrations (0.6%) of methacryloyloxyundecylpyridinium bromide (MUPB) reduced the resin's flexural strength.[454]

The incorporation of PTBAEMA proved its antimicrobial activity.[455] On the other hand, the addition of TBAEMA decreased the resin's flexural strength.[456]

2.4.4. Critical Analysis

This systematic review reports a wide range of heterogeneity in the data obtained from the included articles. Even though 47 articles out of the 57 obtained studied the loaded resins' antimicrobial activity, the results were not consistent.

Most of the studies did not follow an adequate methodology for antimicrobial testing of dental devices, such as proposed by Camilleri *et al.* (2020)[457], namely, replying on pre-testing parameters like pre-sterilization of the device or aging of the samples. Most of the studies included performed pre-sterilization but did not submit the specimens to any aging process to mimic the resin's behavior after its integration in the oral cavity.

C. albicans was the microorganism most frequently used in microbiological tests. This opportunistic pathogen, essential for the development of denture stomatitis, was described by Camilleri *et al.* (2020)[457] as the relevant strain of fungi to test in non-implantable dental devices. However, the data obtained was limited since several other species have already been identified in a complex biofilm that colonizes the mucosa. Further studies including several species or even a clinical strain should be conducted.

Colony-forming units (CFUs) counting, minimum inhibitory concentration (MIC), and the direct contact test were the most common antimicrobial testing methods published in the papers. According to the recommendations by Camilleri *et al.* (2020)[457], antimicrobial testing should include at least two combined methods, while

only one is required for evaluating the properties. In this systematic review, only nine of the 19 reviewed articles with antimicrobial characterization performed just one antimicrobial testing method.

All the other data of interest (properties, drug release, and cytotoxicity) were obtained in a few articles. A paper that studies all the parameters of interest for this review, namely, antimicrobial activity, resins' properties, drug release, and cytotoxicity, is still missing. The lack of data on drug release and cytotoxicity is of particular relevance for validating this therapeutic modality. Thus, it is important to develop more *in vitro* drug delivery system studies characterizing various properties. It is necessary to prove the data *in vitro* before *in vivo* studies.

Most articles did not assess the drug delivery systems' influence on the material's mechanical or surface properties. Moreover, the property and the method to study it varied between studies, making data comparison very difficult. Drug concentrations were also very different between studies. This heterogeneity reinforces the need to establish protocols with appropriate concentrations for the different drugs of interest.

All studies included in this systematic review were *in vitro* and showed different methodologies and protocols. Therefore, direct comparison or extrapolation of the findings of several studies with the same drug could not be performed.

In addition to the lack of experimental evidence, there is a strong need for clinical studies on drug delivery systems using acrylic resins. The conclusions of these studies could be directly clinically applicable to the prevention and treatment of denture stomatitis. The fact that many authors describe the applicability of drugs by these systems highlights this theme as of global interest.

2.5. Conclusions

In conclusion, introduction a drug delivery system with an antimicrobial agent can be a treatment option for denture stomatitis, even though it can lead to changes in the acrylic resin's properties. Further experimental studies must be conducted with similar and validated methodologies to establish conclusions and expand clinical studies.

Chapter 3

A Novel Drug Delivery System to Treat Denture Stomatitis

3. A Novel Drug Delivery System to Treat Denture Stomatitis

Based on the conclusions of Chapters 1 and 2, the drug and the biomaterial for a novel drug delivery system to use in removable prosthodontics were selected. The innovative system proposed for *C. albicans*-associated denture stomatitis control and treatment consists of incorporating chlorhexidine (CHX) into reline acrylic resin.

3.1. Chlorhexidine (CHX)

CHX has been used as a mouthwash since 1953 and is considered a gold standard in infection control in dentistry due to its antiseptic and disinfectant capabilities.[61,458] CHX ($C_{22}H_{30}Cl_2N_{10}$) is a synthetic bisbiguanide formulation with cationic properties. The molecule is symmetric with two chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain (Figure 3.1).[459]

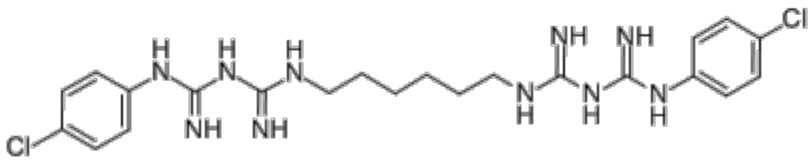

Figure 3.1 – Chemical structure of chlorhexidine. Adapted from Greenstein *et al.* (1985).[459]

The compound has a strong alkaline property and a wide antimicrobial spectrum that includes most microbes, such as gram-positive and gram-negative organisms (less effective with some gram-negative bacteria), including bacterial spores, lipophilic viruses, yeasts, and dermatophytes.[460,461] The CHX has a 625.56 g/mol molecular weight and high water solubility (19 mg/mL).[22]

CHX is available in three forms: digluconate, acetate (diacetate), and hydrochloride salts. The most common preparations are CHX digluconate and CHX diacetate because of their high water solubility.[462] Commercially, it is available as mouthwash, gel, aerosol, spray, disks, and varnish.[462,463]

3.1.1. Advantages

The effect of CHX is dose-dependent: at low concentrations, CHX is bacteriostatic, while in high concentrations, it also has a bactericidal effect.[464,465] Because the bacterial cell is negatively charged, the cationic CHX molecule binds to the cell surface. The integrity of the bacterial cell is thereby altered so that CHX can penetrate the inner cell membrane, leading to increased permeability. This change results in leakage of low-molecular-weight components. At this point, the antimicrobial action is still at the bacteriostatic stage and can still be reversed if CHX is removed and the bacterial cell can recover. Stable or increasing CHX concentrations, however, can lead to irreversible cell damage.[458] When this happens, CHX causes cell death by cytolysis: it increases the permeability of the bacteria cell membrane, resulting in the release of the main intracellular components, thus promoting cell breakage.[466,467]

When CHX is applied as a single rinse in the oral cavity, its action lasts for about 12 hours. Its long-lasting bacteriostatic action, also termed substantivity, is the most notable feature of this molecule. This phenomenon is due to the molecule's adhesion to the salivary glycoproteins that are slowly released over time.[468]

CHX has been reported as having acceptable biocompatibility and a low degree of cytotoxicity.[61,469]

3.1.2. Applications in Dentistry

CHX as an Antiplaque Agent

CHX is the gold standard for chemical control of dental plaque and is commonly used in mouthwashes to combat oral biofilms.[470] It is widely prescribed as an oral antiseptic (as an adjuvant to normal brushing and flossing in subjects unable to maintain proper oral hygiene) and for denture hygiene procedures.[279,298,463]

CHX's role in Dental Caries Prevention

CHX has been frequently investigated for adhesive dentistry applications and has been incorporated in various resin composites.[61,62,471] Since CHX possesses antibacterial properties, its incorporation into bonding agents may be a viable method to improve restorative dentistry's long-term durability, reducing secondary caries.[472] In the last decade, the incorporation of CHX into glass-ionomer cement (GIC) has been shown to confer beneficial antibacterial properties.[473-475]

Okada *et al.* (2016)[476] performed a systematic review and meta-analysis to evaluate CHX varnish's effectiveness in reducing caries incidence during fixed orthodontic treatment. The results suggest that CHX varnish is an effective preventive measure to decrease caries incidence during fixed orthodontic treatment.

CHX as an Adjunct to Nonsurgical and Surgical Periodontal Therapy

Subgingival irrigation with CHX as an adjunct to mechanical periodontal therapy is better than a mouthwash in eliminating microbiota located beneath the gingival margin.[477] After periodontal surgery, a CHX mouthwash enhances wound healing.[478]

CHX as a Root Canal Irrigant

The first use of CHX in dental practice was for washing operation sites and disinfecting root canals, while nowadays, it is widely used as an endodontic irrigant and medicament.[469] Previous studies have demonstrated that incorporating CHX can add some degree of antimicrobial property to epoxy-based resin sealers without significantly altering their physical properties.[109,110]

3.1.3. Side Effects

The most common side effect associated with the use of CHX is brownish discoloration of teeth, restoration and tongue. Staining caused by CHX usually cannot be removed by brushing with normal toothpaste, and the exact reason behind the staining is still being debated. In recent years, research has focused on developing different methods to reduce staining while maintaining CHX efficacy. Mouthwashes with lower concentrations and/or combined with other ingredients, such as herbal extracts, have been suggested as alternatives.[479,480] There is some evidence that regular and frequent application of CHX mouthwashes may temporarily impair the taste sensation.[481] Desquamative lesions in the oral mucosa were observed in a small number of individuals with prolonged use of CHX.[482] However, with minimal and transitory local and systemic side effects, CHX is still considered a safe compound.[463]

3.2. Acrylic Resins

Acrylic resins consist of polymeric biomaterials and are frequently used in daily dental practice for diverse functions, like denture base or denture liners. They can be classified as heat-, light-, or chemically-activated depending on the factor that promotes the polymerization. Heat-activated materials can use the heat generated by a hot water bath or microwave energy, while the light-activated ones use visible light as the energy source. Chemically-activated or autopolymerizing materials involves a chemical activator, like N,N-dimethyl-*p*-toluidine. Since for relining, a low polymerization temperature is desirable to minimize distortion of the remaining denture base, autopolymerizing resins were chosen.[13]

Acrylic resins undergo an addition polymerization, also called chain-growth polymerization. This process adds new monomer units to the growing polymer molecule one at a time through double or triple bonds in the monomer. In general, the three stages of polymerization reaction are initiation, propagation, and termination. In autopolymerizing acrylic resins, the initiator (usually benzoyl peroxide) opens the monomer's double bond by activating the tertiary amine, producing a free radical. Then, more monomer units are propagated and linked to form polymer units until the termination.[483,484]

The conversion monomer-polymer is never fully complete and results in the presence of residual monomers, which can stay trapped in the polymer network (affecting the properties) or diffuse to the surrounding medium (causing an allergic reaction). Comparing the autopolymerizing acrylic resins with heat-activated ones, the first have a higher residual monomer due to a low degree of conversion.[485-488]

Acrylic resins are based on polymethylmethacrylate (PMMA) polymer and methylmethacrylate (MMA) monomer. To allow for polymerization in the oral cavity, hard chairside reline resins were created based on polyethylmethacrylate (PEMA), whereas their liquid composition varies among materials and could contain isobutyl methacrylate (IBMA), butyl methacrylate (BMA), 2-hydroxyethyl methacrylate (HEMA), or 1,6-hexanediol dimethacrylate (1,6-HDMA).[486]

3.2.1. Hard and Soft Materials

Reline acrylic resins can be hard reline resins or soft lining materials, depending on the amount of plasticizer in their composition.[13]

Hard resins are more appropriate for long-term relining. Their composition is based on PMMA or PEMA polymer in powder particles, along with a peroxide initiator and a pigment. One the other hand, their liquid composition varies among materials and could contain IBMA, BMA, HEMA or 1,6-HDMA, and MMA.[486,489-492]

The soft lining materials maintain resilience for some time. However, they present disadvantages, particularly the temporary ones, such as difficult cleaning due to being significantly softer and less resistant to brushing than denture-based acrylic resins, and being porous and incompatible with certain denture cleaning solutions, even in short periods of immersion.[493] The soft lining materials have a short life cycle since they are easily degradable and more prone to microbial colonization than hard acrylic resin materials.[494] *Candida* can easily colonize this surface.[495]

3.2.2. Direct and Indirect Method

Relining with autopolymerizing acrylic resins can be performed through a direct or indirect method.[496-499]

The direct relining is performed directly in the mouth (chairside).[496-499] This method is faster, can reproduce the morphologic features of oral tissues directly on the new denture base, and the patient does not need to be without the denture during the time required for laboratory procedures.[500,501] However, it has been associated with adverse allergic reactions, and it is not easy to produce a relining with optimum material thickness.[502]

Alternatively, an indirect method can be done, in which the denture base is used as an individual tray, and a functional impression of the tissue is made. However, it requires more time and costs.[496,503,504]

3.3. A Chlorhexidine Delivery System Based on Reline Acrylic Resins

Denture stomatitis should be treated because it can progress to more severe infections. A wide range of treatments have been described, and the treatment of choice or the association of more than one treatment are aspects to be considered.[505] In 2011, a group of researchers in Brazil[241] presented a treatment protocol for denture stomatitis. They described three cases of totally edentulous patients submitted to the following treatment: denture rebasing with chemically activated acrylic resin, denture night immersion in 2.5% sodium hypochlorite, and use of topical antifungals for two weeks. The authors considered this protocol effective in denture stomatitis treatment but highly dependent on the patient compliance.

Nowadays, denture stomatitis treatment remains challenging because it is associated with high relapse rates after conventional therapy with topical and/or systemic antifungal drugs.[506] Studies have already shown resistance to antimicrobial agents in *Candida* biofilms.[272,277,507,508] Moreover, most of the individuals affected by denture stomatitis are immunosuppressed or underprivileged elderly who may not have access to antifungals or conditions to use the correct denture cleaning procedures.

The ideal denture stomatitis treatment should simultaneously focus on the removable denture, the microbial biofilm, and the infected mucosa.[377] This therapy can be based on the sustained release of antifungal drugs that may reach sufficient therapeutic concentrations to eliminate *Candida* from both the infected denture surfaces and supporting tissues.[17,157,509]

The authors conclude that autopolymerizing reline acrylic resins should be the biomaterial chosen for loading with CHX. The powder-liquid form should be used due to its low cost, easy manipulation, and potential use with either the direct or indirect method. With this novel drug delivery system involving reline acrylic resins incorporated with anti-infective drugs, one of the treatment targets is the denture surface. The top layer of contaminated old dentures is physically removed before relining to develop a new surface that provides unfavourable conditions for the adhesion and proliferation of *C. albicans*. This treatment is simpler and cheaper than fabricating a new denture.[358,510] In addition to interfering with infectious factors responsible for the etiopathogenesis of denture stomatitis, these autopolymerizing reline acrylic resins

improve denture fitting to the alveolar ridge, providing stability and retention and diminishing trauma occurrence.[511]

The drug to be loaded into acrylic resins in this novel drug delivery system is CHX diacetate monohydrate in its powder form for its incorporation into the biomaterial. This drug seems to be the best choice since it presented higher release rates and substantivity and has topical action in a broad spectrum of microorganisms, including *C. albicans* and bacteria.[145,146,150,153,163,164,299,435] *Candida* inhibition by CHX lasts longer than that by amphotericin B and nystatin, is not associated with resistances (cell death by membrane rupture), and allergic reactions are also rare.[148,463]

CHX has been proposed to treat denture stomatitis as a mouthwash or overnight denture disinfectant[146,151,279,283,298,352,512], as a gel for topical use[371,372], or integrated into soft and hard resins as a drug delivery system.[145,146] The incorporation of CHX into reline acrylic resins increases the drug distribution in the biomaterial.

In this project, we propose to design a novel CHX delivery system based on reline acrylic resins to be used for the prevention or treatment of denture stomatitis. This proposed treatment is a combination and simplification of the treatments already presented in the literature that have shown better results for denture stomatitis.

Loading the acrylic resins' polymeric matrix with 10% (w/w) CHX concentration during its manufacture has shown to be effective and feasible in antimicrobial tests against *C. albicans* and drug release earlier studies.[17,150,153,164] However, this addition of CHX has had a negative effect on porosity.[513] Moreover, decreased flexural strength has been found after the incorporation of nanoparticles as antimicrobial agents.[300,514] Therefore, the incorporation of lower concentrations of this drug into reline acrylic resins should be investigated.

In conclusion, this project's main purpose will be to evaluate the effectiveness of an innovative drug delivery system in the context of *C. albicans*-associated denture stomatitis control and treatment. Before implementing a clinical trial, this novel drug delivery system for denture stomatitis should be investigated in an *in-vitro* study at baseline and after being submitted to a biodegradation process. Our *in-vitro* testing of this novel drug delivery system for denture stomatitis was based on the recommendations for characterization and standardization of antimicrobial testing of dental devices.[457]

Chapter 4

Engineering a Novel Chlorhexidine Delivery System Based on Reline Acrylic Resins to Treat Denture Stomatitis

4. Engineering a Novel Chlorhexidine Delivery System Based on Reline Acrylic Resins to Treat Denture Stomatitis

4.1. Introduction

Denture stomatitis is a common condition among people who wear removable dentures. Treatment of this condition remains challenging since it is associated with a high recurrence rate after the conventional therapy based on topical and/or systemic antifungal drugs.[506] Factors such as low patient compliance and incomplete elimination of the main etiological factor, a *Candida* species biofilm, can explain this phenomena.[277,378]

A complex and organized structure like a biofilm has unique characteristics that confer survival and pathogenicity to the microorganisms.[145] As biofilms hinder the diffusion of drugs, impregnating denture materials with antimicrobials for localized delivery near the infection site is an advanced strategy that can deter the development of adherent biofilms and consecutively the onset of the disease.[144] This approach also overcomes the side effects of a systemic drug and guarantees the agent's availability in the target area at a therapeutic dosage for an extended time.[21,150,153,164] The monitoring is minimal, and the patient compliance dependency is reduced.[162,435]

This drug delivery system was first proposed to be used on denture base resins, with a treatment purpose. However, systems added to reline resins have the advantage of the top layer of contaminated old dentures being physically removed before relining and represent a simpler and cheaper treatment (economic and time advantages) than fabricating a new denture.[241,358]

Numerous previous studies investigated the addition of antimicrobial agents into denture soft lining materials with promising results.[21,23,159,237,513] However, since their core base material is easily degradable by the oral environment, they present a short life cycle (one to two weeks) and are considered temporary. Moreover, due to their porous structure, they are more prone to intense microbial colonization and need cleaning procedures like brushing and cleaning solutions, which also degrade the material.[493] To overcome these disadvantages[494], alternative drug delivery systems based on hard reline materials have been proposed.[17,150,153,435]

Among the various antimicrobials proposed, chlorhexidine (CHX), commonly used as an antiseptic mouthwash in dentistry, has been suggested as a therapeutic supplement to denture stomatitis due to its antimicrobial activity against a broad spectrum of organisms, including *Candida*.[150,153,370,435] In previous studies, the immersion of acrylic dentures into a CHX solution has suppressed the adhesion of *Candida albicans* (*C. albicans*) to the buccal epithelial cells and acrylic denture surfaces.[21,145,153]

Low concentrations of CHX showed substantivity (slowly release from retention sites, maintaining a prolonged antimicrobial activity for several hours) and a high ability to reduce biofilm formation and disorganize a pre-formed biofilm. Compared to antifungals, CHX's *Candida* inhibition last longer than amphotericin B's and nystatin's.[148] CHX also shows better results than fluconazole, both on releasing and microbiological tests.[145,150,153,163-165]

Besides immersion in solutions[21,145,153], CHX can also be added to a coating system[148] or be loaded to acrylic resins' polymeric matrix during manufacture.[153,435,436] Loading acrylic resins with a 10% (w/w) CHX concentration was considered effective and feasible in drug release and microbiological tests against *C. albicans*.[17,150,153,164] However, loading a drug to the polymeric matrix can alter its structure. Adding CHX can affect the porosity of temporary soft liners[22], and the incorporation of antimicrobial agents' nanoparticles may influence the mechanical properties of acrylic resins, decreasing their flexural strength.[300,514] Therefore, the efficacy of this drug delivery system using lower concentrations of CHX should be investigated.

We propose and describe an innovative treatment to prevent and treat denture stomatitis based on loading hard reline acrylic resins with the lowest CHX concentration that has an antimicrobial effect and maintains mechanical, structural, surface, and biological properties. The drug release profile in all resins in artificial saliva is also described.

4.2. Objectives

The main purpose of this work is to design a novel local drug delivery system based on acrylic resins aimed to be used in the prevention or treatment of denture stomatitis associated with *C. albicans*. This purpose will be achieved through an iterative approach with the following specific goals:

- Determine the minimum inhibitory concentration (MIC) of CHX for *C. albicans* and *S. oralis* strains;

- Evaluate the **microbiological** activity of three CHX-loaded reline acrylic resins, according to the following hypotheses:
  - $H0_1$: Loading reline acrylic resins with CHX does not produce antimicrobial action against *C. albicans* and *S. oralis*;
  - $H1_1$: Loading reline acrylic resins with CHX produces antimicrobial action against *C. albicans* and *S. oralis*.

- Evaluate the effect of loading the three reline acrylic resins with CHX, using a previously established concentration with antimicrobial activity, on their **mechanical properties**, according to the following hypotheses:
  - $H0_2$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX does not affect the reline acrylic resin's microhardness;
  - $H1_2$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX affects the reline acrylic resin's microhardness;
  - $H0_3$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX does not affect the reline acrylic resin's flexural strength;
  - $H1_3$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX affects the reline acrylic resin's flexural strength.

- Evaluate the effect of loading the three reline acrylic resins with CHX, using a previously established concentration, on their **structural property**:
  - Observe the total porosity with a 3D reconstruction model by micro-CT;
  - Analyze the chemical structure with FTIR-ATR.

- Evaluate the effect of loading three reline acrylic resins with CHX, using a previously established concentration, on their **surface properties**, according to the following hypotheses:
  - $H0_4$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX does not affect the reline acrylic resin's surface porosity;
  - $H1_4$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX affects the reline acrylic resin's surface porosity;
  - $H0_5$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX does not affect the reline acrylic resin's surface free energy;
  - $H1_5$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX affects the reline acrylic resin's surface free energy;
  - Observe the surface morphology of three reline acrylic resins with scanning electron microscopy (SEM) images;
  - $H0_6$: The reline acrylic resin used does not affect the shear bond strength to the denture base resin;
  - $H1_6$: The reline acrylic resin used affects the shear bond strength to the denture base;
  - $H0_7$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX does not affect the shear bond strength between the denture base and the reline resin;
  - $H1_7$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX affects the shear bond strength between the denture base and the reline resin;

- $H0_8$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX does not affect the type of bonding failure to the denture base resin;
- $H1_8$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX affects the type of bonding failure to the denture base resin.

- Evaluate the **release** of CHX by three reline acrylic resins, according to the following hypotheses:
  - $H0_9$: Materials differences do not affect the CHX release by the reline resin;
  - $H1_9$: Materials differences affect the CHX release by the reline resin.

- Evaluate the effect of loading three reline acrylic resins with a concentration of CHX on the **cell viability** of mouse fibroblast cell line, according to the following hypotheses:
  - $H0_{10}$: The reline acrylic resin used does not influence the cell viability;
  - $H1_{10}$: The reline acrylic resin used influences the cell viability;
  - $H0_{11}$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX does not affect the reline acrylic resin's cell viability;
  - $H1_{11}$: Loading Kooliner with 2.5% CHX, Ufi Gel Hard with 5% CHX, or Probase Cold with 5% CHX affects the reline acrylic resin's cell viability.

4.3. Materials and Methods

Three autopolymerizing hard reline acrylic resins, in the powder-liquid form, with different chemical compositions and physical structures were selected. Two were direct reline resins: **Kooliner** (GCAmerica Inc, Alsip, Illinois, USA), a non-crosslinking material, and **Ufi Gel Hard** (Voco GmbH, Cuxhaven, Germany), a crosslinking material, composed of pre-polymerized polyethylmethacrylate (PEMA) powder particles and the monomers isobutyl methacrylate (IBMA) or 1,6-hexanediol dimethacrylate (1,6-HDMA), respectively. The third one was an indirect reline resin: **Probase Cold** (Ivoclar Vivadent, Liechtenstein), a material based on polymethylmethacrylate (PMMA) that has methylmethacrylate (MMA) as the monomer (Figure 4.1).[487] The name, composition, powder/liquid ratio, polymerization condition, and manufacturer of the products used in the present investigation are listed in Table 4.1 (Appendix 5).

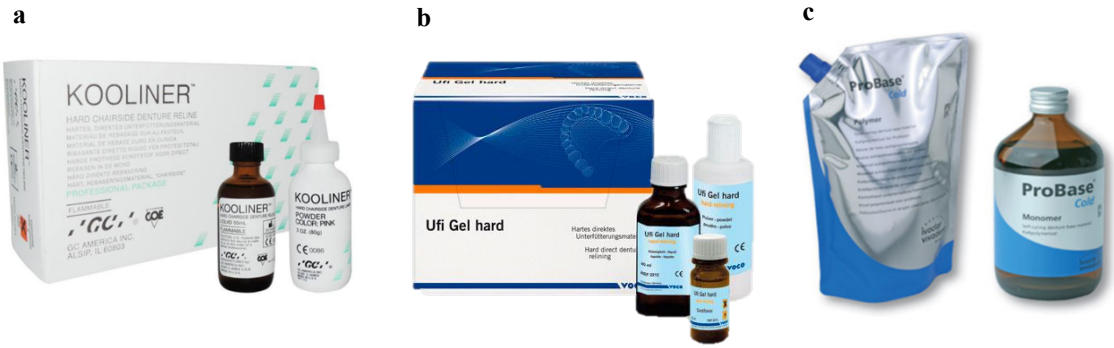

Figure 4.1 – Materials under evaluation in the study. a) Kooliner; b) Ufi Gel Hard; c) Probase Cold.

The CHX diacetate monohydrate (Panreac Applichem, Darmstadt, Germany) was selected to load the reline acrylic resins. The product used had the batch number 8F015944, and its expiration date was 10/2023 (Figure 4.2).

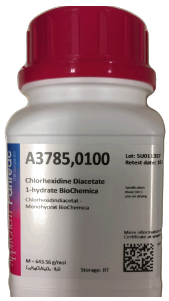

Figure 4.2 – Chlorhexidine diacetate monohydrate (CHX).

Table 4.1 – Materials under evaluation in the study and their characteristics.

MATERIAL	*MANUFACTURER*	*BATCH NUMBER*	*COMPOSITION*	*P/L RATIO (g/mL)*	*CURING CYCLE*
Kooliner (K)	GC America Inc., Alsip, Illinois, USA	1007201(P) 1008101 (L)	PEMA (P) IBMA (L)	1.4 / 1	Autopolymerization 10 minutes 37ºC
Ufi Gel Hard (UGH)	Voco GmbH, Cuxhaven, Germany	1128441 (P) 1134070 (L)	PEMA (P) HDMA (L)	1.77 / 1	Autopolymerization 7 minutes 37ºC
Probase Cold (PC)	Ivoclar Vivadent, Liechtenstein	L49853(P) L43809 (L)	PMMA (P) MMA (L)	1.5 / 1	Autopolymerization 15 minutes 40ºC 4 bar

P = Powder, L = Liquid, PEMA = Polyethylmethacrylate, IBMA = Isobutyl methacrylate, HDMA = Hexanediol dimethacrylate, PMMA = Polymethylmethacrylate, MMA = Methylmethacrylate.

4.3.1. Preparation of the Specimens

For each material, a control group and experimental groups of specimens were settled. Control groups had 0% CHX. In the experimental groups, CHX (concentrations varied among tests) was incorporated in the corresponding reline acrylic resin's powder, using a mortar and pestle for homogenization (Figure 4.3).

Figure 4.3 – Homogenization of chlorhexidine in the powder of the reline acrylic resin, using a mortar and pestle.

Before mixing, the powder of the reline resin and the CHX were each weighed using a precision scale (A&D Company, Limited, Tokyo, Japan) (Appendix 2, Figure 1).

Afterward, the liquid monomer was measured using a graduated pipette and added to the powders' mixture to prepare the specimen. The powders and the liquid monomer were mixed until homogenization was achieved (Figure 4.4).

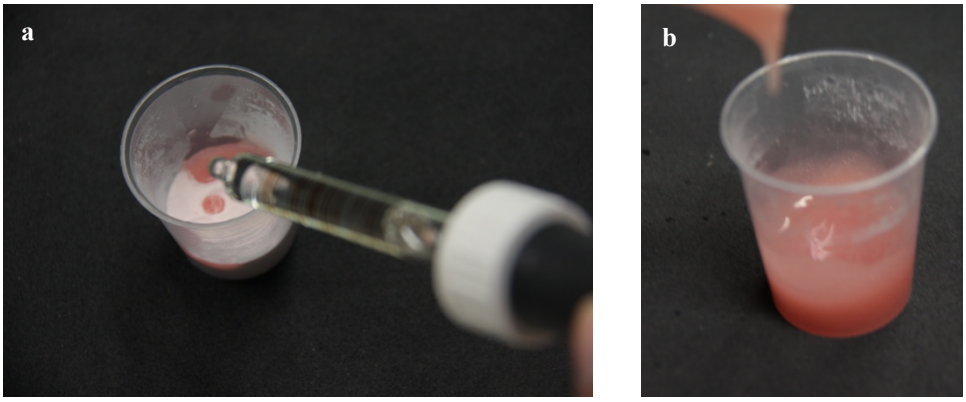

Figure 4.4 – a) Incorporation of the liquid monomer in the powders' mixture; b) Homogeneous mixture.

The mixture was then placed into a specific mold (the specimen's final shape varied with the test and is described ahead) and kept there for the time recommended by the manufacturer's instructions. The mold was maintained under compression at 37±2ºC in an oven (Ehret, Mahlberg, Germany) (Appendix 2, Figure 2) during the recommended polymerization time to simulate the intraoral polymerization of the direct reline resins (Table 4.1). Polymerization of the indirect reline resin was carried out in a pressure device (Ivomat, Ivoclar Vivadent, Liechenstein) at the recommended time, temperature, and pressure (Table 4.1) (Figure 4.5).

Figure 4.5 – Pressure device (Ivomat, Ivoclar Vivadent, Liechtenstein).

The edges of the specimen were polished with a 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL., USA) on a polisher with constant water cooling (DAP-U, Struers, Denmark) to remove irregularities (Figure 4.6) (Appendix 2, Figure 3).

Figure 4.6 – Polishing of sample irregularities.

The specimens were stored in distilled water at 37±2ºC for 24 h in an oven (Ehret, Mahlberg, Germany) before testing (Appendix 2, Figure 2).

4.3.2. Minimum Inhibitory Concentration (MIC) of CHX

Before the materials' tests, the CHX activity was tested against two microorganisms by the broth microdilution method.[515,516] The microorganisms selected for this evaluation were obtained from the American Type Culture Collection (ATCC): *C. albicans* (ATCC® 10231TM) and *S. oralis* (ATCC® 3507). Both microorganisms were taken from frozen stocks at -80ºC and cultured on a GPYA (glucose (Fluka, Sigma Aldrich Co., St Louis, USA)-peptone (Oxoid Ltd, Hampshire, United Kingdom)-yeast extract-agar (Biokar Diagnostics, France)) culture medium for 24 h at 37°C to ensure purity and viability. The inoculum preparation for the three antimicrobial tests followed the direct colony suspension method, with the colonies being picked and suspended in the corresponding medium (Figure 4.7).

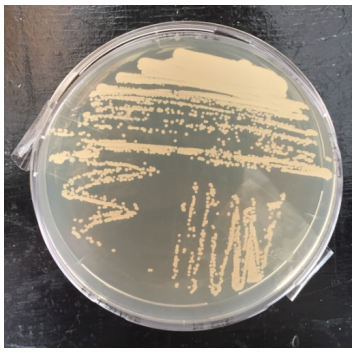

Figure 4.7 – Example of an inoculum with *C. albicans* ATCC® 10231TM.

All assayed samples were two-fold diluted in Roswell Park Memorial Institute (RPMI-1640) broth (Gibco, Thermo Fisher Scientific, USA), and the final CHX concentration ranged from 64 µg/mL to 0.125 µg/mL. The inoculum suspension was adjusted in a spectrophotometer (U-2000, Hitachi) (Appendix 2, Figure 4) in Roswell Park Memorial Institute (RPMI-1640) medium (≅1×10^6 cells/mL for yeast and ≅5×10^5 cells/mL for bacteria per well) (Figure 4.8). After 48 h of incubation at 35±2°C, the MIC was determined as the lowest drug concentration that could inhibit visible growth. All assays were performed with negative controls (not inoculated media) and positive controls (CHX-free inoculated media) and carried out in three independent experiments.

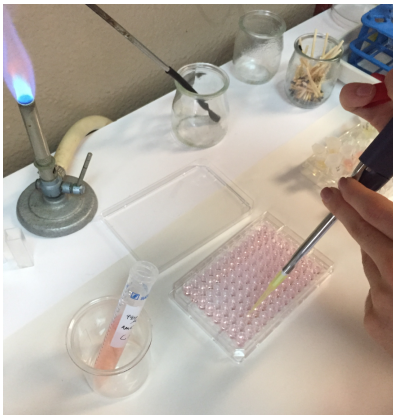

Figure 4.8 – Inoculation with *C. albicans* in the microdilution plate.

4.3.3. Biomaterials' Antimicrobial Activity

The antimicrobial activity was evaluated by two methods: agar disk diffusion (*Kirby-Bauer* test) and biofilm inhibition assay.

4.3.3.1. Agar Disk Diffusion Tests

One control (0% CHX) and five experimental (1%, 2.5%, 5%, 7.5%, and 10% of CHX (w/w)) groups of 5x1-mm disk-shaped specimens (*n*=3) (Figure 4.9) were settled for each of the three resins, with the fabrication of 54 specimens.

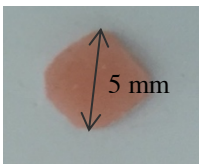

Figure 4.9 – Example of a polymerized Probase Cold disk-shaped specimen.

For the agar test, each strain inoculum was adjusted to 0.5 McFarland units using a spectrophotometer in Mueller-Hinton broth (≅1×10^6 cells/mL at a wavelength of 530 nm for yeast and ≅1×10^8 cells/mL at 600nm for bacteria). The inoculum was swabbed on Mueller-Hinton agar (Biokar Diagnostics, France), followed by specimens' placement (*n*=3). Paper disks with 10-µg CHX and 20-µg amphotericin B (Sigma Aldrich Co., St Louis, USA) (Appendix 2, Figure 5) were used as a positive control.

After 48 h, at 35±2ºC, inhibition zones' presence was observed and measured three times by a vernier calliper (Mitutoyo Digimatic, MFG.Co., Ltd) (Figure 4.10). Assays were performed in three independent experiments (Appendix 2, Figure 6).[517,518]

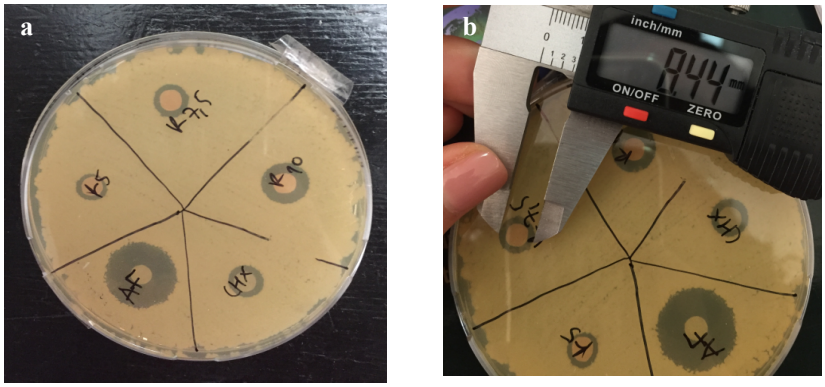

Figure 4.10 – Antifungal activity results. a) Inhibition zones; b) Measurement.

4.3.3.2. Biofilm Inhibition Assay

For biofilm experiments, only the experimental groups whose concentration of CHX was the lowest with antifungal activity against *C. albicans* were tested, i.e., 2.5% CHX-loaded Kooliner (2.5% CHX K), 5% CHX-loaded Ufi Gel Hard (5% CHX UGH), and 5% CHX-loaded Probase Cold (5% CHX PC).

A suspension of *C. albicans* was prepared in RPMI-1640 culture medium supplemented with glucose at 1% (w/V) adjusted to 1.0 McFarland units. In each well of the 24-well microtiter plates containing a previously fixed resin 5x1-mm disk-shaped resin specimen (Figure 4.9), the assayed volume was 1 mL with the final yeast concentration of 1×10^5 CFU/mL (Figure 4.11). After incubation, at 35±2°C for 72 h under static conditions, the specimens were fixed with different ethanol solutions, at 75%, 90%, and 100% (V/V), for 40 min, and the biofilm was observed by SEM analysis (JSM7001F JEOL Ltd., Tokyo, Japan) operated at 10kV. The specimens were coated with a thin layer of a conductive gold film under vacuum in an argon atmosphere (Quorum Technologies, Polaron E5100) to increase their conductivity.[519] Non-inoculated acrylic specimens were used as negative controls.

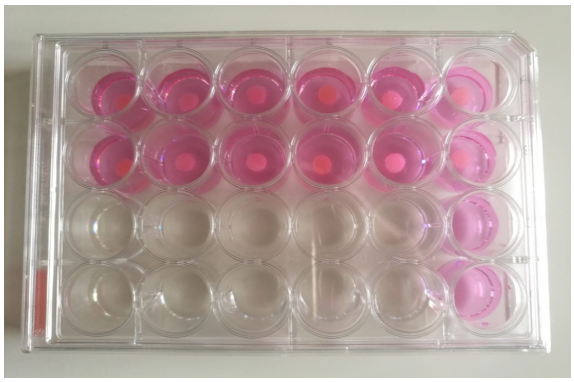

Figure 4.11 – Immersion of disk specimens in an adequate volume of *C. albicans* inoculum.

4.3.4. Mechanical Characterization

Specimens of control (0% CHX) and experimental (2.5% CHX K, 5% CHX UGH, and 5% CHX PC) groups (*n*=8) were prepared from rectangular-shaped stainless-steel molds (64×10×3.3 mm), as recommended by ISO 20795-1: 2013[520], resulting in 48 specimens.

For the specimens' preparation, the stainless-steel mold was placed on a glass plate covered by a polyester sheet. The materials were prepared following the manufacturers' instructions (Table 4.1) and placed into the mold. Another polyester sheet and glass plate were positioned on the top of the mold (Figure 4.12).

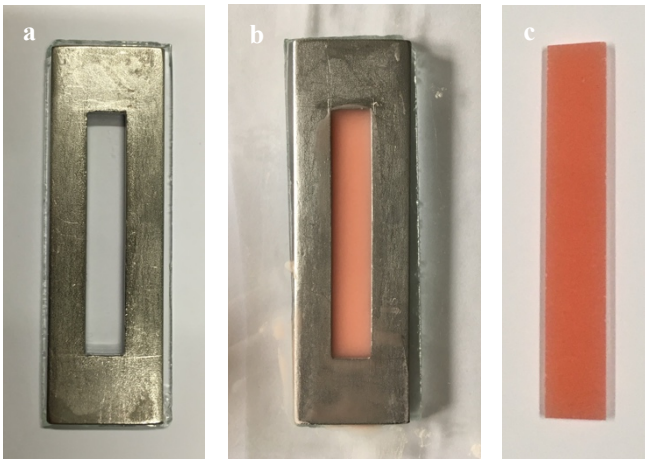

Figure 4.12 – a) Stainless-steel mold placed on glass plate covered by a polyester sheet; b) Mixture and mold between polyester sheets and glass plates; c) Example of a polymerized Probase Cold specimen.

4.3.4.1. Microhardness

The Knoop microhardness test was performed (Duramin, Struers DK 2750, Ballerup, Denmark) (Appendix 2, Figure 7) with a 98.12-mN load for 30 seconds. The operator used the Duramin software to measure the length of the pyramidal indentations immediately after each indentation within a maximum period of ten seconds (Figure 4.13). As there was a short time interval between indentation and measurement, it was assumed that the viscoelastic recovery was minimal. The mean of 12 equidistant measurements in each specimen was used as the reference value.

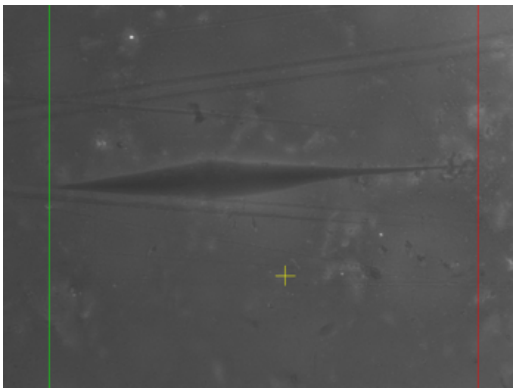

Figure 4.13 – Microscopic image of a Knoop indentation on a specimen of the 2.5% chlorhexidine-loaded Kooliner experimental group.

4.3.4.2. Flexural Strength

Immediately after microhardness testing, the same specimens were submitted to the three-point flexural test using a universal testing machine (Instron model 4502,

Instron Ltd, Bucks, England) (Appendix 2, Figure 8) with 1-kN load cell at a crosshead speed of 5 mm/min and a distance of 50 mm between rods.[520] Load was applied until failure (Figure 4.14), and the fracture load was recorded in Newtons (N). The flexural strength was calculated using the formula FS = 3WL/2bd^2, where *FS* is the flexural strength (MPa), *W* is the maximum load before fracture (N), *L* is the distance between rods (50 mm), *b* is the specimen's width (mm), and *d* is the specimen's thickness (mm).

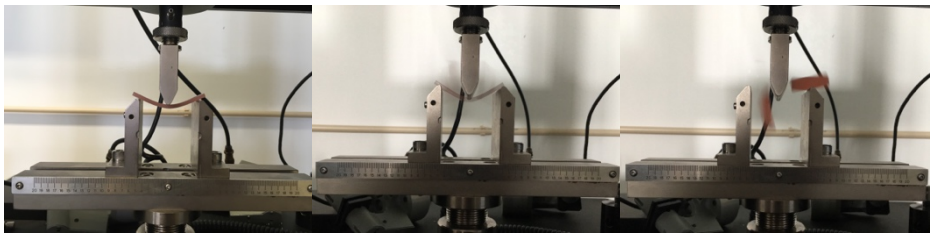

Figure 4.14 – Specimen submitted to the three-point loading flexural strength test in a universal machine.

4.3.5. Structural Characterization

Controls (0% CHX) and CHX-loaded specimens (2.5% CHX K, 5% CHX UGH, and 5% CHX PC) were prepared from cylinder-shaped stainless-steel molds (12x6 mm) to obtain qualitative data regarding the reline resins' morphological and chemical structure (Figure 4.15).

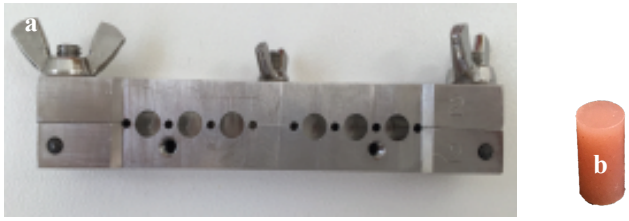

Figure 4.15 – a) Cylinder-shaped stainless-steel mold; b) Example of a polymerized Kooliner specimen.

4.3.5.1. 3D Morphological Structure

The 3D microarchitecture morphology was evaluated by morphometric analysis and 3D model images in a micro-CT equipment (SkyScan 1174; Bruker, Brussel, Belgium) (Figure 4.16). The specimen was mounted on a stub and scanned with the following parameters: 30.1-µm image pixel size; 50 kV; 800 mA; 5500-ms exposure time; 0.9º rotation step; and no aluminum filter. Data were reconstructed with the NRecon software (Bruker, microCT, Kontich, Belgium).

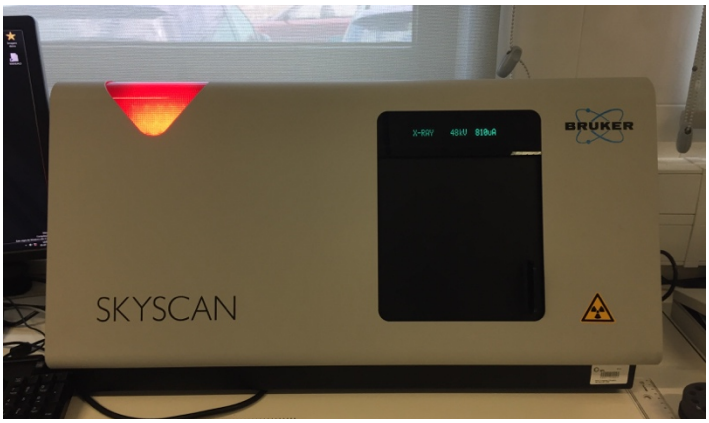

Figure 4.16 – Micro-CT device (SkyScan 1174; Bruker, Brussel, Belgium).

4.3.5.2. Chemical Structure

Fourier Transform Infrared with Attenuated Total Reflectance (FTIR-ATR) spectroscopy was used for the specimens' chemical analysis. FTIR-ATR was carried out using an Alpha-P spectrometer (Bruker, Brussel, Belgium) (Figure 4.17). The tests were performed at room temperature in a spectral range of 400-4000 cm^{-1} at a resolution of 2.00 cm^{-1}. The obtained spectra were averaged over 64 scans. After scanning, characteristic infrared absorption bands of organic functional groups were analyzed using the Opus software 6,5 version.

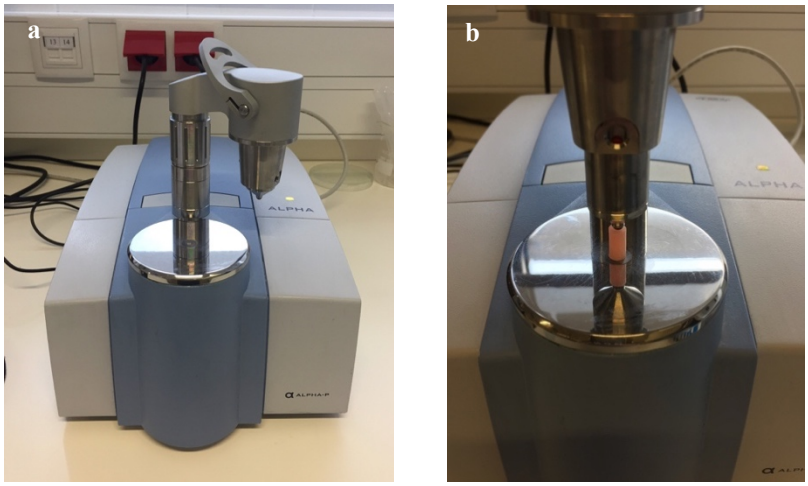

Figure 4.17 – a) Alpha-P spectrometer (Bruker, Brussel, Belgium); b) Specimen of reline acrylic resin in a spectrometer.

4.3.6. Surface Characterization

4.3.6.1. Surface Porosity

The open porosity at the surface of cylinder-shaped (12x6 mm) specimens of the control and experimental (2.5% CHX K, 5% CHX UGH, and 5% CHX PC) groups (n=5) was measured using the Archimedes' principle. Porosity (P) was calculated according to the following equation (1):

$$P\ (\%) = \frac{W_{sat} - W_{dry}}{W_{sat} - W_{sus}} \times 100 \qquad (1)$$

where W_{sat} is the weight of a specimen saturated with water, W_{dry} is the dry weight of a specimen, and W_{sus} is the weight of a specimen suspended in water.

4.3.6.2. Surface Free Energy

Specimens of each group (n=5) with 16×25×1 mm were obtained from rectangular-shaped metallic strips (125×25×1 mm) (Figure 4.18).

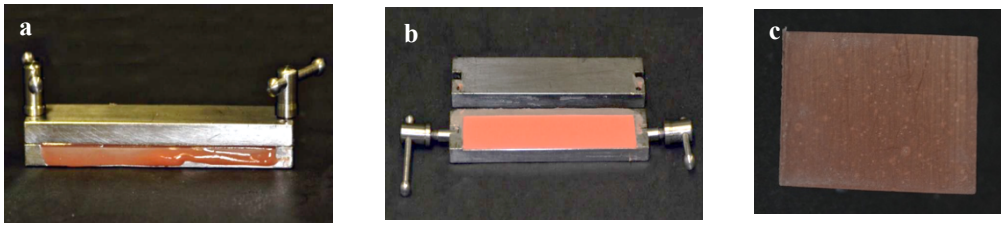

Figure 4.18 – a) Compression of the resin through rectangular-shaped metallic molds; b) After the cure is complete; c) Example of a polymerized Ufi Gel Hard specimen after cut with a cylindrical turbine drill.

With a digital micrometer of ±0.01-mm precision (Mitutoyo Digimatic, MFG.Co, Ltd. Tokyo, Japan) (Appendix 2, Figure 9), each specimen's height, width, and thickness were measured and introduced in the software.

Assays were made with Tensiometer K12 (Kruss, Hamburg, Germany) using the Wilhelmy plate method (Appendix 2, Figures 10 and 11) by immersing plates into water (Milli quality, Merck Millipore, Germany) and 1,2-propanediol (Merck, Germany) (Appendix 2, Figure 12), at a speed of 20 µm/s and 25±0.1ºC (Figure 4.19). To estimate the specimens' surface free energy (γ) and its dispersive (γ^d) and polar (γ^p) components, based on the harmonic mean method[521], advancing contact angles were used. Equations for surface free energy estimation were solved using the equation-handling

KRUSS software program: K121 contact angle measuring system (version 2.05) (Appendix 2, Figures 13, 14, and 15).

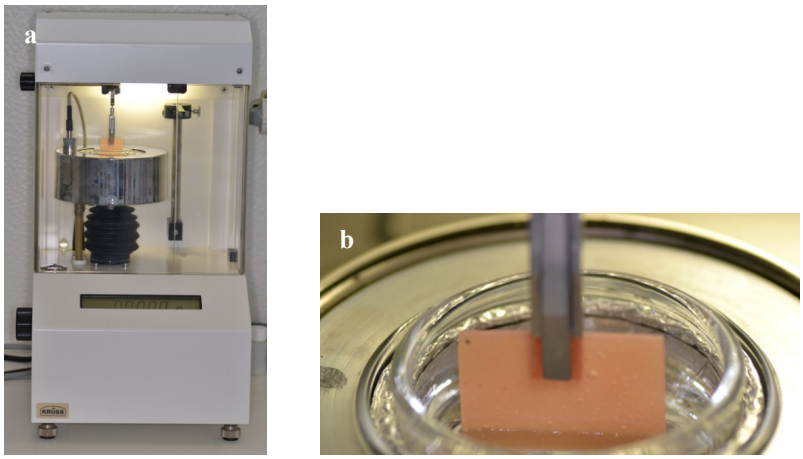

Figure 4.19 – a) Specimen of reline acrylic resin suspended in the equipment's scale**;** b) Specimen of reline acrylic resin immersed in the glass cuvette with distilled water.

4.3.6.3. Surface Morphology

The surface morphology of the 5x1-mm disk-shaped specimens from the control and experimental groups (2.5% CHX K, 5% CHX UGH, and 5% CHX PC) was examined by SEM using the Vega3 Tescan equipment (Tescan, Brno, Czechia). Before the examination, samples were coated with a gold/palladium (Au/Pd) thin film by sputtering using the sputter coater equipment (Quorum Technologies).

4.3.6.4. Shear Bond Strength

For the shear bond strength (SBS) test between the denture base and the reline resin, 60 cubic-shaped specimens (10×10×10 mm) of the heat-polymerizing denture-base acrylic resin Probase Hot (Ivoclar Vivadent, Liechtenstein) (Appendix 2, Figure 16) were produced by a conventional flasking technique, according to manufacturer's instructions (Appendix 5). Their sides were then grounded in a rotational grinding and polishing machine (DAP-U, Struers, Denmark) with 600-grit silicon carbide paper to remove irregularities (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL).

These specimens were submitted to 2 500 thermal cycles, alternating submersions of 20 seconds at 5ºC and 55ºC with a duel time of 5 seconds, on a thermocycling machine (Refri 200-E, Aralab, Cascais, Portugal) in order to simulate a 3-month aging process inside the oral cavity.[205]

Using the same grinding and polishing machine, the denture base specimens' surfaces were finished to a 3-mm thickness to simulate the preparation of the denture base to be relined. Their thickness was confirmed with a digital micrometer (Mitutoyo Digimatic, Mfg. Co., Ltd. Tokyo, Japan) with a ± 0.01-mm precision.

The denture base specimen was stabilized in a single-plane lap SBS device with gypsum type III (Figure 4.20).[522] Afterward, the relining procedure was performed. A perforated adhesive tape (Glossy White Film EA, Xerox) was positioned on the center of the denture base's surface, providing a customized and uniform bonding area (3 mm in diameter). The surface of the Probase Hot was previously conditioned according to the reline resin used. For Kooliner and Probase Cold, the bonding area was scrubbed once with a microbrush soaked with the corresponding monomer. In Ufi Gel Hard groups, a specific conditioner was applied to the area and left to dry for 30 seconds, as recommended by the manufacturer. Then, the other side of the single-plane lap SBS device was placed on the adhesive tape and filled with freshly mixed reline resin (Figure 4.21). The 20 specimens corresponding to each reline acrylic resin were randomly divided into control and experimental (2.5% CHX K, 5% CHX UGH, and 5% CHX PC) groups (*n*=10). Polymerization of the relining materials was carried out as described above (see section 4.1).

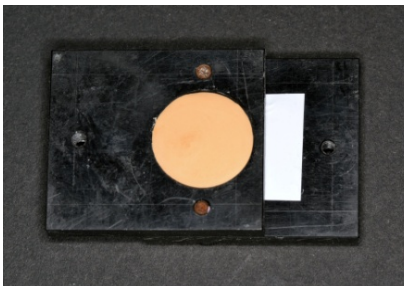

Figure 4.20 – Shear bond strength device with gypsum type III.

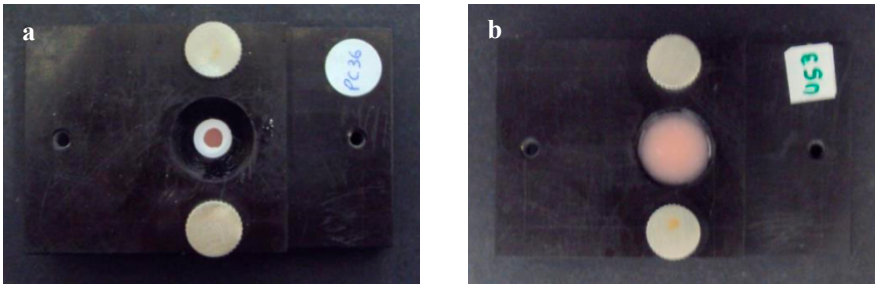

Figure 4.21 – a) Shear bond strength device with adhesive tape; b) Shear bond strength device filled with Ufi Gel Hard.

All specimens were then stored in distilled water at 37±2ºC for 24 h in an oven (Ehret, Mahlberg, Germany) (Appendix 2, Figure 2) before testing.

The SBS test was performed with a universal test machine (Instron model 4502, Instron Ltd, Bucks, England) with a 1-kN load cell and a crosshead speed of 1 mm/min until fracture (Figures 4.22 and 4.23). All tests were performed under uniform atmospheric conditions at room temperature.

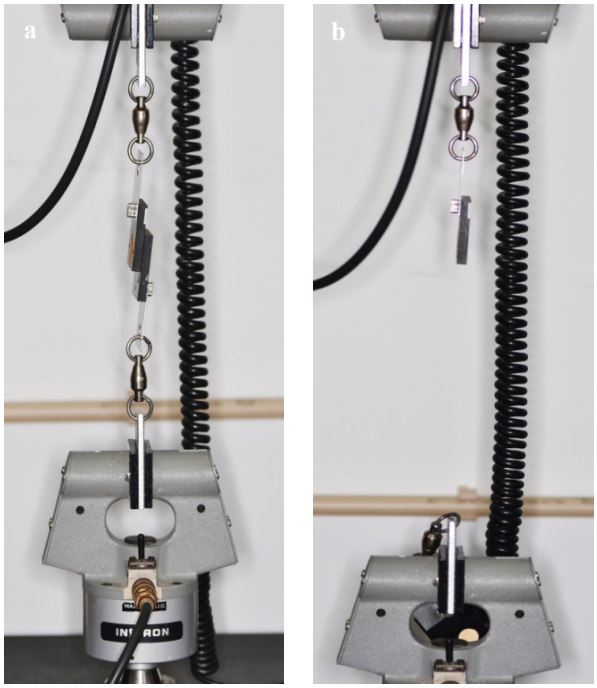

Figure 4.22 – One example of a specimen submitted to the shear bond strength test in a universal testing machine. a) Before test; b) After test.

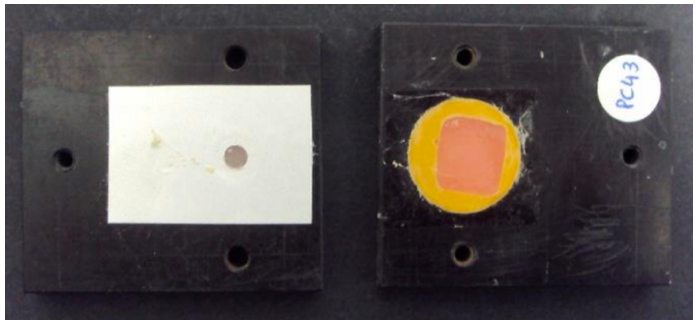

Figure 4.23 – Shear bond strength device after being submitted to the shear bond strength test.

Two calibrated observers assessed the failure mode with a stereomicroscope (EMZ-8TR, Meiji Techno Co, Saitama, Japan) (Appendix 2, Figure 17) and classified it as follows: adhesive if the failure occurred at the adhesive interface, between the reline resin and the denture base resin; cohesive when it occurred exclusively within one of the

resins; or mixed when it occurred in the interface of the two resins but included residues of reline resin (Figure 4.24).

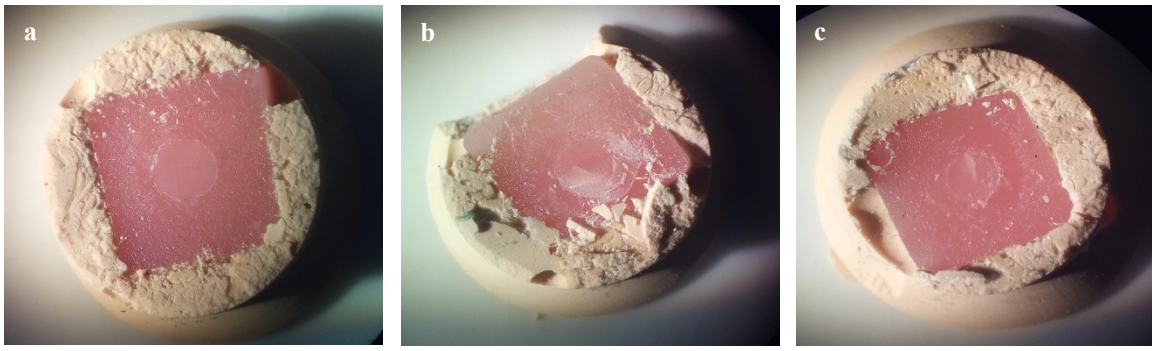

Figure 4.24 – Stereomicroscope's images of the three types of failures. a) Adhesive; b) Cohesive; c) Mixed.

4.3.7. *In-vitro* Drug Release Assay

In-vitro CHX release from cylinder-shaped (12×6 mm) specimens of the experimental (2.5% CHX K, 5% CHX UGH, and 5% CHX PC) groups (n=3) was assessed with each sample incubated in an adequate volume of artificial saliva prepared according to previous studies (Appendix 4) at pH=7, in a ratio of 1 g/5 mL, in a shaking water bath at 37°C for 28 days (Figure 4.25).[208,212,523] At predetermined intervals (1, 2, 4, 7, 24, 48, 72, 96, 168, 240, 360, 528, and 672 hours), aliquots of the supernatant were collected and analyzed in triplicate. The withdrawn aliquots were then replaced with equal volumes of fresh release solution to simulate the constant salivary renovation, and sink conditions were guaranteed during the whole study. CHX content was determined based on a linear calibration methodology by UV spectrophotometry (255 nm) using a microplate reader (FLUOstar Omega, BMGLabtech, Germany) (Figure 4.26) (Appendix 2, Figure 18).[213]

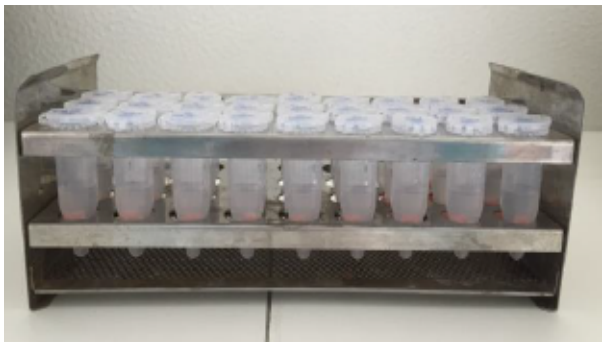

Figure 4.25 – Incubation of the specimens in graduated falcon tubes with artificial saliva at pH=7.

Figure 4.26 – Microplate reader (FLUOstar Omega, BMGLabtech, Germany).

4.3.8. Cytotoxicity

Extracts from disk-shaped (10×1 mm) specimens of the control (0% CHX) and experimental (2.5% CHX K, 5% CHX UGH, and 5% CHX PC) groups (*n*=2) were obtained by immersing the specimens at 37°C for 48 h in an adequate volume of distilled water in a ratio of 1 g/5 mL (Figure 4.27).(524)

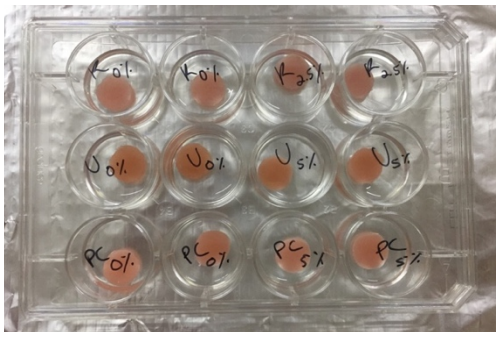

Figure 4.27 – Immersion of disk-shaped specimens in an adequate volume of distilled water.

The extracts' cytotoxicity was assessed using the general cell viability endpoint MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) on L929 (mouse fibroblast cell line, ATCC1 CCL-1TM) (Figures 4.28 and 4.29).(525)

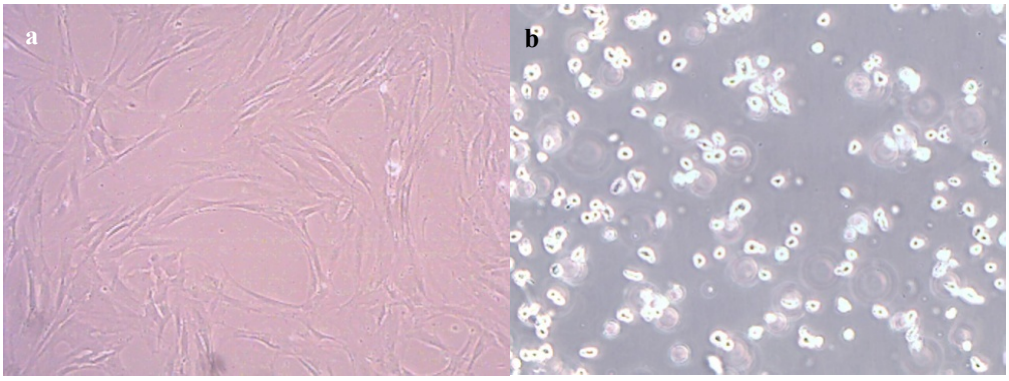

Figure 4.28 – Light microscopy (×100) of mouse fibroblasts cell line incubated at a) after 24 h; b) after trypsinization.

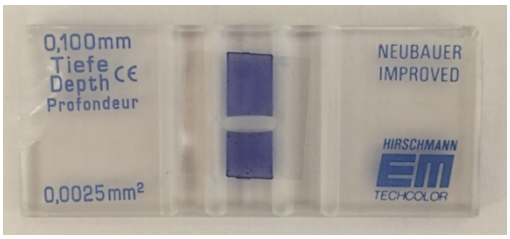

Figure 4.29 – Neubauer camera used to count the cells in a microscope.

In brief, cells were seeded on a 96-well plate (Greiner, Germany) in RPMI 1640 culture medium (Gibco, Thermo Fisher Scientific, USA), supplemented with 10% (V/V) fetal bovine serum, 100 units of penicillin G (sodium salt) (Invitrogen, UK), 100 mg of streptomycin sulfate (Invitrogen, UK), and 2 mM of L-glutamine (Invitrogen, UK), at a concentration that allowed cells to grow exponentially during the time of the assay. All samples to be tested and sodium dodecyl sulfate (SDS) (positive control) were diluted in the culture medium. After 24 h, the cell media was removed and replaced with fresh medium and the cell viability was assessed. In brief, the MTT dye solution was then added to each well (stock solution 5 mg/mL in 10 mM phosphate buffer solution at pH 7.4). After 3 h of incubation, the media was completely removed, and the intracellular formazan crystals were solubilized and extracted with 100-mL dimethylsulfoxide (DMSO) (Figure 4.30). After 15 min at room temperature, the absorbance was measured at 570 nm in the microplate reader (FLUOstar Omega, BMGLabtech, Germany) (Figure 4.20). The relative cell viability (%) compared to control cells was calculated by $\frac{[absorbance_{570nm}]\,sample}{[absorbance_{570nm}]control} \times 100\%$. Assays were performed in three independent experiments.

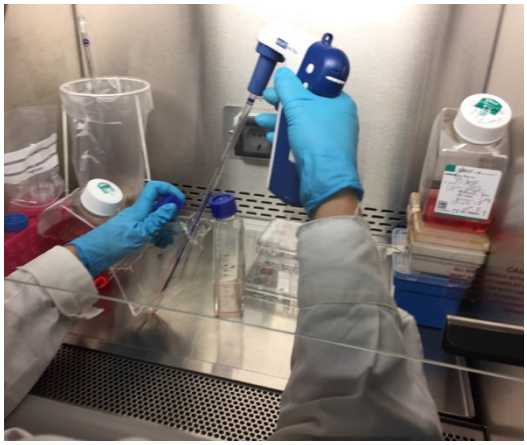

Figure 4.30 – Laminar flow cabinet that allowed working in a sterile environment.

4.3.9. Statistical Analysis

Whenever quantitative data were needed, the sample size (n) was estimated with a power analysis to provide statistical significance (α=0.05) at a power of 80%, based on data from a pilot study. Since normality and homogeneity of variance were not verified in scale variables (Shapiro-Wilk and Levene tests, p<0.05), data were submitted to Kruskal-Wallis (followed by Bonferroni corrections) and Mann-Whitney non-parametric tests. In all statistical tests, a 5% level of significance was considered (α=0.05). Failure mode data were analyzed with chi-square tests (α=0.05).

4.4. Results

4.4.1. MIC of CHX

The MIC values of CHX for *C. albicans* and *S. oralis* were 2 µg/mL (Figure 4.31).

Figure 4.31 – Result of the determination of the minimum inhibitory concentration for *C. albicans* ATCC® 10231TM.

4.4.2. Biomaterials' Antimicrobial Activity

Experimental groups with 1% CHX did not show agar inhibition zones against *C. albicans*. The Kooliner group presented an inhibition zone in specimens with 2.5% CHX, unlike the other two resins. All specimens loaded with 5% CHX showed an inhibition zone against *C. albicans* (Figure 4.32). Agar tests with *S. oralis* showed that all specimens loaded with CHX developed an inhibition zone (Figure 4.33).

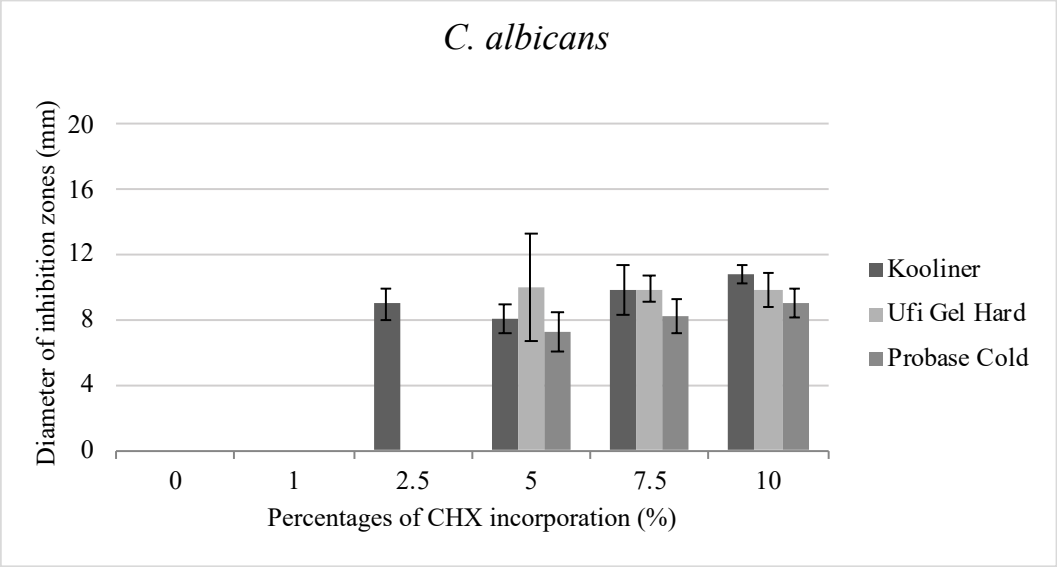

Figure 4.32 – Diameter of the inhibition zone (mm) of chlorhexidine-loaded specimens (*n*=3) from each group of the three reline acrylic resins, using *C. albicans* culture. Data are expressed as mean ± standard deviation of at least three independent experiments.

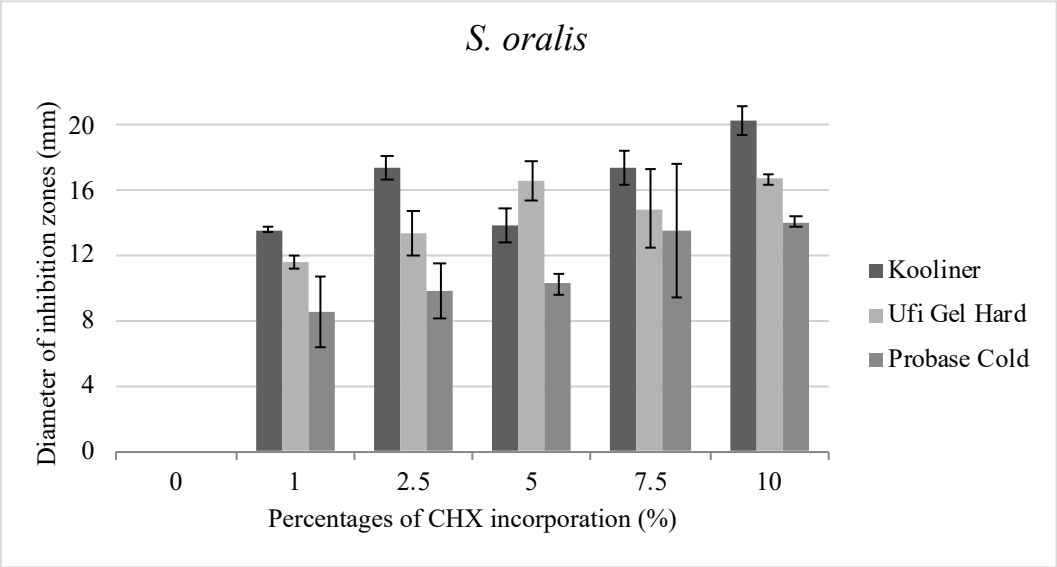

Figure 4.33 – Diameter of the inhibition zone (mm) of chlorhexidine-loaded specimens (*n*=3) from each group of the three reline acrylic resins, using *S. oralis* culture. Data are expressed as mean ± standard deviation of at least three independent experiments.

In the biofilm inhibition assay, the 2.5% CHX-loaded Kooliner and the 5% CHX-loaded Ufi Gel Hard specimens presented no antimicrobial activity since they were covered by a *C. albicans* biofilm similar to that of their control specimens (Figures 4.34 and 4.35).

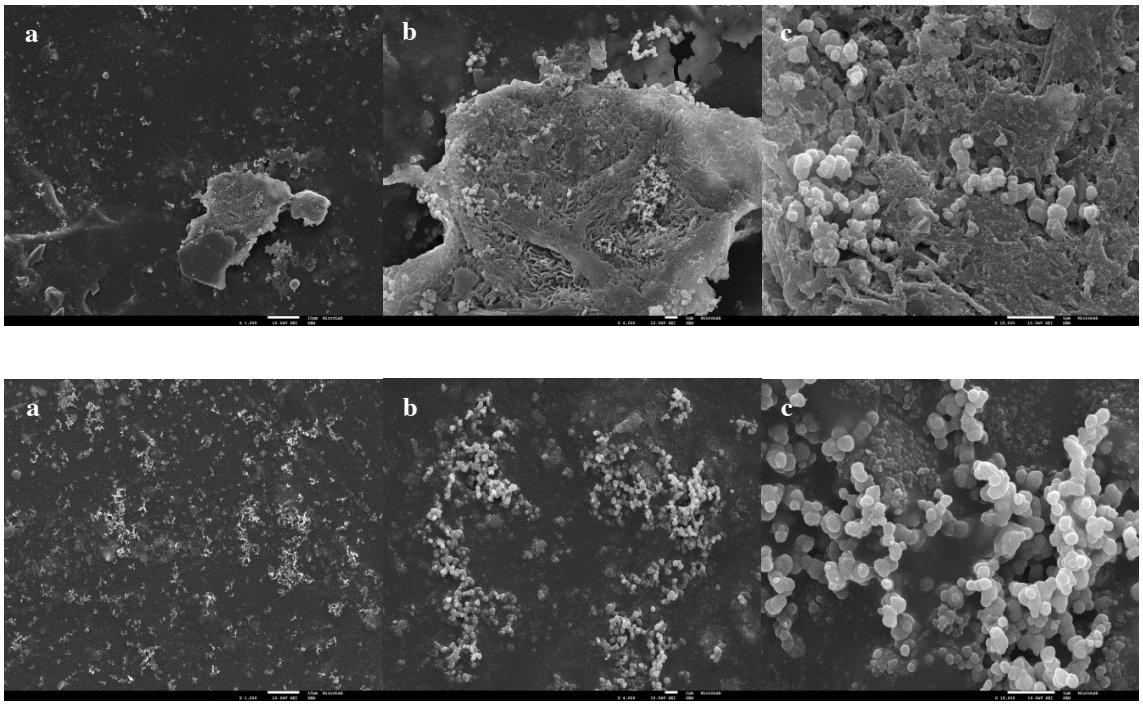

Figure 4.34 – Representative images by SEM of Kooliner's antimicrobial activity against *C. albicans* biofilm formation. Top row: Kooliner – control group; Low row: Kooliner – 2.5% chlorhexidine. Images with different magnification a) 1000x; b) 4000x; c) 15000x.

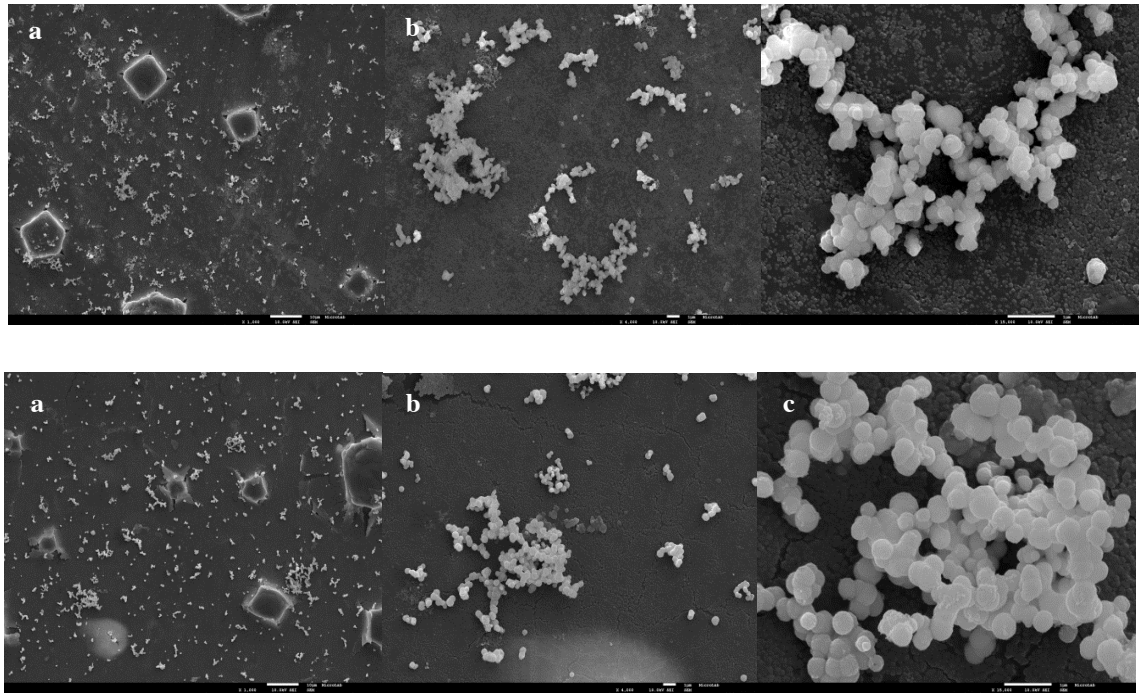

Figure 4.35 – Representative images by SEM of Ufi Gel Hard's antimicrobial activity against *C. albicans* biofilm formation. Top row: Ufi Gel Hard – control group; Low row: Ufi Gel Hard – 5% chlorhexidine. Images with different magnification a) 1000x; b) 4000x; c) 15000x.

However, the 5% CHX-loaded Probase Cold specimen, unlike the control specimen, showed a pronounced antimicrobial response, and no microorganism grew on the surface of the material (Figure 4.36).

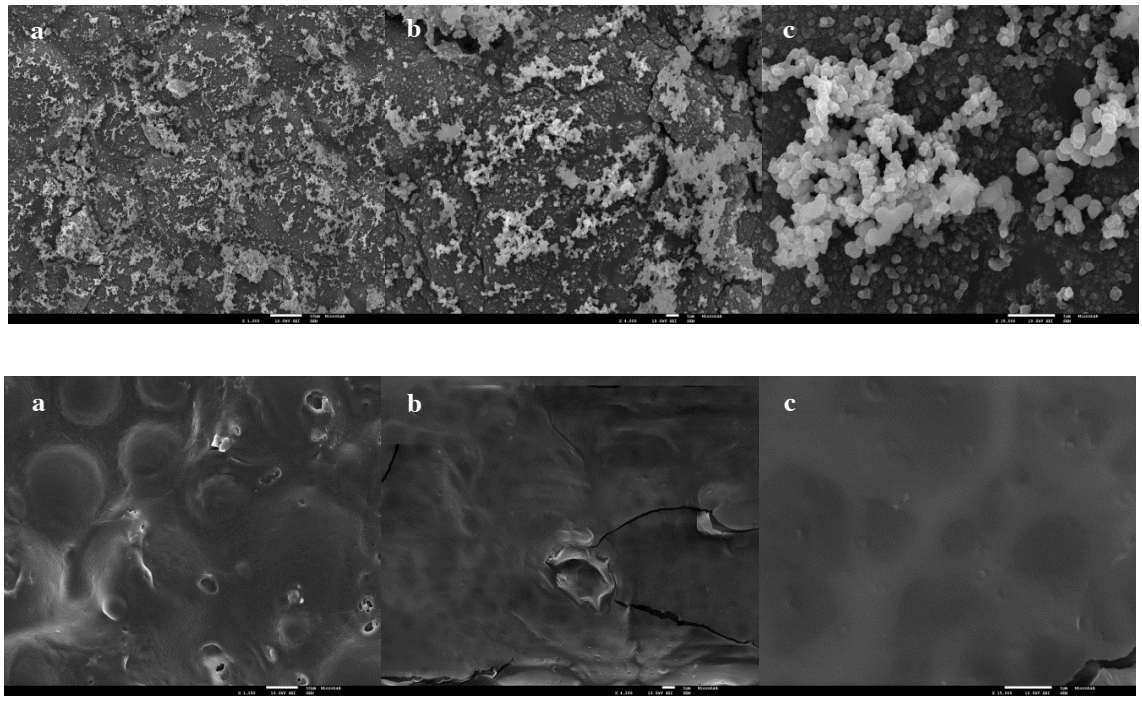

Figure 4.36 – Representative images by SEM of Probase Cold's antimicrobial activity against *C. albicans* biofilm formation. Top row: Probase Cold – control group; Low row: Probase Cold – 5% chlorhexidine. Images with different magnification a) 1000x; b) 4000x; c) 15000x.

4.4.3. Mechanical Characterization

Descriptive analysis of the data obtained was performed for each material. The mean, standard deviation, median, and interquartile range values of microhardness and flexural strength were determined (Table 4.2).

Table 4.2 – Microhardness and flexural strength data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*MICROHARDNESS (KHN)*		*FLEXURAL STRENGTH (MPa)*	
			M ± SD	*MED ± IQR*	*M ± SD*	*MED ± IQR*
Kooliner	**0%**	8	5.4 ± 0.38	5.6 ± 0.26	39.9 ± 5.44	42.1 ± 7.71
	2.5%	8	5.4 ± 0.72	5.1 ± 1.17	38.8 ± 4.88	40.6 ± 6.12
Ufi Gel Hard	**0%**	8	8.5 ± 0.48	8.5 ± 0.95	39.5 ± 6.00	37.0 ± 12.08
	5%	8	9.9 ± 0.93	9.9 ± 1.86	34.2 ± 5.75	33.9 ± 7.91
Probase Cold	**0%**	8	11.8 ± 0.54	11.8 ± 0.68	83.3 ± 12.05	79.2 ± 22.23
	5%	8	11.7 ± 0.35	11.7 ± 0.40	65.0 ± 7.68	62.5 ± 10.08

CHX = Chlorhexidine, M = Mean, SD = Standard deviation, MED = Median, IQR = Interquartile range.

The CHX incorporation did not significantly influence the microhardness of Kooliner (p=0.721) or Probase Cold (p=0.645). However, incorporating 5% CHX into Ufi Gel Hard led to significantly (p=0.003) higher microhardness values than the control (Figure 4.37).

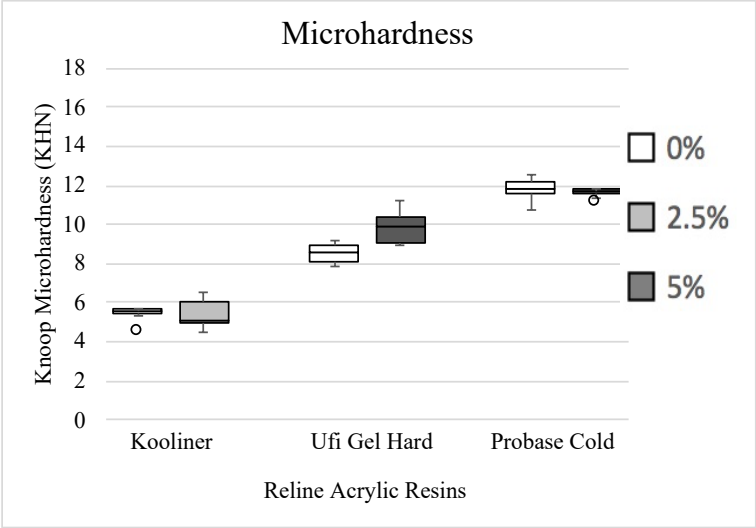

Figure 4.37 – Boxplot of the microhardness (KHN) distribution among experimental groups [Kooliner – 0% vs. 2.5% (p=0.721); Ufi Gel Hard – 0% vs. 5% (p=0.003); and Probase Cold – 0% vs. 5% (p=0.645)].

The flexural strength of the Kooliner (p=0.382) and Ufi Gel Hard (p=0.105) was not significantly affected by the CHX incorporation. However, the 5% CHX-loaded Probase Cold specimens showed significantly (p=0.005) lower flexural strength than the control group (Figure 4.38).

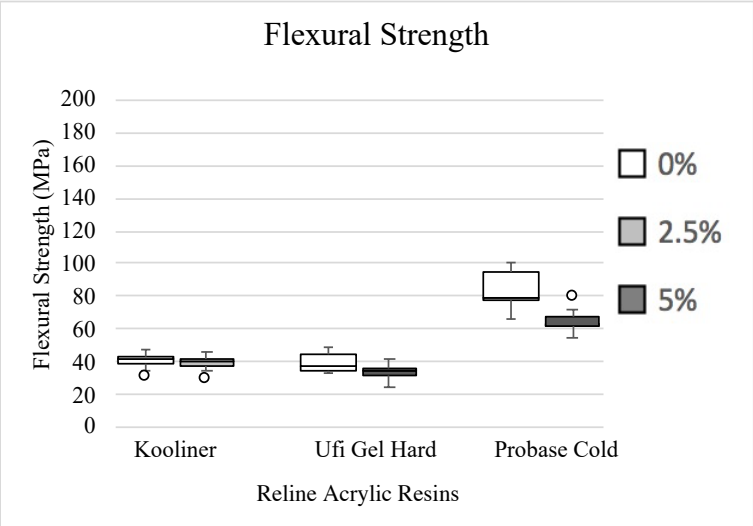

Figure 4.38 – Boxplot of the flexural strength (MPa) distribution among experimental groups [Kooliner – 0% vs. 2.5% (p=0.382); Ufi Gel Hard – 0% vs. 5% (p=0.105); and Probase Cold – 0% vs. 5% (p=0.005)].

4.4.4. Structural Characterization

4.4.4.1. 3D Morphological Structure

Selected 3D reconstruction models by micro-CT allowed a qualitative evaluation of the specimens' total porosity (Figure 4.39). Two images per material were evaluated, with the porosity colored in green and the reline acrylic resin structure in pink. The Ufi Gel Hard control specimen exhibited the most porous structure, and Probase Cold specimens did not show relevant porosity. Representative micro-CT images of CHX-loaded specimens of all materials presented a lower level of porosity than the micro-CT images of the control groups.

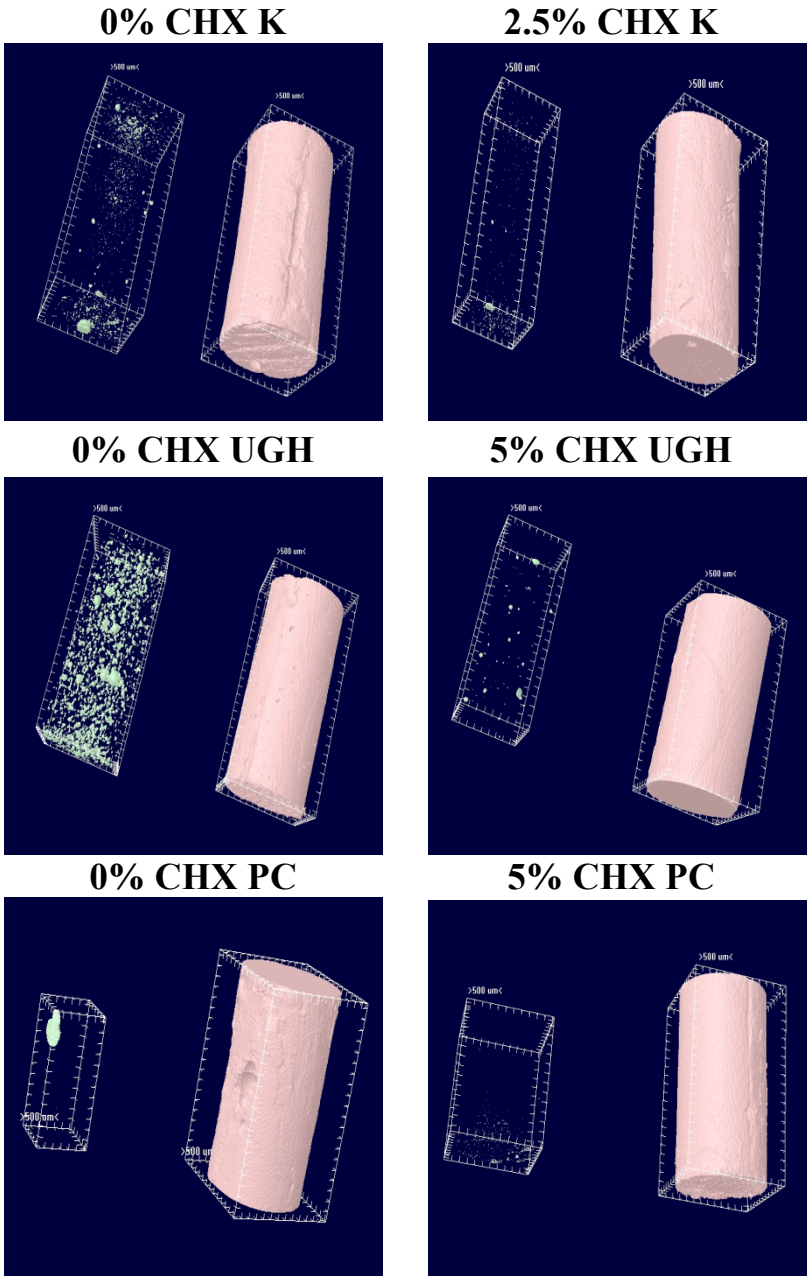

Figure 4.39 – Representative three-dimensional reconstruction models showing the porosity (%) of the reline acrylic resins specimens by micro-CT (CTAn and CTVol, Bruker softwares).
The green color indicates the presence of porosity.
[CHX – Chlorhexidine, K – Kooliner, UGH – Ufi Gel Hard, PC – Probase Cold].

4.4.4.2. Chemical Structure

FTIR-ATR spectra of the specimens and CHX diacetate were produced for chemical structure analysis and are exposed in Figures 4.40 until 4.44.

The CHX diacetate spectrum is characterized by two bands at 1260 and 1520 cm^{-1}, corresponding to the C-N and N-H bonds, respectively (Figure 4.40).

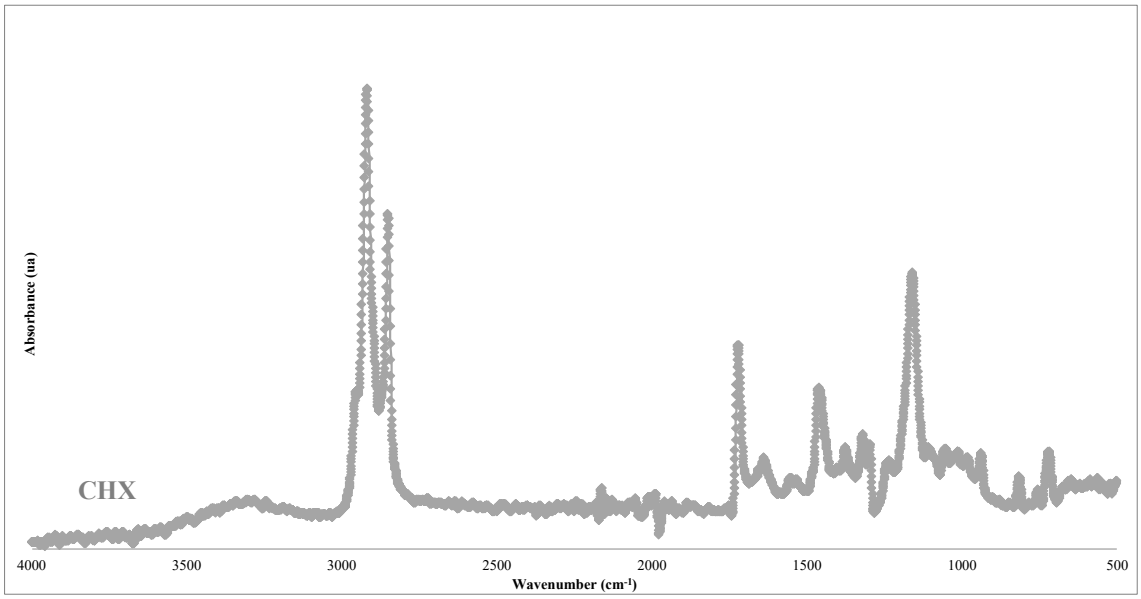

Figure 4.40 – FTIR-ATR spectrum of chlorhexidine diacetate.

The FTIR spectrum of the CHX-loaded Kooliner specimen maintained PEMA's characteristic bands observed in the control specimen and showed the CHX spectra. The presence of CHX was indicated by characteristic CHX bands, which were not present in the control specimen, such as C-N (at 1260 cm^{-1}) and N-H (at 1520 cm^{-1}) (Figure 4.41).

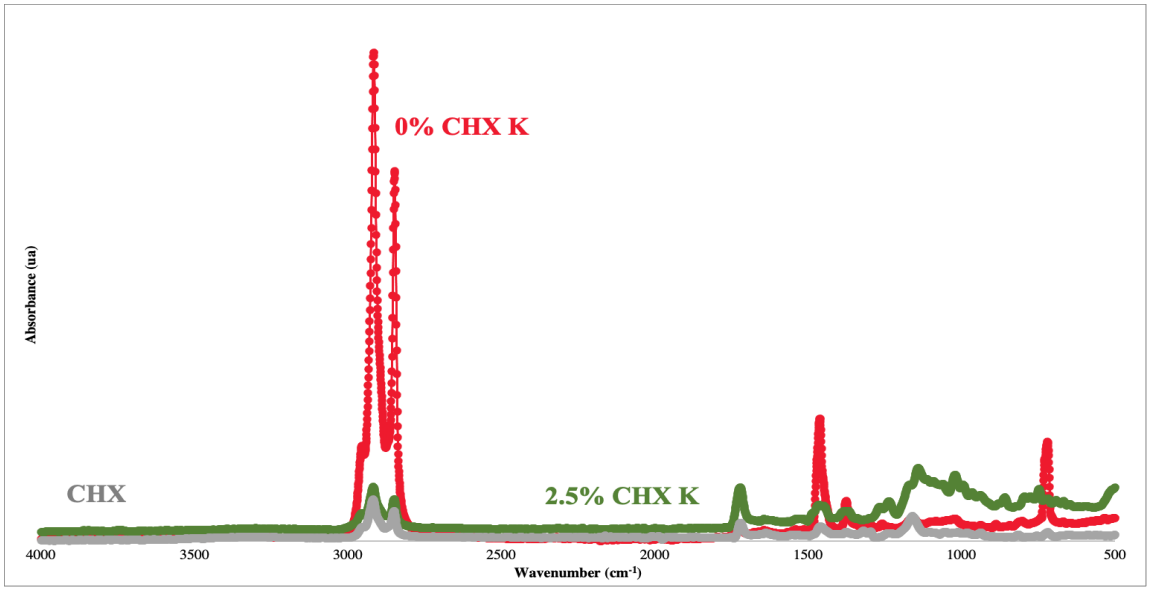

Figure 4.41 – FTIR-ATR spectra of Kooliner control and 2.5% CHX experimental groups [CHX – Chlorhexidine, K – Kooliner].

The control and CHX-loaded Ufi Gel Hard's spectra are almost identical, with bands in the same range of wavenumber. CHX-loaded Ufi Gel Hard spectrum showed no new bands, maintaining the CHX and Ufi Gel Hard bands (Figure 4.42).

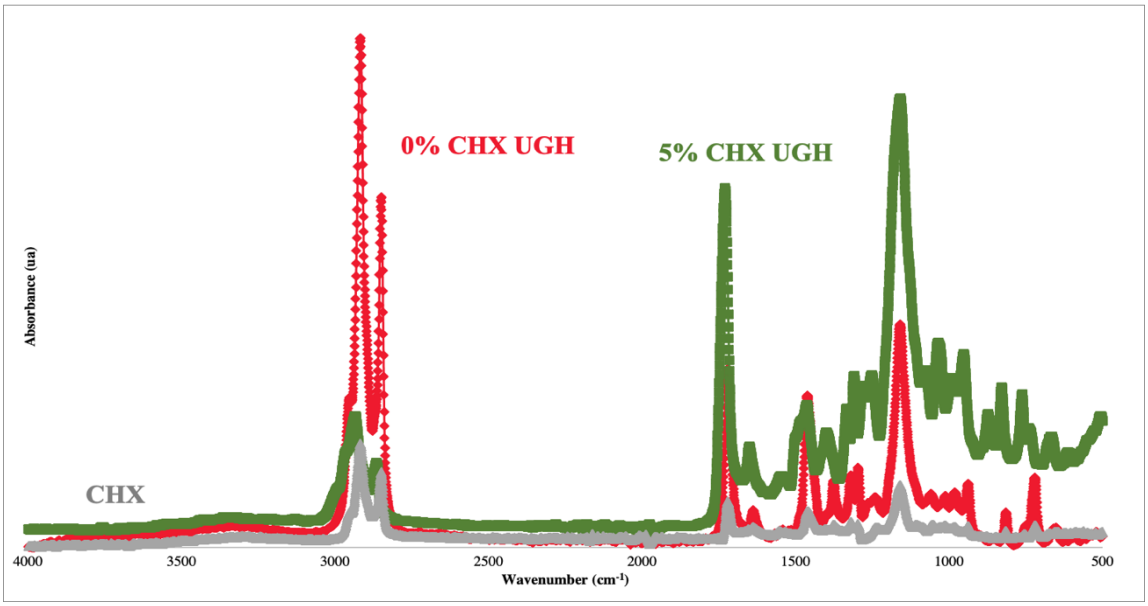

Figure 4.42 – FTIR-ATR spectra of Ufi Gel Hard control and 5% CHX experimental groups [CHX – Chlorhexidine, UGH – Ufi Gel Hard].

The CHX-loaded Probase Cold spectrum showed the two characteristic CHX bands (at 1260 and 1520 cm^{-1}) (Figure 4.43).

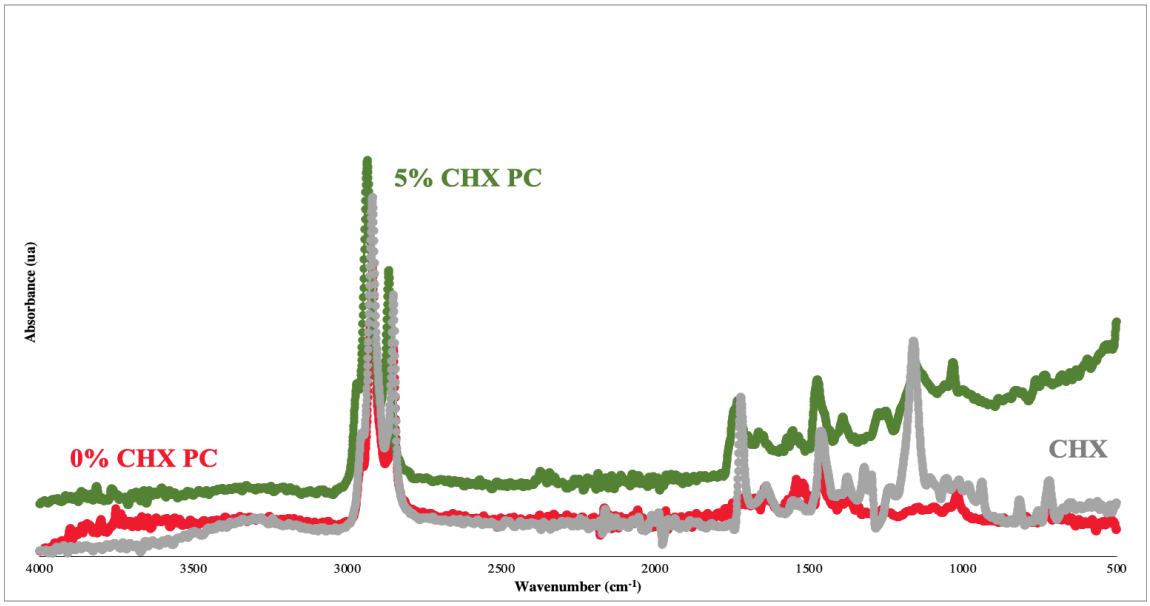

Figure 4.43 – FTIR-ATR spectra of Probase Cold control and 5% CHX experimental groups [CHX – Chlorhexidine, PC – Probase Cold].

4.4.5. Surface Characterization

4.4.5.1. Surface Porosity

The mean open porosity at the surface ranged between 1.8E-02 %, in the 5% CHX-loaded Ufi Gel Hard group, and 6.6E-01 %, in the 2.5% CHX-loaded Kooliner group (Table 4.3).

Table 4.3 – Descriptive analysis of the open porosity results at the specimen's surface (%) by the Archimedes' principle (n=5). Vertically identical superscripted letters denote no significant differences among groups (p>0.05).

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*POROSITY (%)*	
			M ± SD	*MED ± IQR*
Kooliner	**0%**	5	4.3E-02 ± 2.77E-02[a]	6.2E-02 ± 4.70E-02
	2.5%	5	6.6E-01 ± 9.92E-01[b]	2.5E-01 ± 1.25E00
Ufi Gel Hard	**0%**	5	3.7E-02 ± 3.31E-02[a]	3.1E-02 ± 4.60E-02
	5%	5	1.8E-02 ± 6.53E-02[a]	3.0E-02 ± 9.00E-02
Probase Cold	**0%**	5	1.2E-01 ± 8.87E-02[a]	1.5E-01 ± 1.63E-01
	5%	5	1.8E-02 ± 3.22E-02[a]	3.0E-02 ± 4.22E-02

CHX = Chlorhexidine, M = Mean, SD = Standard deviation, MED = Median, IQR = Interquartile range.

The CHX incorporation did not significantly change the porosity of Ufi Gel Hard (p=0.690) or Probase Cold (p=0.310). However, loading Kooliner with CHX resulted in significantly (p=0.008) higher porosity values compared to the control group (Table 4.3).

4.4.5.2. Surface Free Energy

Data descriptive analysis was carried out for each material, including mean, standard deviation, median, and interquartile range for contact angle (Appendix 1, Table 3). The mean, standard deviation, median and interquartile range values of total surface free energy (γ) and its dispersive (γ^d) and polar (γ^p) components were determined (Table 4.4).

Table 4.4 – Surface free energy data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*SURFACE FREE ENERGY (mN/m)*					
			γ		γ^d		γ^p	
			M ± SD	*MED ± IQR*	*M ± SD*	*MED ± IQR*	*M ± SD*	*MED ± IQR*
Kooliner	**0%**	5	24.5 ± 1.82	24.3 ± 2.85	14.5 ± 5.85	12.4 ± 9.70	10.1 ± 6.56	12.0 ± 11.85
	2.5%	5	27.9 ± 3.83	29.9 ± 5.65	19.6 ± 1.16	19.0 ± 1.80	10.3 ± 1.90	11.1 ± 3.60
Ufi Gel Hard	**0%**	5	32.4 ± 1.69	32.5 ± 2.70	22.8 ± 1.54	23.2 ± 2.80	9.5 ± 2.12	9.1 ± 4.10
	5%	5	36.2 ± 1.73	35.6 ± 3.10	22.2 ± 2.26	23.1 ± 4.40	14.0 ± 3.83	11.8 ± 7.15
Probase Cold	**0%**	5	24.6 ± 1.79	24.4 ± 3.05	14.6 ± 1.21	14.4 ± 2.00	10.0 ± 2.54	10.0 ± 4.65
	5%	5	30.8 ± 1.20	30.9 ± 2.05	16.0 ± 1.30	15.7 ± 2.25	14.9 ± 2.18	14.3 ± 3.75

CHX = Chlorhexidine, M = Mean, SD = Standard deviation, MED = Median, IQR = Interquartile range.

The total surface free energy of experimental Kooliner specimens was not significantly (p=0.151) different from the control specimens. However, CHX-loaded Ufi Gel Hard and Probase Cold showed a significantly (p=0.008) higher total surface free energy values than the control groups (Figure 4.44).

No differences were found (p>0.05) between the dispersive and polar components in Ufi Gel Hard. However, in Probase Cold, the CHX-loaded group presented significantly (p=0.032) higher values in the polar component of surface free energy than the control group, but no significant (p>0.05) differences were found in the dispersive component.

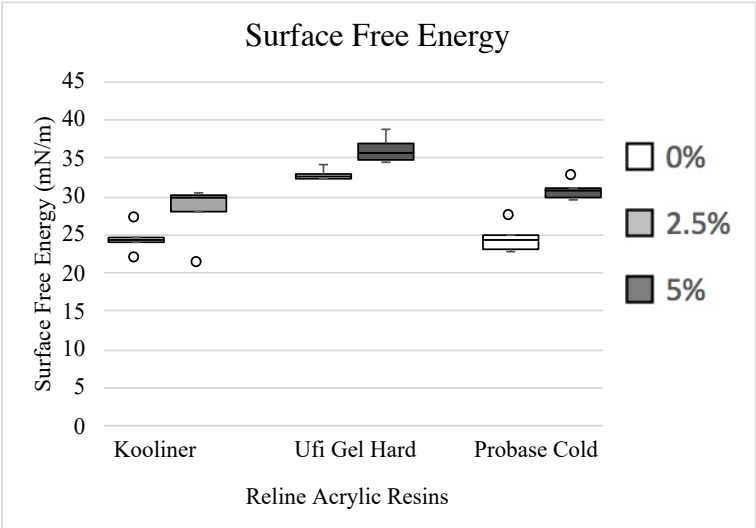

Figure 4.44 – Boxplot of the surface free energy total (mN/m) distribution among experimental groups [Kooliner – 0% vs. 2.5% (p=0.151); Ufi Gel Hard – 0% vs. 5% (p=0.008); and Probase Cold – 0% vs. 5% (p=0.008)].

4.4.5.3. Surface Morphology

Probase Cold presented a more spherical nature than the other two reline resins. Specimens loaded with CHX were similar to the control in Kooliner and Ufi Gel Hard. However, loading Probase Cold with CHX produced bigger spaces between smaller and more irregular spherical particles (Figure 4.45).

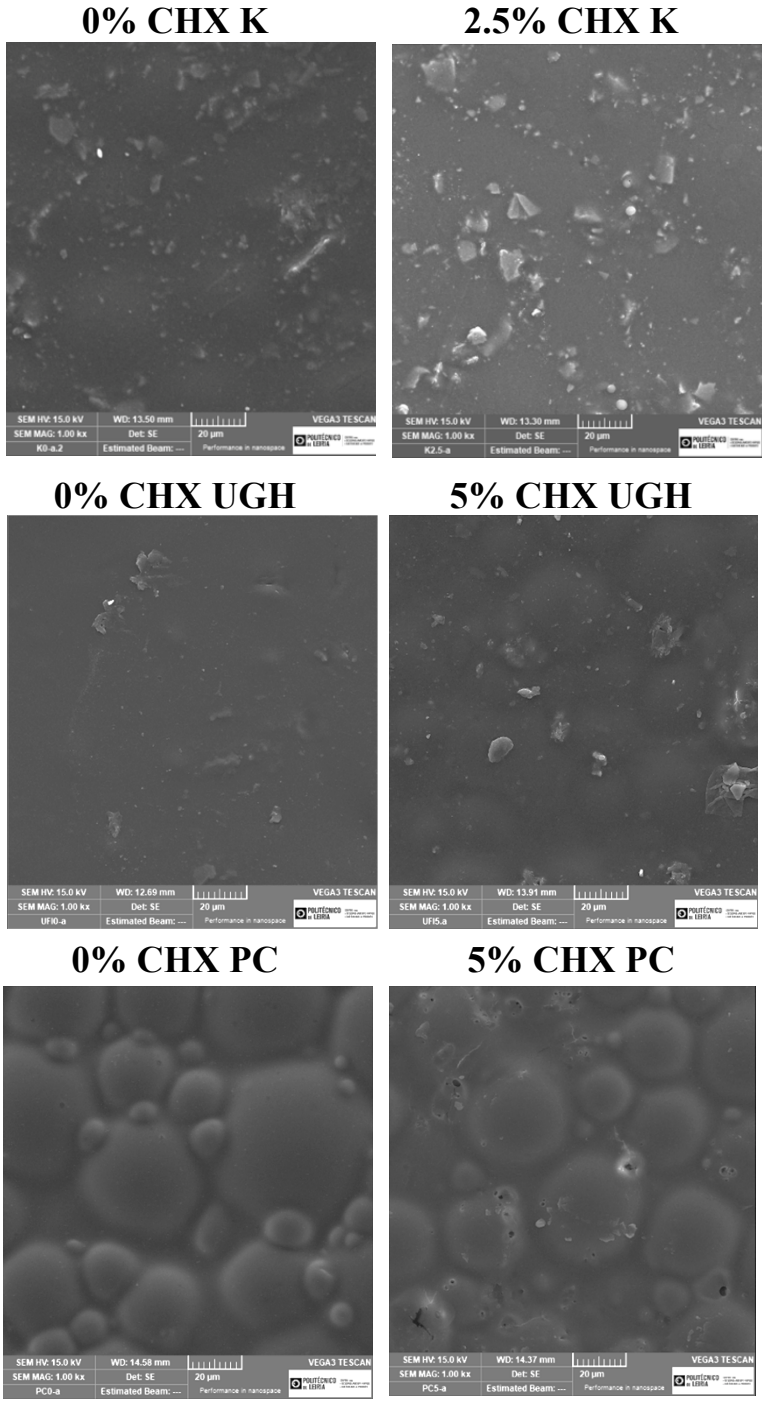

Figure 4.45 – Representative images by SEM of the reline acrylic resin without and with CHX at one 1000x magnifications assay [CHX – Chlorhexidine, K – Kooliner, UGH – Ufi Gel Hard, PC – Probase Cold].

4.4.5.4. Shear Bond Strength

Descriptive analysis of the obtained data was performed for each material. The mean, standard deviation, median, and interquartile range values of SBS were determined (Table 4.5). The SBS mean values ranged between 40.2 MPa, in Probase Cold without CHX, and 13.1 MPa, in Kooliner without CHX (Table 4.5).

Table 4.5 – Shear bond strength data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*SHEAR BOND STRENGTH (MPa)*	
			M ± SD	*MED ± IQR*
Kooliner	**0%**	10	13.1 ± 3.44	12.7 ± 4.10
	2.5%	10	18.8 ± 4.40	17.2 ± 4.67
Ufi Gel Hard	**0%**	10	24.5 ± 1.94	24.2 ± 2.42
	5%	10	28.1 ± 2.46	28.7 ± 3.47
Probase Cold	**0%**	10	40.2 ± 2.52	40.8 ± 1.76
	5%	10	23.5 ± 3.90	25.7 ± 5.91

CHX = Chlorhexidine, M = Mean, SD = Standard deviation, MED = Median, IQR = Interquartile range.

Statistical differences (p<0.001) were found between resins. Probase Cold showed a higher (p<0.001) SBS than the other two resins. Also, Ufi Gel Hard showed higher (p<0.001) SBS to denture base than Kooliner.

CHX-loaded Kooliner (p=0.001) and Ufi Gel Hard (p=0.004) showed a higher SBS than the control groups. However, CHX-loaded Probase Cold resulted in lower (p<0.001) bond strength to the denture base than the control group (Figure 4.46).

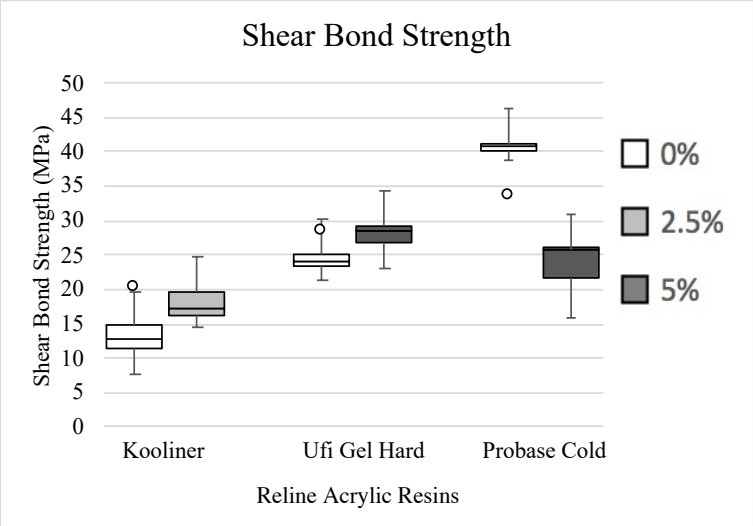

Figure 4.46 – Boxplot of the shear bond strength (MPa) distribution among experimental groups [Kooliner – 0% vs. 2.5% (p=0.001); Ufi Gel Hard – 0% vs. 5% (p=0.004); and Probase Cold – 0% vs. 5% (p<0.001)].

The percentages of failure inside each group of the three reline acrylic resins are stated in Table 4.6. The failure mode was predominantly adhesive (60%). In Ufi Gel Hard, the CHX incorporation affected the failure mode (p<0.001). One the other hand, in Probase Cold, the failure mode was not affected by CHX incorporation (p=0.350).

Table 4.6 – Percentage of failure data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*Failure mode n (%)*		
			Adhesive	*Mixed*	*Cohesive*
Kooliner	**0%**	10	10 (100%)	-	-
	2.5%	10	10 (100%)	-	-
Ufi Gel Hard	**0%**	10	9 (90%)	1 (1%)	-
	5%	10	-	10 (100%)	-
Probase Cold	**0%**	10	2 (20%)	8 (80%)	-
	5%	10	5 (50%)	5 (50%)	-

CHX = Chlorhexidine.

4.4.6. *In-vitro* Drug Release Assay

The results of CHX release from the different materials are shown in Figure 4.47. For all the evaluated reline resins, the greatest CHX release occurred within the first 24-48 h of incubation. This high initial release rate was followed by a slower and steadier release during the entire study period of 28 days.

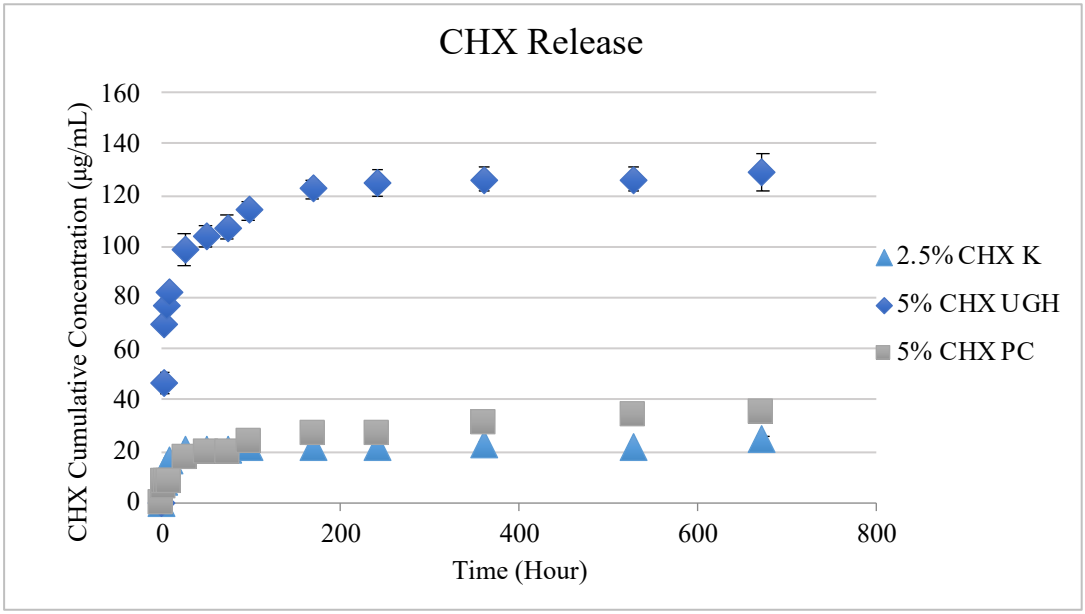

Figure 4.47 – Cumulative chlorhexidine (µg/mL) release profile for 28 days. The data was obtained by UV spectrophotometry at 255 nm. Data are expressed as mean ± standard deviation (*n*=3).

Data analysis comparing the three materials showed that the release of CHX by Ufi Gel Hard was significantly ($p<0.001$) higher than Kooliner's and Probase Cold's, with no significant differences between the last two ($p>0.05$). The maximum cumulative release (% *w/w*) from the three loaded materials after the 28 days was low for all resins. The results showed that Ufi Gel Hard had the highest CHX release (1.3 ± 0.56), followed by Kooliner (0.5 ± 0.14) and Probase Cold (0.4 ± 0.16) (Table 4.7).

Table 4.7 – Chlorhexidine release (µg/mL) at 48 h and 672 h and maximum cumulative release of chlorhexidine (% w/w) (mean ± standard deviation) by chlorhexidine-loaded Kooliner, Ufi Gel Hard, and Probase Cold (*n*=3).

RELINE ACRYLIC RESIN	*CHX LOADING*	*CHX RELEASE (µg/mL)*		*MAXIMUM CUMULATIVE RELEASE (% w/w)*
		48h	*672h*	
		M ± SD	*M ± SD*	*M ± SD*
Kooliner	**2.5%**	20.5 ± 3.87	25.2 ± 6.79	0.5 ± 0.14
Ufi Gel Hard	**5%**	103.9 ± 52.27	129.0 ± 55.52	1.3 ± 0.56
Probase Cold	**5%**	19.6 ± 4.95	35.5 ± 8.13	0.4 ± 0.16

CHX = Chlorhexidine, M = Mean, SD = Standard deviation.

4.4.7. Cytotoxicity

Cell viability was determined by the ability of cells to metabolically reduce MTT to a formazan dye. CHX-loaded Probase Cold was the least cytotoxic loaded resin (70.6 ± 6.17%), and CHX-loaded Ufi Gel Hard the most cytotoxic (16.6 ± 5.24%). The incorporation of the drug decreased cell viability in the three tested resins (p<0.001). (Figure 4.48).

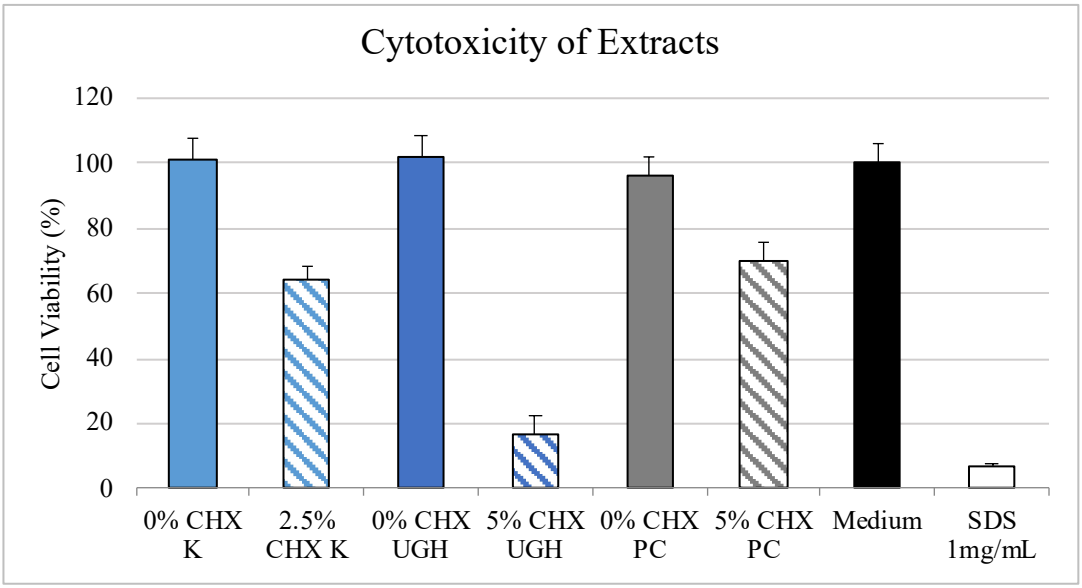

Figure 4.48 – Cell viability MTT assays of L929 mouse fibroblast cells exposed to Kooliner (0% CHX K), 2.5% chlorhexidine-loaded Kooliner (2.5 CHX K), Ufi Gel Hard (0% CHX UGH), 5% CHX-loaded Ufi Gel Hard (5% CHX UGH), Probase Cold (0% CHX PC), and 5% CHX-looaded Probase Cold (5% CHX PC) extracts, culture medium as the negative control, and sodium dodecyl sulfate (SDS) as the positive control. Data are expressed as mean ± standard deviation of at least three independent experiments.

4.5. Discussion

The present study investigated the creation of a drug-releasing device for denture stomatitis prevention and treatment by incorporating CHX in reline acrylic resins. Loading acrylic resins (soft and hard) with 10% CHX has an inhibitory effect on *C. albicans*.[17,150,153] In the clinical context, a continuous CHX release into the surrounding fluid media helps saturate the salivary film that bathes the tissue surface of a denture base.[153] This interesting finding should encourage the use of antimicrobial drugs in low concentrations, maintaining substantial antifungal potential but reducing the chance of developing local side effects (such as allergic reaction).

No studies evaluating the addition of a minor CHX concentration into long-term reline resins to prevent or treat denture stomatitis were found in the literature. In our study, different parameters of CHX-loaded acrylic resins were tested and monitored, including their mechanical, structural, surface, drug release, and biological characterization and its potential to be used in the context of *C. albicans*-associated denture stomatitis.

Salim *et al.* (2013)[163] reported 5.03 mg/L as the MIC of CHX after 48 hours. However, they used 32 different isolated specimens of *C. albicans*. Since there are no publications in the literature showing the MIC value for the selected strains for these microbiological studies, this test was conducted in our study. The MIC of the CHX for *C. albicans* ATCC® 10231TM and *S. oralis* ATCC® 3507 was 2 µg/mL.

Different methods have been used to assess the antimicrobial properties of dental materials.[457] Since CHX is soluble in the culture mediums used, the agar diffusion test could be performed. This test is not quantitative and does not distinguish between bacteriostatic and bactericidal effects, but it is a simple test and is the most commonly used for assessment of materials.[25,526]

Our microbiological tests' results showed that incorporation CHX influences the antimicrobial activity against *C. albicans* and *S.oralis*. The etiology of denture stomatitis is multifactorial, but the presence of a biofilm with *Candida* species (mainly *C. albicans*) on the surface of the denture is considered the mechanism that precipitates the disease.[22,277] *S. oralis* was included in this study because it is believed to have an important role in the formation of the complex biofilm that colonizes the denture-baring

mucosa.[22] Antibacterial approaches should be adopted in addition to the antifungal strategies to effectively eliminate biofilms and thus control stomatitis.[527]

In the present study, the minimal concentration of CHX that promoted antimicrobial activity in the agar assay was 2.5% for Kooliner and 5% for Ufi Gel Hard and Probase Cold. These results differed from those obtained by Bertolini *et al.* (2014)[21], who reported inhibition zones against *C. albicans* with the incorporation of 1% CHX. These disparities might be due to the weak interchain structure of short-term liners loaded with CHX used by those authors[21] that promoted the CHX release and, consequently, the formation of important inhibition zones in the agar.

Along with the characteristics that promote the drug release, their structure is also importance for the biofilm formation on the material's surface. Our SEM images of antimicrobial activity against *C. albicans* biofilm formation revealed that only CHX-loaded Probase Cold inhibited microorganism growth on the material's surface. This finding can be explained by the indirect resin's lower porosity, as confirmed in our micro-CT images, since microorganisms' adhesion is enhanced in porous surfaces.[528] Our SEM antibiofilm results seems to disagree with agar assays, which might be due to methodology differences. The agar disk diffusion test is a simple method that relies on the solubility of the drug from the material in the medium, unlike the biofilm inhibition test, which measures the microorganism growth in direct contact with the material.[457] However, the treatment success depends not only on methods that eliminate or reduce the microorganisms from the material's surface but also on the biofilms in the infected mucosa, which are more susceptible to the CHX released from the material to the medium.[377] Therefore, the study's first hypothesis is rejected since adding CHX produces antimicrobial action against relevant fungal (*C. albicans*) or bacterial (*S. oralis*) strains.

Having established the minimal antimicrobial concentrations of CHX required to load the reline acrylic resins, it was essential to determine whether the addition of CHX influenced the properties of the biomaterial. Some studies reported that loading composite resins with CHX caused detrimental mechanical and physical properties, including decreased strength, increased surface porosity, and water sorption.[59,213,529,530] The Knoop microhardness test is used in acrylic resins because it minimizes the elastic recovery of these biomaterials due to the Knoop indenter's geometry.[402,531] Flexural strength simulates the mastication forces and gives good information about the clinical performance of the reline resin.[300,402,412,436] CHX incorporation into Ufi Gel Hard

increased microhardness values. In the other two reline resins, Kooliner and Probase Cold, CHX incorporation did not influence the results. However, CHX incorporation in Probase Cold specimens led to decreased flexural strength, as found in other studies using other antimicrobial agents.[300,514,532-536] Thus, it was concluded that both null hypotheses could be rejected.

The incorporation of CHX changes intermolecular distances among polymers chains after polymerization, which can reduce flexural strength values.[514] Since CHX is incorporated in a powder formed by PMMA pre-polymerized particles, adding the CHX molecules can hamper the polymerization process by preventing interparticle chains. This reasoning was corroborated by our SEM surface morphology study that showed a higher interparticle distance in the CHX-loaded Probase Cold than in the control group. The polymerization process change also results in a higher amount of residual monomer, which will have adverse consequences on the resins' mechanical properties due to having a plasticizing effect.[533,537]

Even though our results showed decreased flexural strengths when CHX was added to Probase Cold specimens, the values obtained were still above the limit of 60 MPa defined by ISO requirements for an adequate function of the material.[520]

The micro-CT analysis is used in different dentistry applications to observe and understand innovative biomaterials with detailed 3D information about the size and location of open and closed pores.[538-541] With this technique, one sample can be analyzed many times, as it does not need any cutting or treatment.[542] Many articles report this methodology in endodontics and restorative dentistry investigations[542-544], but its use with reline acrylic resins is new. Although there were some differences between the resins tested, with Ufi Gel Hard exhibiting the most porous structure and Probase Cold showing almost no porosity, micro-CT images allowed us to verify that CHX loading decreased the total porosity of all resins. It seems that CHX particles fill the resin's pores, which can be explained by chemical analysis. FTIR-ATR revealed that adding CHX did not change the polymer's main absorption bands, suggesting that CHX did not chemically bond to the polymer chains and remained apart from the chemical structure of the PMMA.

In the present study, the surface porosity of reline resins was also investigated by a classic method based on the volume of water absorbed by the material. This method presented lower porosity values than the micro-CT morphometric evaluation of total porosity because it is limited to evaluating the open pores at the biomaterial's surface.

CHX incorporation did not influence the resins' surface porosity, except for Kooliner, so the fourth null hypothesis is rejected. This result can be explained by the differences in these resins' chemical composition and structural arrangement. Kooliner has a simple polymer structure that is less complex than the crosslinking net formed in Ufi Gel Hard, and Probase Cold, in turn, is an indirect reline resin with a higher level of monomer-polymer conversion and less distance between particles.[487,545]

Loading Ufi Gel Hard and Probase Cold with CHX increased the total surface free energy, so the fifth hypothesis null is rejected. Although the total surface free energy differed among Ufi Gel Hard groups, the absence of differences in the dispersive and polar components means that the two components reached a balance. In Probase Cold, CHX loading increased the polar component, but the dispersive component suffered no significant changes, which means that incorporating CHX slightly increased the Probase Cold's tendency to become polar and, consequently, more hydrophilic.

Increasing the acrylic resins' surface free energy will directly improve their surface wettability, affecting their clinical performance. The retention and stability of removable dentures are improved because high surface free energy provides the necessary conditions for saliva to spread more easily over the denture surfaces. The wettability's effect on the adherence of microorganisms such as *Candida* is not consensual. Studies with denture base materials have shown a relationship between the cell numbers of *Candida* species adhering per unit area and the contact angle measurement. Some of these studies postulate that the more hydrophobic the surface, the less the cell adherence.[537,546] One the other hand, a greater adherence of *Candida* species in hydrophobic surfaces has been suggested.[153]

The three reline acrylic resins studied are not used independently in the oral cavity since they are associated with a denture base material. Thus, the bond strength of this novel CHX delivery system to the denture base was investigated. The SBS test has been widely used in acrylic resins because it represents a shear load directly to the interface between reline resin and denture base, considered more accurate regarding what happens in the oral cavity.[497,547-551]

In the present study, differences were found between the three reline resins' SBS, so the sixth null hypothesis is rejected. Probase Cold showed the highest bond strength to the denture base, followed by Ufi Gel Hard and Kooliner. Bond strength depends on both materials' chemical composition and seems to be achieved by monomer's penetration and diffusion into the denture base polymeric

matrix.[124,547,548,550] In our study, Probase Cold demonstrated higher bond strength to the PMMA base denture resin than non-PMMA reline resins because its chemical composition was similar to the denture base's. In turn, Kooliner showed lower values than Ufi Gel Hard, and this can be explained by the presence of IBMA with higher molecular weight, which might have limited penetration into the denture base.[547] These results agree with other studies.[547,552,553]

Loading the Kooliner and Ufi Gel Hard with CHX did not negatively affect their SBS to the denture base. However, the same did not happen with Probase Cold. So, the seventh null hypothesis is rejected. Other studies also described that incorporating antimicrobial agents into resins affects their bond strength.[162] This phenomenon can be explained by the agent's physical presence within the polymeric matrix, which introduces more spaces and less homogeneity to the polymerized materials, weakening the bond strength. Nevertheless, CHX-loaded Probase Cold specimens presented higher SBS values than the other materials in the study, and the values seem to be sufficient and durable for clinical use.[554,555] This reasoning is corroborated by the absence of cohesive failures observed in all three reline resins.

Regarding the failure mode, all Kooliner specimens showed adhesive failures, which indicates that this reline resins's bond strength to the denture base is weaker, as confirmed by the lower values observed in the present study. Concerning Ufi Gel Hard, mixed failures increased, probably due to the increased bond strength promoted by the specific adhesive used before relining. In Probase Cold, adhesive and mixed failures were observed, again corroborating that these results agree with the present study's SBS values. The null hypothesis that loading reline acrylic resins with CHX affects the type of bonding failure to the denture base resin is thus rejected since CHX-loaded Ufi Gel Hard only showed mixed failures.

The release profile of the CHX incorporated in the acrylic resins was also added as an important evaluation. Our *in-vitro* drug release assay results showed that the CHX release from the three tested acrylic resin materials exceeded the MIC of CHX in the agar tested *C. albicans* and *S. oralis*. The greatest amount of CHX release occurred within the first 24-48 hours of incubation; this rapid elution phase indicates a surface release process. This finding agrees with a previous study[153] that revealed a high initial 4-day CHX release. The release of high levels of CHX over a longer period can be explained by a higher concentration of the drug (10%) used in that study and by a different composition of the medium, which can enhance the release. Most studies use

distilled water as the media solution.[17,21,60] However, in the present study, artificial saliva at pH 7 was used to simulate the oral cavity conditions. In all reline resins, the most intense initial release was followed by a slower and steadier diffusion until the end of the study period (28 days). This subsequent slow phase may be caused by the drug diffusion from the core of the polymer by complex processes involving fluid cluster formation around the CHX molecules and the interaction of these clusters with the fluid uptake process.[153]

The ninth hypothesis is rejected since, in experimental groups, Ufi Gel Hard released the highest amount of CHX, followed by Kooliner and Probase Cold. These results are consistent with micro-CT studies that revealed that Ufi Gel Hard had the most porous structure, and support the theory that the drug eluation may also be enhanced by porosity.[150] This reasoning can also help explain the findings in the cytotoxicity test. Ufi Gel Hard was the most cytotoxic resin, and Probase Cold was the least cytotoxic resin. Differences between materials can be associated with different compositions and structural arrangements of the acrylic resins.[487,545,556,557] Both direct acrylic resins are PEMA-based materials, known from their anomalous water uptake behaviour[17,557,558], which leads to greater drug release compared to PMMA-based materials such as Probase Cold.[558]

The tenth null hypothesis is also rejected since the incorporation of CHX decreased cell viability in the three tested resins. This result is in accordance with other studies[559-561] that stated that CHX was cytotoxic to fibroblast cells, having a concentration-dependent behavior. However, fibroblasts are in direct contact with these resins only in cases of ulceration of the epithelium (oral ulcers).[562,563] It has already been established that the cytotoxic effect differs between unpolished and polished acrylic resins.[564] Therefore, if we added a polishing procedure before testing, it could reduce inflammation and increase cell viability. Furthermore, there is no non-cytotoxicity acrylic resin available in the dental market[565] and, according to the ISO standard 10993-5[566], only a cell viability reduction by more than 30% is considered a cytotoxicity effect, which happened in Kooliner and Ufi Gel Hard.

Reline acrylic resins are in intimate contact with a large oral mucosa area, so cytotoxicity testing was considered. MTT assay was selected due to being a simple, fast, objective, and accurate method to determine cell viability.[563] Since mouse fibroblast cell line is more sensitive than epithelial cells, as already reported by several authors, we opted for the former.[567-569]

Probase Cold presented antimicrobial activity against *C. albicans* and *S. oralis*, including a high effect against the fungus. It was also the less cytotoxic resin under evaluation. Thus, its use may be a potential approach in the prevention or treatment of denture stomatitis. However, further studies with thermal and chemical aging should be performed for a better clinical application.

4.6. Conclusions

Within the limitations of this *in-vitro* experimental study, it can be established that:

- The MIC of CHX for *C. albicans* and *S. oralis* is 2 μg/mL.

- 2.5% CHX incorporated in Kooliner and 5% CHX in Ufi Gel Hard and Probase Cold were the minimal concentrations for antimicrobial activity against *C. albicans* and *S. oralis*.

- Loading Kooliner and Probase Cold with CHX does not affect these reline acrylic resins' microhardness. However, loading Ufi Gel Hard with CHX affects its microhardness, presenting higher values than the control group.
- Loading Kooliner and Ufi Gel Hard with CHX does not affect these reline acrylic resins' flexural strength. However, loading Probase Cold with CHX affects that property, presenting lower values than the control group.

- In the micro-CT images, Ufi Gel Hard without CHX exhibited the most porous structure, while Probase Cold (control and with CHX) showed almost no porosity. Loading with CHX decreased total porosity in all resins.
- CHX was included in the polymeric network without affecting the chemical structure of the polymer.

- Loading Kooliner with CHX increased the surface porosity compared with the control group. However, loading Ufi Gel Hard or Probase Cold with CHX did not negatively affect their surface porosity.
- Loading Kooliner with CHX did not affect the surface free energy. However, loading Ufi Gel Hard and Probase Cold with CHX affected their surface properties, with these groups presenting higher values than the controls.
- According to SEM, Probase Cold resin presented a different surface of spherical nature than the other two reline acrylic resins.

- According to the SEM images, no changes were observed in CHX-loaded Kooliner and Ufi Gel Hard. However, the incorporation of CHX in Probase Cold produced spaces between smaller and more irregular spherical particles.
- The reline acrylic resins used influenced the SBS to the denture base resin. Probase Cold revealed higher values of SBS, followed by Ufi Gel Hard and Kooliner.
- Loading Kooliner and Ufi Gel Hard with CHX caused higher bond strength to the denture base. However, loading Probase Cold with CHX negatively affected its SBS to the denture base.
- The predominant type of failure was adhesive. Loading Ufi Gel Hard with CHX influenced the type of failure between it and the denture base resin.

- In all the evaluated reline acrylic resins, a high initial release rate was followed by a slower and steadier release until the end of the study period.
- The greatest CHX release occurred within the first 24- 48h of incubation in all materials.
- The reline acrylic resins' different compositions affected the drug release. Ufi Gel Hard revealed the highest amounts of CHX released.

- The reline acrylic resin used influenced cell viability. Ufi Gel Hard was the most cytotoxic, and Probase Cold the least cytotoxic resin to mouse fibroblasts.
- The incorporation of CHX decreased cell viability in the three tested reline resins.

A CHX delivery system based on reline acrylic resins was developed and characterized. Under our experimental conditions, the addition of 5% CHX to Probase Cold resin presented antifungal, antibacterial, and antibiofilm activity and revealed CHX release in a slower and steadier diffusion over one month, with no serious detriment of their mechanical, structural, surface and biological properties.

Considering the overall results, loading CHX into reline acrylic resins appears to be a potential approach in the treatment of denture stomatitis.

Chapter 5

Color and Mechanical Properties' Stability in a Novel Chlorhexidine Delivery System Based on Reline Acrylic Resins

5. Color and Mechanical Properties' Stability in a Novel Chlorhexidine Delivery System Based on Reline Acrylic Resins

5.1. Introduction

A sustained-release drug delivery system using chlorhexidine (CHX) incorporated in reline acrylic resins has been suggested for the prevention of microbiological infections in the oral cavity. This novel treatment option for denture stomatitis involves relining a denture base with resins loaded with an antimicrobial agent. It has shown advantages such as the preservation of therapeutic levels by continuous drug release at the infection site, minimal risk of systemic toxicity, decreased need for patient compliance, and simultaneous treatment of ill-fitting dentures and *Candida*-related infection.[21,150,265,570] Furthermore, these carriers prevent the initial adhesion of microorganisms to the denture base and inhibit biofilm formation, thus considerably interfering in the mechanism of infection.[21,164,571] However, this drug delivery system has some possible disadvantages, like the risk of promoting microbial tolerance or resistance to the drugs and potentially compromise biomaterial's properties when the drug is incorporated.[21-24] The latter may restrict the use of a biomaterial as a carrier. Many *in-vitro* studies were conducted to determine the suitability of biomaterials by simulating the oral biodegradation processes.[221-224]

CHX is an agent with antimicrobial properties against a large number of microorganisms, including *Candida spp*.[150,153,370,435] When loaded into resins, its release is high in the first two to seven days and then decreases and remains constant for at least 28 days.[17]

A previous study (Chapter 4) showed promising results from loading reline acrylic resins with antimicrobial CHX concentrations that did not negatively influence their mechanical, structural, and surface properties. A concentration of 2.5% of CHX in Kooliner seems to be enough to prevent fungus development, while for Ufi Gel Hard and Probase Cold, a 5% concentration was required. However, in that study, the tests were performed without aging, remaining some concerns about the long-term effect the resins' properties may suffer while they are in function and exposed to the intraoral

environment.[13,486,514] Furthermore, an adverse and undesired effect of CHX, when used as a mouthwash or gel, is a brown staining of the teeth, the oral mucosa, and the acrylic dentures.[371,572]

Denture resins in the oral cavity have constant contact with saliva, foods, and hot and cold liquids, undergoing thermal changes than can affect their properties.[563,573-575] Several studies use distilled water or artificial saliva at pH 7 as the media solution to investigate drug release[17,21,60]; however, drug release is known to have different behavior when submitted to acidic conditions.[151] Since the materials are exposed to endogenous and exogenous acids, it is important to simulate pH variations and temperature changes that mimic the oral cavity environment. Also, pathological conditions such as denture stomatitis promote a lower pH environment and should also be simulated when testing properties of the biomaterials used for treatment.[291]

5.2. Objectives

The main purpose of this work is to evaluate the effect of loading three reline acrylic resins (Kooliner, Ufi Gel Hard and Probase Cold) with CHX, using a previously established concentration (Chapter 4), on their microhardness, flexural strength, surface free energy, and color stability, after undergoing a thermal or 28-day chemical aging process, according to the following hypotheses:

- $H0_1$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX does not affect the reline acrylic resin's microhardness after thermal aging;
- $H1_1$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX affects the reline acrylic resin's microhardness after thermal aging;

- $H0_2$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX does not affect the reline acrylic resin's flexural strength after thermal aging;
- $H1_2$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX affects the reline acrylic resin's flexural strength after thermal aging;

- $H0_3$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX does not affect the reline acrylic resin's microhardness after chemical aging;
- $H1_3$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX affects the reline acrylic resin's microhardness after chemical aging;

- $H0_4$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX does not affect the reline acrylic resin's flexural strength after chemical aging;
- $H1_4$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX affects the reline acrylic resin's flexural strength after chemical aging;

- $H0_5$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX does not affect the reline acrylic resin's surface free energy after thermal aging;
- $H1_5$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX affects the reline acrylic resin's surface free energy after thermal aging;

- $H0_6$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX does not affect the reline acrylic resin's surface free energy after chemical aging;
- $H1_6$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX affects the reline acrylic resin's surface free energy after chemical aging;

- $H0_7$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX does not affect the reline acrylic resin's color stability after thermal aging;
- $H1_7$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX affects the reline acrylic resin's color stability after thermal aging;

- $H0_8$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX does not affect the reline acrylic resin's color stability after chemical aging;
- $H1_8$: Loading Kooliner, Ufi Gel Hard, or Probase Cold with CHX affects the reline acrylic resin's color stability after chemical aging.

5.3. Materials and Methods

Three autopolymerizing hard reline acrylic resins, with differences in their chemical composition and physical structure, were selected, as mentioned in section 4.3: Kooliner, Ufi Gel Hard, and Probase Cold. CHX diacetate monohydrate (Panreac Applichem, Darmstadt, Germany) was selected to load the reline acrylic resins, as mentioned in section 4.3.

5.3.1. Preparation of the Specimens

Two groups of specimens were established for each material: a control group, with a 0% concentration of CHX; and an experimental group, where a concentration of 2.5% CHX was incorporated in the Kooliner and a 5% CHX in both the Ufi Gel Hard and the Probase Cold, based on a previous study (Chapter 4). The number of specimens per group varied with the type of data required in the assay.

The powders of the reline resin and the CHX were each weighed using a precision scale (A&D Company, Limited, Tokyo, Japan) (Appendix 2, Figure 1). Each specimen was prepared by incorporating CHX and mixing it with the corresponding reline acrylic resins's powder, using a mortar and a pestle for homogenization. Then, the powder was mixed with the liquid monomer, measured using a graduated pipette, and the final mixture was placed into a specific mold (final shape depends on the test and is described ahead) and left there during the time recommended by the manufacturer's instructions, as mentioned in section 4.3.1. (Table 4.1).

The edges of the specimens were polished with a 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL., USA) on a polisher with constant water cooling (DAP-U, Struers, Denmark) to remove irregularities (Appendix 2, Figure 3).

5.3.2. Aging

Specimens from each of the resins' two groups (control and experimental) were randomly divided into the following groups: thermal (TA) and chemical aging (CA).

The thermal aging process included 1000 cycles of thermal fluctuations between 5°C and 55°C (20 seconds in each bath) with 5 seconds of dwell time, in a thermocycling machine (Refri 200-E, Aralab, Cascais, Portugal) (Figure 5.1).

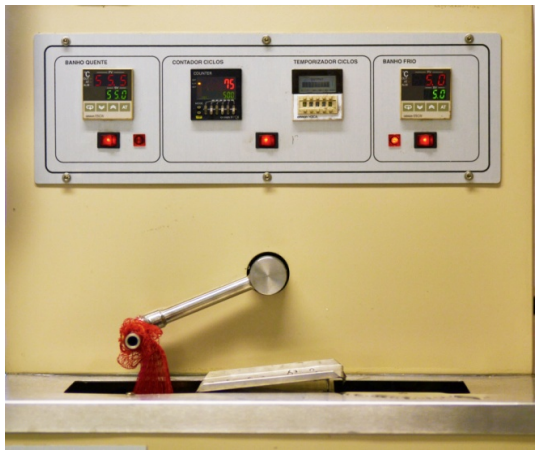

Figure 5.1 – Thermocycling equipment (Refri 200-E, Aralab, Cascais, Portugal).

For the chemical aging process, each specimen was weighed on a precision scale (A&D Company, Limited, Tokyo, Japan) (Appendix 2, Figure 1) and immersed in artificial saliva[208,212,523] with a ratio of 1 g/5 mL in individual graduated falcon tubes (Appendix 4). The specimens were stored at 37°C (Memmert, Schwabach, Germany) with constant gentle shaking (300 rpm) for 28 days. To simulate oral conditions, the samples were subjected to a protocol of chemical aging based on 6-hour cycles in artificial saliva at pH=3 interchanging with 18-hour cycles in artificial saliva at pH=7 (Appendix 2, Figure 19). Between each cycle, the samples were washed with distilled water and dried with absorbent paper (Figure 5.2).

Figure 5.2 – Chemical aging protocol. a) Incubation of the specimens in graduated falcon tubes with artificial saliva; b) Incubator (Memmert, Schwabach, Germany).

5.3.3. Mechanical Properties

Specimens from each material were prepared from rectangular-shaped stainless-steel molds (64×10×3.3 mm), as recommended by ISO 20795-1: 2013.[520] Six groups of specimens (n=8) were prepared: one control and one experimental group for each resin, as mentioned above in section 5.3.1., in a total of 96 specimens. After polymerization, all specimens were submitted to one of the aging processes before testing.

5.3.3.1. Microhardness

The Knoop microhardness test was performed (Duramin, Struers DK 2750, Ballerup, Denmark) (Appendix 2, Figure 7) with a 98.12 mN load for 30 seconds. The operator measured the length of the pyramidal indentations using the Duramin software immediately after each indentation, within a maximum period of ten seconds. As there was a short time interval between indentation and measurement, it was assumed that the viscoelastic recovery was minimal. Twelve equidistant measurements were made in each specimen, and the mean value was used as the specimen's Knoop microhardness (KHN – kg/mm^2).

5.3.3.2. Flexural Strength

After checking each specimen's width and thickness with a digital micrometer of ±0.01-mm precision (Mitutoyo Digimatic, MFG.Co, Ltd. Tokyo, Japan), the same specimens were submitted to the three-point flexural test in a universal machine (Instron model 4502, Instron Ltd, Bucks, England) (Appendix 2, Figure 8) with 1-kN load cell at a crosshead speed of 5 mm/min and a distance of 50 mm between rods.[520] Load was applied until failure, and the fracture load was recorded in Newtons (N). The flexural strength was expressed in megapascal (MPa) and calculated using the formula $FS = 3WL/2bd^2$, where FS is the flexural strength, W is the maximum load before fracture (N), L is the distance between rods (50 mm), b is the specimen's width (mm), and d is the specimen's thickness (mm).

5.3.4. Surface Property

5.3.4.1. Surface Free Energy

Rectangular-shaped metallic molds were used to prepare strips with 125×25×1 mm that were then cut with a cylindrical turbine drill into 60 specimens with approximately 16×25×1 mm (n=5). The specimens were submitted to one of the aging processes before contact angle measurement. Each specimen's width, thickness, and

height were measured with a digital micrometer of ±0.01-mm precision (Mitutoyo Digimatic, MFG.Co, Ltd. Tokyo, Japan) (Appendix 2, Figure 9) and introduced in the software. Assays were made with Tensiometer K12 (Kruss, Hamburg, Germany) using the Wilhelmy plate method (Appendix 2, Figures 10 and 11) by immersing plates into two test liquids: water (Milli quality, Merck Millipore, Germany) or 1,2-propanediol (Merck, Germany) (Appendix 2, Figure 12), at a speed of 20 μm/s and 25±0.1ºC. To estimate the specimens' surface free energy (γ), as well as its dispersive (γ^d) and polar (γ^p) components, based on the harmonic mean method[521], advancing contact angles were used. Equations for surface free energy estimation were solved using the equation handling KRUSS software program: K121 contact angle measuring system (version 2.05) (Appendix 2, Figures 13, 14, and 15) (Figure 5.3).

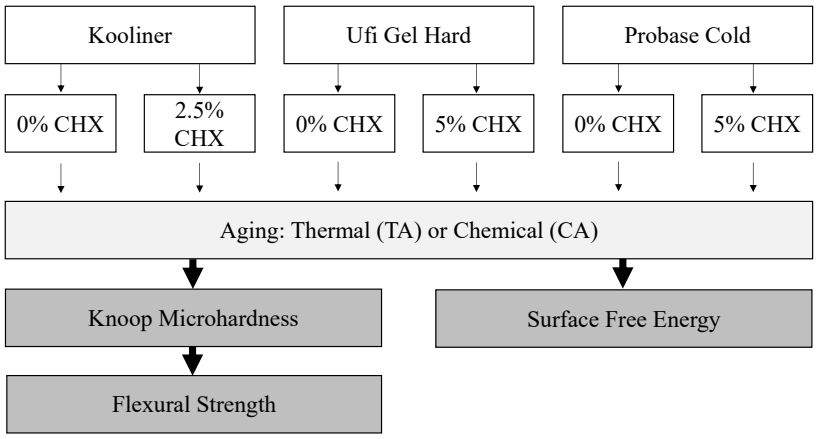

Figure 5.3 – Experimental design protocol for mechanical and surface properties [CHX – chlorhexidine].

5.3.5. Color Stability

A total of 60 cylindric specimens (12×6 mm) were prepared as recommended by ISO 7491:2000[576] and mentioned above in section 4.1. Five cylindric specimens per group (one control and one experimental for each resin) (*n*=5) were subjected to aging (Appendix 2, Figure 20).

Color measurements were performed before and after aging using two spectrophotometers: VITA Easyshade (ES) and Spectroshade Micro (SS) (Figures 5.4 and 5.5). With ES, the acrylic resin specimens and the reader were placed into a dark chamber so that the specimens were not exposed to light. The color was measured by placing the specimen at the center of the reader. Both ends of the specimens were assessed, and three measurements were made for each end.

Figure 5.4 – Color measurement of the specimens. a) VITA Easyshade (ES) spectrophotometer; b) Dark chamber.

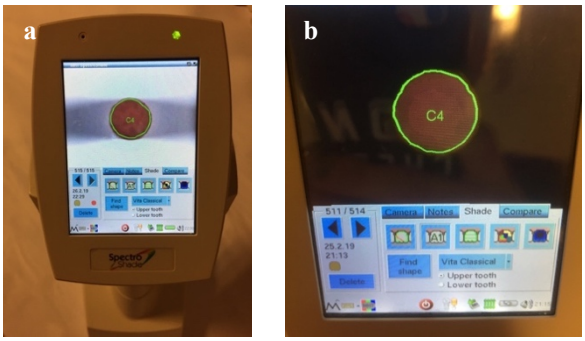

Figure 5.5 – Color measurement of the specimens. a) Spectroshade (SS) spectrophotometer; b) Registration of L (lightness), C (chroma), and h (hue) values.

The L (lightness), C (chroma), and h (hue) values were registered, and an average value was calculated and converted to the CIELab system. L values were equal in both systems, $c=(a^2+b^2)^{1/2}$, and $h=tang^{-1}(b/a)$.[577,578] The overall color change ($\Delta E=[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$) was calculated and converted to NBS (National Bureau of Standards) units, using the formula NBS units=ΔE^* x 0.92, to denote the color differences in a clinical perspective (Appendix 1, Table 4) (Figure 5.6).[577,579,580]

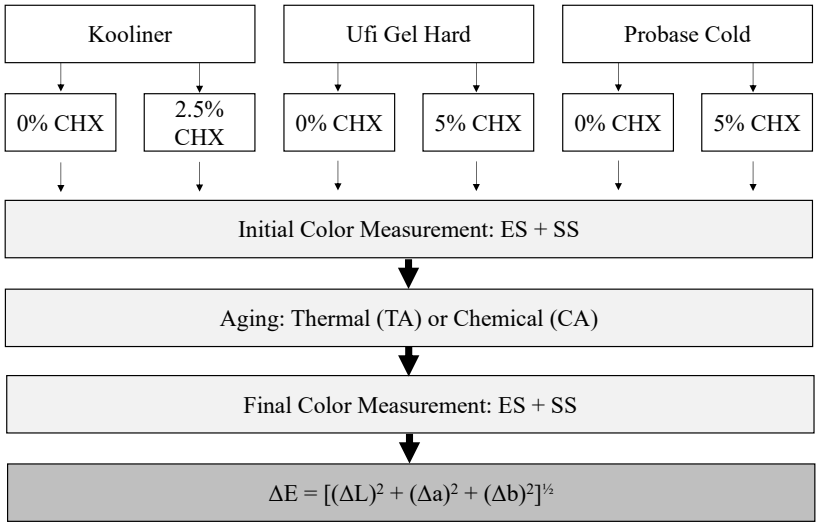

Figure 5.6 – Experimental design protocol for color stability [CHX – chlorhexidine, ES – VITA Easyshade, SS – Spectroshade Micro].

5.3.6. Statistical Analysis

The sample size (n) was estimated with a power analysis to provide statistical significance (α=0.05) at a power of 80%, based on data from a pilot study. Since normality and homogeneity of variance were not verified in scale variables (Shapiro-Wilk and Levene tests, p<0.05), data were submitted to Mann-Whitney non-parametric tests. In all statistical tests, the 5% level of significance (α=0.05) was considered.

5.4. Results

5.4.1. Mechanical Properties

Descriptive analysis of the data obtained was performed for each material. The mean, standard deviation, median, and interquartile range values of microhardness and flexural strength were determined (Table 5.1).

Table 5.1 – Microhardness and flexural strength data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*Aging*	*MICROHARDNESS (KHN)*		*FLEXURAL STRENGTH (MPa)*	
				M ± SD	*MED ± IQR*	*M ± SD*	*MED ± IQR*
Kooliner	**0%**	8	TA	7.5 ± 1.95	7.9 ± 2.73	81.3 ± 14.19	78.9 ± 26.00
	2.5%	8		7.5 ± 1.43	7.9 ± 1.60	92.9 ± 8.51	91.1 ± 17.63
	0%	8	CA	7.0 ± 1.77	7.2 ± 2.97	42.5 ± 7.82	42.0 ± 14.88
	2.5%	8		6.9 ± 1.78	7.0 ± 3.44	42.9 ± 7.51	42.1 ± 12.89
Ufi Gel Hard	**0%**	8	TA	8.2 ± 0.92	8.2 ± 1.85	66.7 ± 10.97	67.0 ± 20.08
	5%	8		8.3 ± 0.35	8.4 ± 0.54	74.2 ± 9.38	75.6 ± 13.60
	0%	8	CA	7.6 ± 1.18	7.6 ± 2.25	37.5 ± 4.41	36.5 ± 6.51
	5%	8		7.6 ± 0.93	7.9 ± 1.94	37.7 ± 4.89	37.4 ± 5.60
Probase Cold	**0%**	8	TA	13.7 ± 0.72	13.5 ± 0.58	163.6 ± 39.48	180.0 ± 57.31
	5%	8		12.9 ± 0.43	12.8 ± 0.53	124.9 ± 18.15	124.6 ± 38.75
	0%	8	CA	13.6 ± 2.27	13.1 ± 4.17	82.1 ± 12.17	87.3 ± 19.04
	5%	8		12.7 ± 2.12	12.4 ± 3.94	65.8 ± 4.96	65.6 ± 9.74

CHX = Chlorhexidine, TA = Thermal aging, CA = Chemical aging, M = Mean, SD = Standard deviation, MED = Median, IQR = Interquartile range.

After thermal aging, Kooliner and Ufi Gel Hard resins' microhardness was not (Kooliner – p=1.000; Ufi Gel Hard – p=0.878) affected by the CHX incorporation. However, the CHX group of Probase Cold showed decreased microhardness values (p=0.010) (Figure 5.7) compared to the control. Concerning flexural strength, the CHX incorporation did not influence Ufi Gel Hard resin (p=0.130) but increased Kooliner's values (p=0.050) and decreased (p=0.038) Probase Cold's (Figure 5.8).

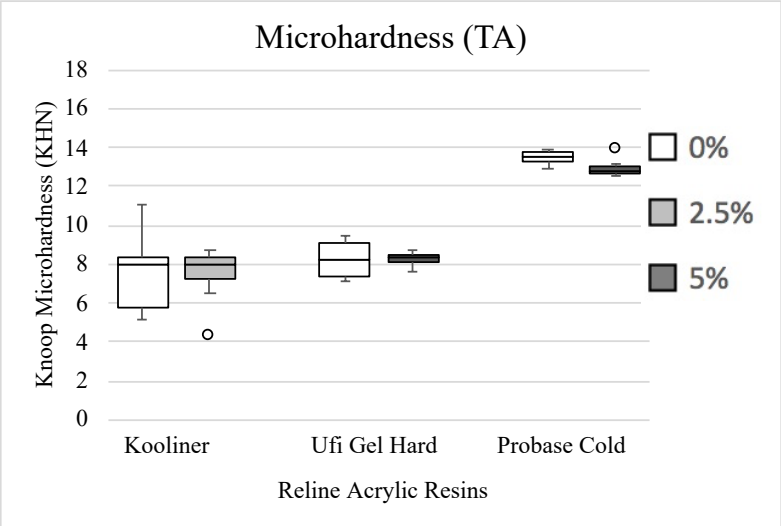

Figure 5.7 – Boxplot of the microhardness (KHN) distribution among experimental groups after a thermal aging process [TA – Thermal Aging: Kooliner – 0% vs. 2.5% (p=1.000); Ufi Gel Hard – 0% vs. 5% (p=0.878); and Probase Cold – 0% vs. 5% (p=0.010)].

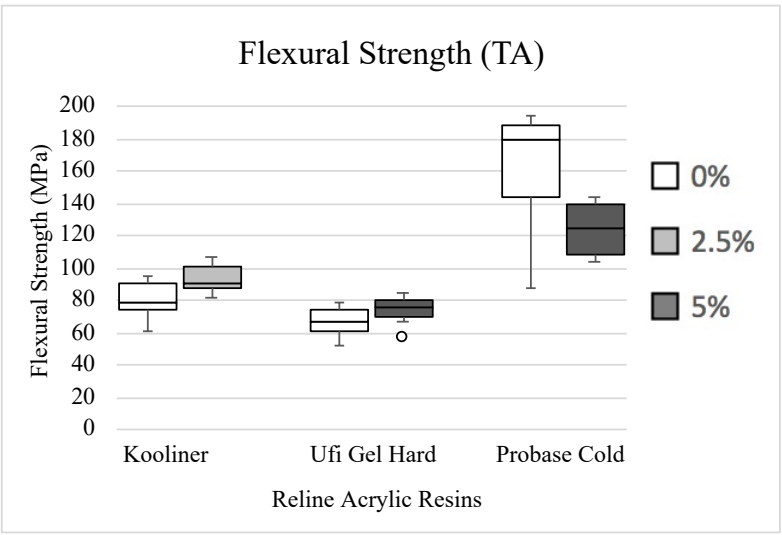

Figure 5.8 – Boxplot of the flexural strength (MPa) distribution among experimental groups after a thermal aging process [TA – Thermal Aging: Kooliner – 0% vs. 2.5% (p=0.050); Ufi Gel Hard – 0% vs. 5% (p=0.130); and Probase Cold – 0% vs. 5% (p=0.038)].

After chemical aging, none of the three reline acrylic resins' microhardness (Kooliner, p=0.798; Ufi Gel Hard, p=0.878; Probase Cold, p=0.195) was affected by the CHX incorporation (Figure 5.9). The CHX incorporation did not influence the flexural strength of Kooliner (p=0.959) or Ufi Gel Hard (p=0.645), but decreased (p=0.021) Probase Cold's flexural strength (Figure 5.10).

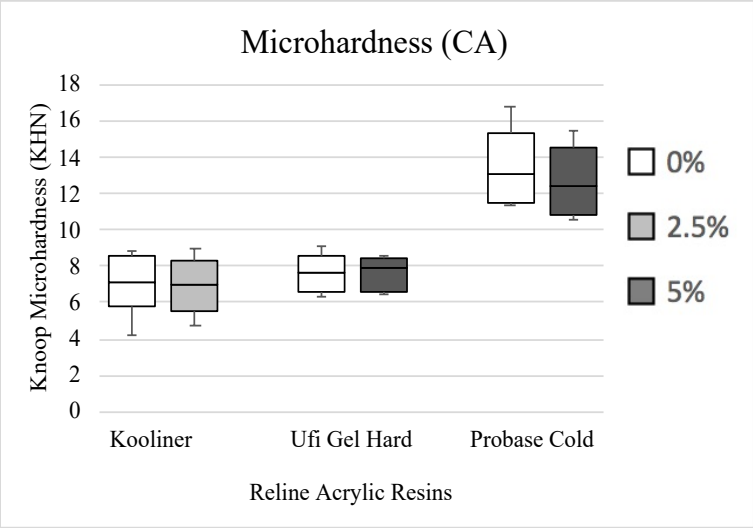

Figure 5.9 – Boxplot of the microhardness (KHN) distribution among experimental groups after a chemical aging process [CA – Chemical Aging: Kooliner – 0% vs. 2.5% (p=0.798); Ufi Gel Hard – 0% vs. 5% (p=0.878); and Probase Cold – 0% vs. 5% (p=0.195)].

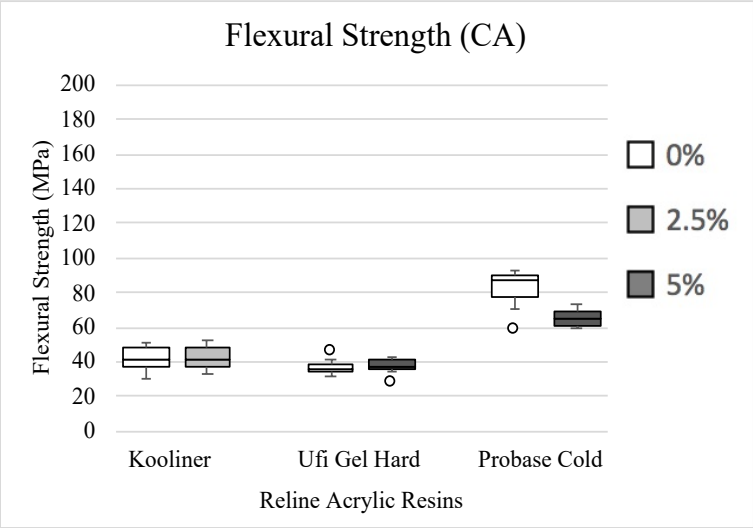

Figure 5.10 – Boxplot of the flexural strength (MPa) distribution among experimental groups after a chemical aging process [CA – Chemical Aging: Kooliner – 0% vs. 2.5% (p=0.959); Ufi Gel Hard – 0% vs. 5% (p=0.645); and Probase Cold – 0% vs. 5% (p=0.021)].

5.4.2. Surface Free Energy

Descriptive analysis of the data was carried out for each material, including the mean and standard deviation for contact angle and the dispersive (γ^d) and polar (γ^p) components of surface free energy (Appendix 1, Table 5). The mean, standard deviation, median, and interquartile range values of total surface free energy (γ) were determined (Table 5.2).

Table 5.2 – Surface free energy data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*Aging*	*SURFACE FREE ENERGY (mN/m)*	
				γ	
				M ± SD	*MED ± IQR*
Kooliner	**0%**	5	TA	28.8 ± 2.30	27.9 ± 4.45
	2.5%	5		26.5 ± 1.01	27.0 ± 1.85
	0%	5	CA	31.7 ± 1.61	31.8 ± 2.95
	2.5%	5		33.2 ± 1.73	33.4 ± 3.05
Ufi Gel Hard	**0%**	5	TA	25.4 ± 2.94	24.0 ± 5.65
	5%	5		30.4 ± 3.37	32.0 ± 6.50
	0%	5	CA	41.6 ± 1.67	41.8 ± 2.70
	5%	5		42.4 ± 0.82	42.5 ± 0.28
Probase Cold	**0%**	5	TA	26.3 ± 0.21	26.3 ± 0.40
	5%	5		30.0 ± 1.26	30.5 ± 2.40
	0%	5	CA	37.4 ± 1.75	37.2 ± 3.30
	5%	5		37.2 ± 1.20	36.6 ± 2.25

CHX = Chlorhexidine, TA = Thermal aging, CA = Chemical aging, M = Mean, SD = Standard deviation, MED = Median, IQR = Interquartile range.

No influence of the CHX incorporation was detected in the surface free energy of the two direct reline resins, Kooliner and Ufi Gel Hard, after thermal or chemical aging (Kooliner, p=0.222 p=0.222; Ufi Gel Hard, p=0.095 p=0.548). In Probase Cold, the surface free energy values were not (p=0.841) affected by the CHX incorporation after chemical aging. However, after thermal aging, incorporating CHX led to an increase (p=0.008) in surface free energy, with no differences (p>0.05) in the dispersive and polar components (Figures 5.11 and 5.12).

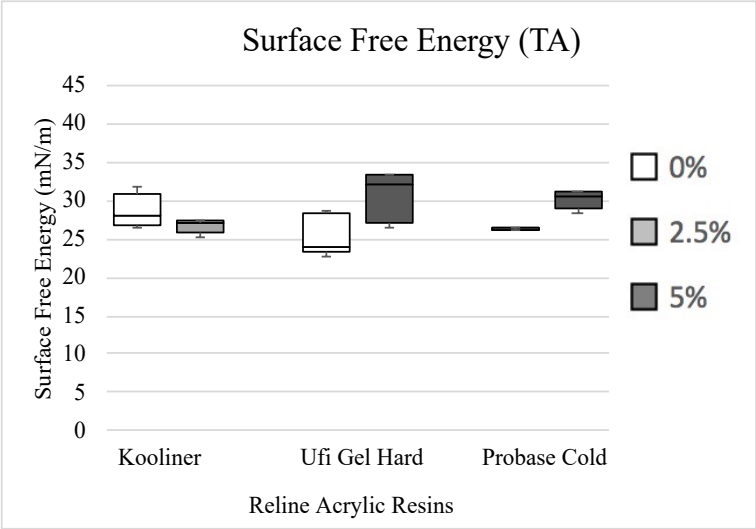

Figure 5.11 – Boxplot of the surface free energy total (mN/m) distribution among experimental groups after a thermal aging process [TA – Thermal Aging: Kooliner – 0% vs. 2.5% (p=0.222); Ufi Gel Hard – 0% vs. 5% (p=0.095); and Probase Cold – 0% vs. 5% (p=0.008)].

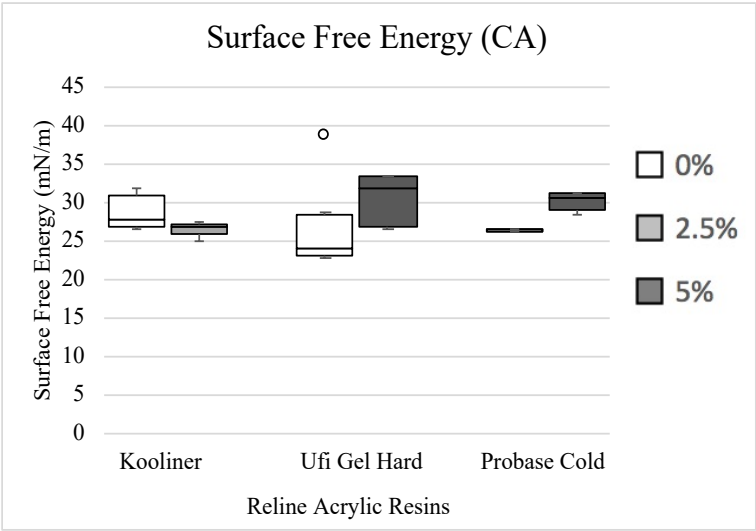

Figure 5.12 – Boxplot of the surface free energy total (mN/m) distribution among experimental groups after a chemical aging process [CA – Chemical Aging: Kooliner – 0% vs. 2.5% (p=0.222); Ufi Gel Hard – 0% vs. 5% (p=0.548); and Probase Cold – 0% vs. 5% (p=0.841)].

5.4.3. Color Stability

After converting the CieLCh system to the CIELab system, ΔL, Δa and Δb values were calculated, and ΔE was measured. Descriptive analysis of the data was carried out for each group. The mean, standard deviation, median, and interquartile range values of color stability were determined (Table 5.3).

Table 5.3 – Color stability data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*Aging*	*Color Stability (ΔE)*			
				ES		*SS*	
				M ± SD	***MED ± IQR***	***M ± SD***	***MED ± IQR***
Kooliner	**0%**	5	TA	3.8 ± 0.92	4.1 ± 1.77	2.4 ± 0.30	2.5 ± 0.56
	2.5%	5		9.2 ± 2.40	10.3 ± 3.00	4.1 ± 1.00	3.8 ± 1.75
	0%	5	CA	4.1 ± 0.54	4.1 ± 1.08	1.9 ± 0.29	1.8 ± 0.50
	2.5%	5		15.1 ± 0.63	15.0 ± 1.15	9.0 ± 0.59	9.0 ± 1.02
Ufi Gel Hard	**0%**	5	TA	2.4 ± 0.60	2.5 ± 1.15	3.3 ± 1.11	3.1 ± 2.01
	5%	5		3.6 ± 2.80	2.6 ± 3.57	4.7 ± 2.80	3.9 ± 4.53
	0%	5	CA	3.1 ± 0.72	3.1 ± 1.33	1.2 ± 0.26	1.2 ± 0.48
	5%	5		6.0 ± 1.30	6.3 ± 2.51	2.0 ± 0.34	1.8 ± 0.52
Probase Cold	**0%**	5	TA	1.8 ± 0.88	2.3 ± 1.59	1.2 ± 0.90	0.9 ± 1.25
	5%	5		2.0 ± 1.25	1.4 ± 1.62	1.7 ± 2.18	0.8 ± 2.84
	0%	5	CA	2.7 ± 1.80	1.8 ± 3.36	0.9 ± 0.55	0.6 ± 0.99
	5%	5		16.0 ± 1.17	16.3 ± 2.13	10.6 ± 0.58	10.5 ± 1.05

CHX = Chlorhexidine, TA = Thermal aging, CA = Chemical aging, ES = VITA Easyshade, SS = Spectroshade Micro, M = Mean, SD = Standard deviation, MED = Median, IQR = Interquartile range.

After thermal aging, loading Kooliner with CHX showed higher ΔE values (p=0.008), as evaluated by ES and SS. On the other hand, Ufi Gel Hard and Probase Cold were not affected by the CHX incorporation [Ufi Gel Hard, p=0.548 (ES) and p=0.421 (SS); Probase Cold, p=1.000 (ES) and p=0.841 (SS)], as measured by the two spectrophotometers (Figure 5.13). After chemical aging, loading all reline acrylic resins with CHX showed higher ΔE values (p=0.008), as evaluated by the two spectrophotometers ES and SS (Figure 5.14).

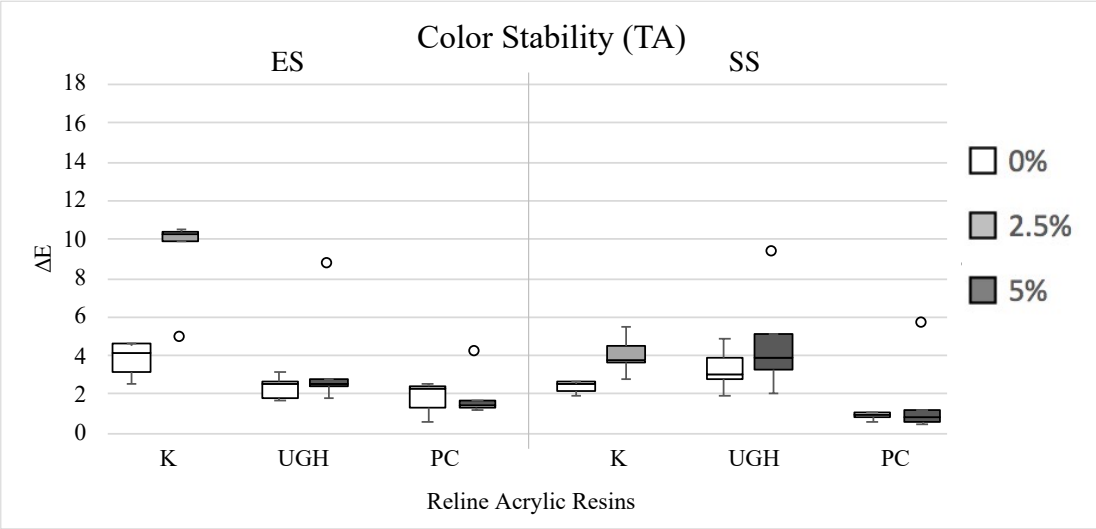

Figure 5.13 – Boxplot of the color stability (ΔE) distribution among experimental groups after a thermal aging process [TA – Thermal Aging: Kooliner – 0% vs. 2.5% (ES+SS: p=0.008); Ufi Gel Hard – 0% vs. 5% (ES: p=0.548, SS: p=0.421); and Probase Cold – 0% vs. 5% (ES: p=1.000, SS: p=0.841)].

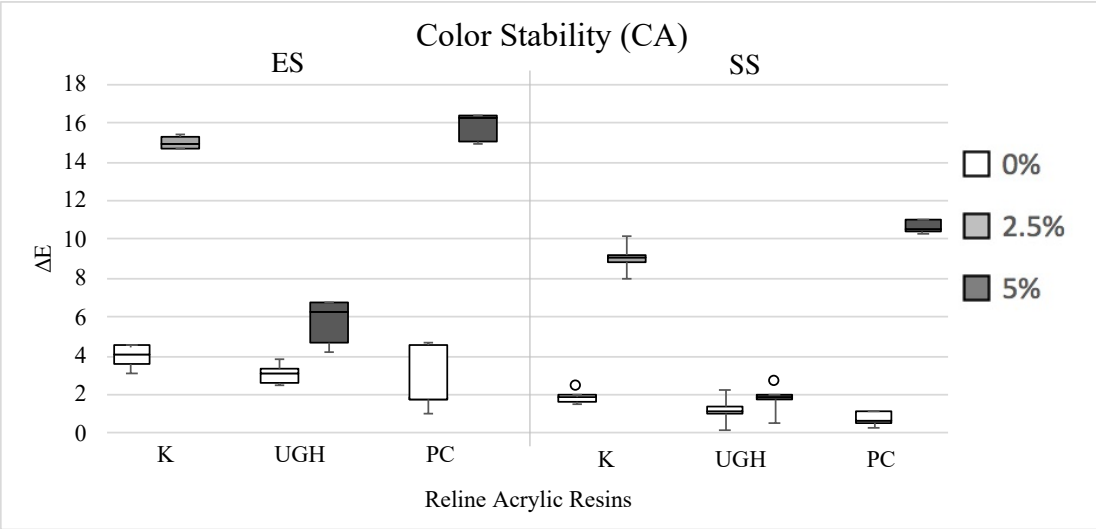

Figure 5.14 – Boxplot of the color stability (ΔE) distribution among experimental groups after a chemical aging process [CA – Chemical Aging: Kooliner – 0% vs. 2.5% (ES+SS: p=0.008); Ufi Gel Hard – 0% vs. 5% (ES+SS: p=0.008); and Probase Cold – 0% vs. 5% (ES+SS: p=0.008)].

To better relate the ΔE to clinical implications according to NBS, the NBS unit and the color differences were determined (Table 5.4).

Table 5.4 – National Bureau of Standards data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*Aging*	*NBS units*			
			ES		*SS*	
			M ± SD	***Color Differences***	***M ± SD***	***Color Differences***
Kooliner	**0%**	TA	3.5 ± 0.85	Appreciable change	2.2 ± 0.27	Perceivable change
	2.5%		8.5 ± 2.21	Much appreciable	3.7 ± 0.92	Appreciable change
	0%	CA	3.7 ± 0.50	Appreciable change	1.7 ± 0.27	Perceivable change
	2.5%		13.9 ± 0.58	Change to another color	8.3 ± 0.54	Much appreciable
Ufi Gel Hard	**0%**	TA	2.2 ± 0.56	Perceivable change	3.1 ± 1.02	Appreciable change
	5%		3.4 ± 2.58	Appreciable change	4.4 ± 2.58	Appreciable change
	0%	CA	2.9 ± 0.66	Appreciable change	1.1 ± 0.24	Slight change
	5%		5.5 ± 1.19	Appreciable change	1.8 ± 0.31	Perceivable change
Probase Cold	**0%**	TA	1.7 ± 0.82	Perceivable change	1.1 ± 0.83	Slight change
	5%		1.8 ± 1.15	Perceivable change	1.6 ± 2.00	Perceivable change
	0%	CA	2.4 ± 1.65	Perceivable change	0.8 ± 0.51	Slight change
	5%		14.8 ± 1.08	Change to another color	9.8 ± 0.53	Much appreciable

CHX = Chlorhexidine, TA = Thermal aging, CA = Chemical aging, ES = VITA Easyshade, SS = Spectroshade Micro, M = Mean, SD = Standard deviation.

After thermal aging, loading Probase Cold with CHX caused a “perceivable change”. One the other hand, the other two acrylic resins, Kooliner and Ufi Gel Hard, showed “much appreciable” and “appreciable change”, respectively, after CHX loading.

Considering chemical aging, as evaluated by the ES spectrophotometer, loading the acrylic resins Kooliner and Probase Cold with CHX caused “change to another color”, while Ufi Gel Hard showed an “appreaciable change”. With the SS spectrophotometer, loading the acrylic resins Kooliner and Probase Cold with CHX showed a “much appreaciable” change, while in Ufi Gel Hard it caused a “perceivable change”.

5.5. Discussion

A CHX delivery system was proposed and characterized in a previous study in the context of *Candida albicans*-associated denture stomatitis control and treatment. The reline acrylic resins (Kooliner, Ufi Gel Hard, and Probase Cold) loaded with CHX for the delivery system showed no changes in their antimicrobial, mechanical, structural, and surface properties at baseline. These drug delivery systems' drug release was slower and steadier over one month, with no detrimental biological properties. Adding 2.5% CHX to Kooliner and 5% CHX to Ufi Gel Hard and Probase Cold seems to be enough, and between these three, the CHX-loaded Probase Cold seems to be the delivery system with the highest effect against the fungus and the least cytotoxic (Chapter 4).

Some problems have been described regarding drug delivery systems, such as the deterioration of the matrix properties or the potential tissue toxicity in cases of excessive drug release.[27] In dentistry, it is particularly important to study the drug delivery system's behavior under the biodegradation processes of the oral medium, where biomaterials are exposed to a harsh environment, subject to temperature and pH changes, food, and cleaning methods adopted by the patient.[232,237] There are no investigations about this novel CHX delivery system based on reline acrylic resins that study resins properties' changes after undergoing aging processes (thermal or chemical). Thus, in the present study, color, mechanical, and surface properties were characterized to evaluate the long-term effect of CHX loading on this drug delivery system.

A laboratory testing procedure of thermal cycling was used to simulate the temperature fluctuations that occur in the oral cavity with mouth breathing and whenever food and beverages with different temperatures are consumed.[573] In the present study, the specimens were submitted to 1000 cycles of thermal fluctuations between 5°C and 55°C, corresponding to 6 weeks in function.[205] Numerous studies reported that thermal fluctuations negatively influenced the microhardness and strength of relined denture bases, depending on the relining material used.[204,581-583] The water entering the spaces between the resins' polymer chains causes separation, resulting in the formation of micro-fractures, degrading its infrastructure.[500,577,584,585] Temperature increases cause a more quick diffusion of the water molecules, resulting in dimensional changes and modifications on the surface of acrylic resins.[584,586]

The pH of the aqueous medium to which the materials are exposed also plays a significant role in microbial colonization.[587,588] Studies have reported a saliva's pH of 5.2 in patients with dentures presenting surface *C. albicans* adherence.[589] The maximum cumulative CHX release reaches higher levels at pH=3 than at pH=7.[217] In the present study, all specimens were subjected to a chemical aging process by immersion in artificial saliva with a cyclic procedure of 6 hours at pH=3 interchanging with 18 hours at pH=7 for 28 days. Studies have described the CHX release by loaded acrylic resins into the oral environment in the first 28 days.[150,151,153] After this period, the therapeutic dose of CHX becomes gradually less efficient since reline acrylic resins are considered semi-permanent materials.

It is important to know whether the loaded resins maintain their properties even after the release of CHX, maintaining their support function. No differences were found in the microhardness values between control and experimental groups of Kooliner and Ufi Gel Hard in thermal and chemical aging. Probase Cold was the only resin whose microhardness decreased after thermal aging since the CHX group had lower values than the control. In our previous study (Chapter 4), the microhardness of the same acrylic resin at baseline was not affected by the CHX loading. Our result is in agreement with the findings of Addy and Handley (1981)[534], who showed that CHX incorporation significantly reduced the hardness values of acrylic resins after 8 days of soaking in water. Probase Cold as a CHX delivery carrier will probably be less resistant to external agents like toothbrushes and food.[590] However, in the chemical aging, no differences were found between experimental groups of Probase Cold. Since CHX-loaded Probase Cold showed lower values than the control after thermal aging, we concluded that this study's first null hypotheses could be rejected. One the other hand, the third null hypotheses are not rejected because no differences were detected in microhardness values after chemical aging.

The flexural strength of Kooliner and Ufi Gel Hard was not influenced by the CHX incorporation. On the other hand, the CHX-loaded specimens of Probase Cold revealed decreased flexural strength compared to the controls, in both aging methods. This result had already been found when the test was made without aging processes (Chapter 4). Some studies[300,514,532-536] that evaluated the influence of incorporating antimicrobial agents on reline acrylic resins' flexural strength showed that this property decreased with increased concentrations of the antimicrobial agents added to acrylic resins.

The different chemical compositions of indirect reline materials could explain why the flexural strength was affected. Probase Cold has a more robust polymer matrix organization than Kooliner and Ufi Gel Hard. It is composed of polymethylmethacrylate, and its polymerization cycle is achieved with high temperature and pressure.[487,545] Once again, the fact that CHX is continuously released from the acrylic resins may cause a significantly decreased flexural strength values when CHX is incorporated.[17,60,150] This explanation can be supported by SEM images (Chapter 4) that showed that Probase Cold with CHX produces spaces between smaller and more irregular spherical particles. Thus, this flexural strength reduction in the 5% CHX-loaded Probase Cold group at baseline and with thermal and chemical aging may be related to the increased of intermolecular distance between the polymer chains after the CHX incorporation, associated with a greater amount of residual monomer by a lower degree of conversion.[514,533,537,591]

Since the 5% CHX-loaded Probase Cold group had a significantly lower flexural strength than the control in both aging processes, the second and fourth hypotheses could be rejected. However, although Probase Cold showed a decrease in flexural strength with the incorporation of CHX, under current experimental conditions, its mean value was higher than that obtained with Kooliner and Ufi Gel Hard. Even so, in this particular situation, the 5% CHX Probase Cold group still reached a flexural strength value that is clinically accepted by the ISO 20795 standard (60 MPa).[412,520] Furthermore, the tested reline acrylic resins are not used independently in the oral cavity, they are always associated with a denture base material, so this result should be interpreted with caution.

Besides mechanical properties, the effect of CHX loading on the reline resins' surface properties was evaluated since the surface energy plays an important role in the adhesion of fungal cells.[592] In the present study, after undergoing thermal aging, CHX-loaded Probase Cold showed higher surface free energy values than the control group, so the fifth null hypothesis is rejected. This tendency was also observed in previous studies without aging (Chapter 4), where this indirect resin became more hydrophilic through the increase of its polar component. After this aging process, the total surface free energy differed among groups without differences in the dispersive and polar components, which means that the two components reached a balance.

The results showed no differences in total surface free energy between Kooliner and Ufi Gel Hard experimental groups after aging. These specimens' exposure to a wet

environment with the test medium suggested a complete elution of CHX from the reline resin, remaining only resin. This finding agrees with the results from the first study without aging (Chapter 4), where the sample did not face any water solution and the reline resin preserved CHX, which led to higher surface free energy values in Ufi Gel Hard and Probase Cold groups. Since loading the three reline acrylic resins with CHX did not change their total surface energy after chemical aging, the sixth null hypothesis could not be rejected.

The success of removable dentures depends on their mechanical and surface properties but also aesthetic appearance. CHX solutions may cause staining of removable dentures when used as a cleaning agent.[593] Thus, it was important to evaluate the effect of adding CHX on the reline acrylic resins' color stability.

The CIELab system, used in this study, is the most widely used system for measuring instrumental color.[594] Our study, used two spectrophotometers (ES and SS) to minimize the error associated with each and because they are used for the white color scale.[595] For color measurements with ES, an x-ray chamber was used to avoid reflected light, which practically does not contain color information and may adversely affect the color measurement.

Some authors have shown that the incorporation of antimicrobial compounds, like silver, zinc oxide, ethanol, and methacryloyloxyundecylpyridinium bromide (MUPB), caused drastic color changes in resins.[410,413,437,596] In our study, after the thermal aging process, only Kooliner showed differences in color stability between specimens without CHX and with 2.5% CHX. Therefore, since CHX incorporation caused a higher color change in this reline acrylic resin, the seventh null hypothesis could be rejected. In the other two resins, the same did not happen. After chemical aging, loading the three reline acrylic resins with CHX affected the color stability, so the eighth hypothesis could be rejected.

Hydrophilic materials exhibit greater color change than hydrophobic materials.[597] However, that did not happen in this study, because after chemical aging, loading the three reline acrylic resins with CHX did not change their total surface energy. This result can be explained by color changes not depending only on this factor. The discoloration of acrylic resins after a long-term use depends on several factors, including stain accumulation, water sorption, dissolution of the ingredients, and surface roughness.[598]

According to previous studies, color differences with corresponding ΔE values lower than 1.0 are not visually detectable by the human eye, and 3.3 NBS units are acceptable in clinical dentistry.[577,579,580,599] According to NBS units[579,599], after thermal aging, loading Probase Cold with CHX caused "perceivable change", while the other two acrylic resins, Kooliner and Ufi Gel Hard, showed "much appreciable" and "appreciable change", respectively. These two direct reline resins showed ΔE values not clinically acceptable (ΔE > 3.3).

All CHX-loaded specimens showed a high color change considered clinically unacceptable after chemical aging, regardless of the type of resin used. Loading CHX to Kooliner and Probase Cold caused "change to another color" and in Ufi Gel Hard caused a "appreciable change". Pero *et al.* (2013)[579] also concluded that incorporating an antimicrobial polymer into an acrylic resin increased the roughness of surfaces and wettability and produced clinically relevant color changes.

It seems that chemical aging with saliva and an acidic medium leads to drastic higher color change than thermal aging in water, probably due to the different aging environments.[13,600] These results are in accordance with a previous study with soft lining material[601] where the authors stated that an ionic solution like artificial saliva might affect the tested biomaterials more than water. Saliva has more components than water, such as inorganic and organic substances like immunoglobulins, proteins, enzymes, and mucins.[602]

In general, we can conclude that after aging processes there were clinically relevant color changes and therefore more studies are needed. However, considering that this novel drug delivery system is used under acrylic resins, the impact on the color stability may not be important to patients. The benefit of a long-term therapeutic effect will likely outweigh the disadvantage of the lack of color stability.

In Kooliner and Ufi Gel Hard, CHX loading did not cause detrimental mechanical and surface properties in either aging method. These findings can be explained by the results of a previous study where Ufi Gel Hard had the highest CHX release because it was the most porous resin. Therefore, the specimens with CHX aged while releasing all the drug through pores and cracks, not affecting their properties.

Future investigations should be carried out combining these two aging methods or other method (e.g., mechanical) to better reproduces the oral environment. After the *in-vitro* investigations, *in-vivo* studies should be designed and conducted to confirm the

potential of this novel chlorhexidine delivery system in the treatment of denture stomatitis.

5.6. Conclusions

Within the limitations of this *in-vitro* experimental study, it can be established that:

- Loading Probase Cold with CHX negatively affected its microhardness compared to the control after a thermal aging process. The microhardness of the other two reline resins was not affected by CHX loading.
- After chemical aging, loading with CHX did not affect the microhardness of any of the reline acrylic resins.
- After thermal aging, loading Kooliner with CHX increased flexural strength values compared to the control, while in Probase Cold, the CHX group showed lower flexural strength values than the control.
- Loading Kooliner and Ufi Gel Hard with CHX did not affect their flexural strength after chemical aging. However, loading Probase Cold with CHX affected its flexural strength, presenting lower values than the control group.
- Loading Kooliner and Ufi Gel Hard with CHX did not affect the surface free energy after thermal aging, while Probase Cold with CHX showed higher values compared to the control.
- After undergoing a chemical aging, loading all reline acrylic resins with CHX did not affect the surface free energy.
- After thermal aging, loading Kooliner with CHX affected the color stability and caused the highest color change. However, loading CHX did not affect the color stability of the two other reline acrylic resins.
- CHX incorporation into all reline acrylic resins led to a higher color change after chemical aging.

Under our experimental conditions, the incorporation of CHX in direct reline resins (Kooliner and Ufi Gel Hard) did not show negative effects on the properties evaluated (microhardness, flexural strength, and surface free energy) with either aging method. However, regarding color stability, loading Kooliner with CHX showed higher ΔE values after thermal and chemical aging, and Ufi Gel Hard only after chemical aging.

In Probase Cold, loading with 5% CHX negatively affected its microhardness, after a thermal aging correspondent to 1 month of exposure to the oral environment, and its flexural strength after both aging methods. No other negative effects were observed on this CHX-loaded indirect reline acrylic resins' surface free energy. The color stability was only affected after 28 days of a chemical aging process.

Considering the overall results, loading CHX into reline acrylic resins continues to be a potential approach in the prevention or treatment of DS. However, thermal fluctuations should be considered when using Probase Cold as the CHX delivery carrier.

Chapter 6

Concluding Remarks and Prospects

6. Concluding Remarks and Prospects

6.1. Concluding Remarks

Within the limitations of the *in-vitro* studies, the following conclusions were made:

- The microbiological investigation confirmed the antimicrobial efficacy of the drug-supplemented delivery systems and favored the use of polymeric systems with an antifungal drug in small concentrations.
- The ideal concentration of chlorhexidine to be loaded was 2.5% (w/w) for Kooliner and 5% for Ufi Gel Hard and Probase Cold.
- Chlorhexidine could be successfully incorporated into reline acrylic resins without affecting the polymer's chemical structure.
- Chlorhexidine release studies indicate that it leaks out of the polymer into the surrounding fluid medium in an environment similar to that of the oral cavity. Drug release profiles showed a high initial release rate (24-48 hours), followed by a slower and steadier release that continued until the end of the evaluated period (28 days).
- Different reline acrylic resins' compositions affect the drug release. Ufi Gel Hard revealed the highest amounts of chlorhexidine released.
- The incorporation of chlorhexidine decreased cell viability, with Probase Cold being the least cytotoxic resin and Ufi Gel Hard the most cytotoxic to mouse fibroblasts.
- Loading chlorhexidine into reline acrylic resins did not change their mechanical and surface properties immediately after being prepared.

- After undergoing thermal aging, loading Kooliner and Ufi Gel Hard with chlorhexidine did not affect the resins' mechanical and surface properties. However, Probase Cold showed decreased microhardness and flexural strength and increased surface free energy values compared to the controls.

- The incorporation of chlorhexidine did not affect color stability in Ufi Gel Hard and Probase Cold. However, chlorhexidine caused changes in the chromatic stability of Kooliner.
- After undergoing chemical aging, loading Probase Cold with the drug decreased its flexural strength, while chlorhexidine-loaded Kooliner and Ufi Gel Hard kept their properties.
- Chlorhexidine incorporation caused a significant color change after chemical aging in all the evaluated reline acrylic resins.

This thesis represents the first evidence of antimicrobial activity of a chlorhexidine delivery system based on reline acrylic resins with lower concentrations and after aging processes. Based on these studies' results, loading chlorhexidine into reline acrylic resins as a drug delivery system can remove *C. albicans* biofilm without affecting the materials' properties and may be a useful innovative method to prevent and treat denture stomatitis.

Regarding drug release properties, Ufi Gel Hard could be an efficient choice for acute denture candidiasis because it will provide a higher chlorhexidine release. It could then be replaced by Kooliner or Probase Cold to maintain the release and prevent relapses. Moreover, results suggest that the best option for a chlorhexidine delivery-system is Probase Cold with 5% of the drug because it presented a high antibiofilm effect against the fungus and low cytotoxicity. Besides the drug delivery system, rigorous hygiene habits for both the denture surface and the oral mucosa are needed to maintain oral health and prevent the appearance of denture stomatitis in denture wearers.

The results pave the way for a controlled *in-vivo* evaluation of a novel chlorhexidine delivery system. The study designs and standardized conditions corroborate the internal validity of the study. As for external validity, there may be some bias, mainly associated with the fact that the aging conditions may not represent those existing in the clinical context in the oral cavity. Therefore, these findings cannot be generalized without conducting a clinical study.

6.2. Prospects

Considering the etiology and contributing factors, the management of denture stomatitis remains challenging. In the future, the incorporation of antifungal agents into acrylic resins' materials may be considered a reliable therapeutic option.

The proposed novel drug delivery system consists of incorporating chlorhexidine into three different reline acrylic resins by mixing the drug with the acrylic powder. For this novel drug delivery system to be used routinely in clinical practice, several previous scientific studies are needed. This drug delivery system has shown to be effective and feasible in all the presented *in-vitro* studies, at baseline and after undergoing thermal and chemical aging processes.

This present work described the *in-vitro* behavior of this novel drug delivery system at baseline (Chapter 4) and after aging (Chapter 5) to simulate clinical situations. Future investigations simultaneously combining the two aging methods should be carried out. Moreover, since correct denture hygiene and disinfection are essential for denture stomatitis prevention, future studies should compare the effects of immersion cleansers on the properties of this novel chlorhexidine delivery system. Studies about materials' surface characteristics, like surface free energy, allow for the association of such characteristics with microbial adhesion susceptibility. Thus, future investigation should be done on the roughness of these chlorhexidine-loaded reline acrylic resins. Provided the good performance of this chlorhexidine drug delivery system in *in-vitro* studies, the need for an *in-vivo* study emerges. Animal or human experiences in a controlled environment should be performed in the future to expose the materials to the complexity of the oral cavity and, thus, guide the implementation of this novel drug delivery system in clinical practice.

After approval by the research and ethics committee, a future clinical study can be designed as follow:

1. Choose participants according to inclusion criteria (presenting denture stomatitis);
2. Sign a written informed consent;
3. Randomly divide patients into two groups: A) chlorhexidine delivery system – relining a denture base with chlorhexidine-loaded reline acrylic resins;

B) control group – relining a denture base with the same reline acrylic resins without chlorhexidine;

4. Determine the levels of *Candida albicans* species from swab samples obtained from denture fitting surfaces and oral mucosa (affected and unaffected);
5. Quantify at various time points (baseline and six weeks, for example).

This type of randomized controlled clinical trial is still necessary in the future to improve the efficacy of chlorhexidine drug delivery systems for denture stomatitis patients.

If the results of further *in-vivo* testing are favorable, this novel chlorhexidine delivery system based on reline acrylic resins can be used as a preventive or therapeutic agent against *Candida*-associated denture stomatitis. Either way, our recommendation for the prevention and treatment of denture stomatitis is an association of more than one treatment, as follows:

- Incorporation of chlorhexidine into reline acrylic resin (drug delivery system);
- Correct denture and oral hygiene;
- Remove denture at night and avoid continuous denture wear;
- Regular dental visits.

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Appendices

Appendix 1 – Tables

Table 1 – Articles excluded from the systematic review by exclusion criteria.

EXCLUSION CRITERIA	***ARTICLE*** *Author (Date of publication)*
Full texts unavailable	Yang Y *et al.* (2015); Haghi HR *et al.* (2015); Kumar MN *et al.* (2012); Tao JX *et al.* (2012); Savabi O *et al.* (2013); Sujitha K *et al.* (2018); Maluf CV *et al.* (2019); Amin F *et al.* (2014); Suganya S *et al.* (2014)
Other types of studies	Varela S *et al.* (2017) [systematic review]
	Sivakumar I *et al.* (2014); Raj P & Dentino A (2013); Rickman LJ *et al.* (2012) [reviews]
Not related to the topic	Dorocka-Bobkowska B *et al.* (2017); Hashem M (2015); Matos A *et al.* (2018); Goiato M *et al.* (2013); Hayran Y *et al.* (2018); Lira A *et al.* (2012); Neppelenbroek K *et al.* (2015); Tsuji M *et al.* (2016); O'Donnell *et al.* (2017); Panariello B *et al.* (2015); Liu S *et al.* (2016); Kamochi G *et al.* (2018); Pero A *et al.* (2016); Albrecht N *et al.* (2018); Silva P *et al.* (2011); Izumi S *et al.* (2016); Goiato M *et al.* (2014); Quishida C *et al.* (2015); Li B *et al.* (2016); Wu T *et al.* (2013); Lima J *et al.* (2016); Bertolini M *et al.* (2014); Hotta J *et al.* (2019); Machado A *et al.* (2011); Silva M *et al.* (2013); Fatemi F *et al.* (2019); Zoccolotti J *et al.* (2018); Sharan S *et al.* (2012); Campanha N *et al.* (2012); Zoccolotti J *et al.* (2019); Ohno T *et al.* (2019); Kurtulmus-Yilmaz S & Deniz S (2014); Alzayer Y *et al.* (2020); Urban V *et al.* (2015); Wiench R *et al.* (2019); Alcântara C *et al.* (2012); Menini *et al.* (2015); Machado A *et al.* (2012); Zamperini C *et al.* (2013); Matthes R *et al.* (2015); Davi L *et al.* (2012); Nakahara T *et al.* (2013); Senna P *et al.* (2013); Vasconcelos L *et al.* (2013); Brożek R *et al.* (2011); Salim N *et al.* (2012); Zamperini C *et al.* (2010); You W *et al.* (2011); Srivatstava A *et al.* (2013); Yu S *et al.* (2012); Paranhos H *et al.* (2014); Neves C *et al.* (2013); Hong G *et al.* (2012); Mariotto L *et al.* (2020); Mutluay M *et al.* (2010); Piskin B, *et al.* (2014); Pisani M *et al.* (2012); Felipucci D *et al.* (2011); Salim N *et al.* (2012); Chopde N *et al.* (2012); Sharma S & Hegde V (2014); Kanathila H, *et al.* (2011); Freire T *et al.* (2014); Radnai M *et al.* (2010); Silva M *et al.* (2016); Procópio A *et al.* (2018); Masseti P *et al.* (2018); Kurt A *et al.* (2018); Basavarajappa S *et al.* (2016); Fernandes F *et al.* (2013); Yildirim-Bicer A *et al.* (2014); Goiato M *et al.* (2013); Karakis D *et al.* (2016); Paranhos H *et al.* (2013); Orsi I *et al.* (2011); Meriç G *et al.* (2017); Basavarajappa S *et al.* (2017); Duyck J *et al.* (2013); Porta S *et al.* (2015); Acosta E *et al.* (2015); Li L *et al.* (2010); Fernandes F *et al.* (2011); Uludamar A *et al.* (2011); da Silva P *et al.* (2011); Orsi I *et al.* (2011); Carvalho C *et al.* (2012); Rossato M *et al.* (2011); Odagiri K *et al.* (2012); Gusmão J & Pereira R (2013); Antunes D *et al.* (2015); Polyzois G *et al.* (2013); Gondim B *et al.* (2018)

Table 2 – Summary of the characteristics in the articles included in the systematic review.

AUTHOR(S)	*YEAR*	*DRUG*	*METHOD*	*ACRYLIC RESIN*	*CHARACTERISTICS STUDIES*	*MICROBIAL SPECIES*
Cartagena *et al.*	2017	Miconazole	Coating	Vipi Wave	Antifungal activity Tensile strength Cytotoxicity	*C. albicans*
Basunbul *et al.*	2018	Miconazole	Coating	Vipi Wave	Tensile strength	----
Feng *et al.*	2017	Silver	Coating	New Century, Nissin, Basis-Hi, Meliodent	Elastic modulus Flexural strength Surface characteristics	----
Kamikawa *et al.*	2014	Silver	Coating	Acron	Antifungal activity	*C. albicans* *C. glabrata*
Shinogaga & Arita	2012	Silver	Coating	Clarex	Antimicrobial activity Superficial characteristics	*Staphylococcus aureus*
Cierech *et al.*	2016	Zinc oxide	Coating	Superacryl Plus	Antifungal activity	*C. albicans*
Su *et al.*	2010	Titanium dioxide	Coating	PMMA sheet obtained from Shangai Dingfanghong Acrylic Factory	Antimicrobial activity Superficial characteristics	*Staphylococcus aureus* *Escherichia coli*
Almeida *et al.*	2018	Equisetum giganteum Punica granatum	Coating	Lucitone 550	Antifungal activity	*C. albicans*
Namangkalakul *et al.*	2020	Chitosan	Coating	Meliodent	Antifungal activity	*C. albicans* *C. glabrata* *C. Krusei* *C. parapsilosis* *C. tropicalis*
Sun *et al.*	2013	Miconazole Chlorhexidine	Immersion	Lucitone 199	Antifungal activity Drug release	*C. albicans*
Cao *et al.*	2010	Miconazole Chlorhexidine	Immersion	Methacrylic acid (MMA) + diurethane dimethacrylate (DUDMA)	Antifungal activity Drug release	*C. albicans*
Gebremedhin *et al.*	2014	Miconazole	Immersion	Heat-cured PMMA obtained from Hing Lung Engineering, Inc.	Antifungal activity	*C. albicans* *C. glabrata* *C. tropicalis* *C. parapsilosis*
Malakhov *et al.*	2016	Miconazole	Immersion	Lucitone 199 + PNVP [poly(N-vinyl-2-pyrrolidinone)]	Antifungal activity Drug release	*Candida isolates*
Dalwai *et al.*	2016	Chlorhexidine Fluconazole Tea tree oil	Immersion	Trevalon	Antifungal activity	*C. albicans*

Ellepola *et al.*	2015	Chlorhexidine Fluconazole Nystatin	Immersion	PMMA, Dentsply	Antifungal activity	*C. dubliniensis*
Al-Dwairi *et al.*	2012	Fluconazole Nystatin	Immersion	PMMA, Meliodent	Antifungal activity Hardness Surface roughness Contact angle	*C. albicans*
Francisconi *et al.*	2020	Nystatin Tea tree oil	Immersion	Vipi Colorless Wave	Antifungal activity	*C. albicans*
Hua *et al.*	2010	Peptide mimetic compounds	Immersion	Lucitone 199	Antifungal activity	*C. albicans*
Zou *et al.*	2020	Cellulose	Immersion	Acron	Antimicrobial activity Flexural strength Cytotoxicity	*Streptococcus mutans* *Porphyromonas gingivalis*
Da Silva *et al.*	2017	Equisetum giganteum	Immersion	Vipi Cril plus	Antifungal activity	*C. albicans*
Koroglu *et al.*	2016	Silver	Incorporation	PMMA, Meliodent + Acron	Flexural strength Elastic modulus	----
Li *et al.*	2016	Silver	Incorporation	Type 1 denture base acrylic resin	Antifungal activity Superficial characteristics	*C. albicans*
Wady *et al.*	2012	Silver	Incorporation	Vipi Wave	Antifungal activity	*C. albicans*
Nam *et al.*	2012	Silver	Incorporation	Lucitone 199	Antifungal activity Color	*C. albicans*
Acosta-Torres *et al.*	2012	Silver	Incorporation	Nature-Cryl	Antifungal activity Flexural strength	*C. albicans*
Monteiro *et al.*	2012	Silver	Incorporation	Lucitone 550	Antifungal activity Drug release	*C. albicans*
Munikamaiah *et al.*	2018	Silver	Incorporation	Lucitone 199	Flexural strength	----
Kurt *et al.*	2017	Silver	Incorporation	PMMA, Meliodent	Antifungal activity Cytotoxicity	*C. albicans*
Zhang *et al.*	2017	Silver	Incorporation	PMMA resin	Antifungal activity Flexural strength Cytotoxicity	*C. albicans*
Malic *et al.*	2019	Silver	Incorporation	Pegasus Plus	Antifungal activity Drug release Cytotoxicity	*C. albicans* *Streptococcus mutans* *Fusobacterium nucleatum*

De Castro *et al.*	2016	Silver	Incorporation	Dêncor Lay + Clássico	Antimicrobial activity Impact strength	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Bacali *et al.*	2019	Silver	Incorporation	Vertex Dental	Antimicrobial activity Flexural strength Cytotoxicity	*Escherichia coli* *Staphylococcus aureus* *Streptocococcus mutans*
Ryalat *et al.*	2011	Chlorhexidine	Incorporation	Paladur	Antifungal activity Drug release	*C. albicans*
Salim *et al.*	2013	Chlorhexidine Fluconazole	Incorporation	Lucite	Antifungal activity	*C. albicans*
Al-Haddad *et al.*	2014	Chlorhexidine Fluconazole	Incorporation	PMMA resin	Fracture toughness	----
Darwish *et al.*	2011	Fluconazole	Incorporation	Paladur	Antifungal activity Drug release	*C. albicans*
Kamonkhantikul *et al.*	2017	Zinc oxide	Incorporation	SR Triplex Hot	Antifungal activity Flexural strength Color	*C. albicans*
Raj *et al.*	2018	Zinc oxide	Incorporation	PMMA, Alfa Aesar	Antimicrobial activity Fracture resistance Cytotoxicity	*Streptococcal mutans* *C. albicans*
Sawada *et al.*	2010	Titanium dioxide	Incorporation	Natural Resin	Antifungal activity	*C. albicans*
Nazirkar *et al.*	2014	Titanium dioxide	Incorporation	PMMA resin	Flexural strength	----
Totu *et al.*	2017	Titanium dioxide	Incorporation	E-Dent 100	Antifungal activity	*C. scotti*
Gad *et al.*	2017	Zirconia	Incorporation	Major.Base.20	Antifungal activity	*C. albicans*
Nezu *et al.*	2017	Ethanol	Incorporation	Procast DSP Clear Shade	Elastic modulus Color	----
Sadeghi *et al.*	2016	Chitosan	Incorporation	Transbond XT	Antifungal activity	*C. albicans* *C. glabrata* *C. tropicalis* *C. Krusei*
Gad *et al.*	2020	Timoquinona	Incorporation	Major base 20	Flexural strength Elastic modulus Roughness Hardness	----

Nawasrah *et al.*	2016	Henna	Incorporation	Trevalon	Antifungal activity	*C. albicans*
Nawasrah *et al.*	2018	Henna	Incorporation	Major base 20	Rugosidade Hardness	----
Yassin *et al.*	2016	Sodium fluoride	Incorporation	Wintercryl Rapid Repair	Antimicrobial activity Drug release	*C. albicans* *Lactobacillus casei* *Streptococcus mutans*
Lee *et al.*	2016	Mesoporous silica	Incorporation	Orthocryl	Antimicrobial activity Flexural strength Roughness Hardness Cytotoxicity	*C. albicans* *Streptococcus oralis*
Chen *et al.*	2017	Zirconia dioxide	Incorporation	Batch No. 201103, Shanghai Medical Instruments	Antimicrobial activity Flexural strength Hardness Cytotoxicity	*C. albicans* *S. mutans*
Tsutsumi *et al.*	2016	Prereacted glass ionomer (S-PRG) fillers	Incorporation	Urban	Antifungal activity Roughness	*C. albicans*
Mirizadeh *et al.*	2018	Quaternized ammonium	Incorporation	Vertex	Antimicrobial activity Flexural strength Drug release	*C. albicans* *E. coli* *S. aureus*
Herman *et al.*	2017	DABCO	Incorporation	Ivocap High Impact	Antifungal activity Cytotoxicity	*C. albicans* *C. dubliniensis* *C. glabrata* *C. parapsilosis* *C. tropicalis*
Regis *et al.*	2012	MUPB	Incorporation	Lucitone 550	Antimicrobial activity Cytotoxicity	*C. albicans* *C. dubliniensis* *C. glabrata* *Lactobacillus casei* *Staphylococcus aureus* *Streptococcus mutans*
Regis *et al.*	2011	MUPB	Incorporation	Lucitone 550	Hardness Roughness Flexural strength Color	----
Marra *et al.*	2011	PTBAEMA	Incorporation	Lucitone 550	Antimicrobial activity	*C. albicans* *Staphylococcus aureus* *Streptococcus mutans*
Paleari *et al.*	2012	TBAEMA	Incorporation	Lucitone 550	Flexural strength	----

Table 3 – Contact angle data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*ADVANCE CONTACT ANGLE (°)*			
			WATER		*1,2-PROPANEDIOL*	
			M ± SD	*MED ± IQR*	*M ± SD*	*MED ± IQR*
Kooliner	**0%**	5	96.1 ± 10.01	92.1 ± 17.93	64.2 ± 2.18	63.3 ± 4.01
	2.5%	5	88.7 ± 3.62	87.3 ± 6.78	42.4 ± 2.42	41.9 ± 4.01
Ufi Gel Hard	**0%**	5	88.0 ± 4.13	88.4 ± 8.22	33.8 ± 4.72	33.2 ± 7.17
	5%	5	79.1 ± 5.96	82.6 ± 10.98	26.0 ± 2.47	26.3 ± 4.03
Probase Cold	**0%**	5	94.0 ± 4.80	93.5 ± 8.53	85.6 ± 2.57	59.2 ± 4.79
	5%	5	82.4 ± 3.06	83.7 ± 4.99	47.1 ± 2.39	47.8 ± 3.58

CHX = Chlorhexidine, M = Mean, SD = Standard deviation, MED = Median, IQR = Interquartile range.

Table 4 – National Bureau of Standards units for expressing color differences.

NBS Units	*Color Differences*
0.0 – 0.5	Extremely slight change
0.5 – 1.5	Slight change
1.5 – 3.0	Perceivable change
3.0 – 6.0	Appreciable change
6.0 – 12.0	Much appreciable
12.0 – +++	Change to another color

Table 5 – Contact angle and surface free energy data by reline acrylic resin.

RELINE ACRYLIC RESIN	*CHX LOADING*	*n*	*Aging*	*ADVANCE CONTACT ANGLE (º)*		*SURFACE FREE ENERGY (mN/m)*	
				WATER	*1,2-PROPANEDIOL*	γ^d	γ^p
				M ± SD	*M ± SD*	*M ± SD*	*M ± SD*
Kooliner	**0%**	5	TA	82.8 ± 4.28	61.0 ± 5.77	11.3 ± 2.36	17.5 ± 3.75
	2.5%	5		86.7 ± 1.71	63.7 ± 7.33	11.0 ± 2.74	15.5 ± 3.16
	0%	5	CA	81.9 ± 5.00	43.4 ± 5.55	18.1 ± 4.28	13.6 ± 4.83
	2.5%	5		78.0 ± 5.35	45.1 ± 6.00	16.9 ± 3.41	16.3 ± 4.57
Ufi Gel Hard	**0%**	5	TA	90.4 ± 4.34	65.4 ± 14.79	11.9 ± 6.70	13.6 ± 4.68
	5%	5		83.7 ± 7.96	59.0 ± 11.36	11.8 ± 3.96	18.7 ± 4.83
	0%	5	CA	64.0 ± 2.70	29.1 ± 7.18	18.1 ± 1.93	23.5 ± 2.77
	5%	5		62.7 ± 1.15	28.1 ± 9.05	18.1 ± 2.49	24.4 ± 2.72
Probase Cold	**0%**	5	TA	88.0 ± 1.71	73.6 ± 14.68	8.1 ± 4.72	18.3 ± 4.81
	5%	5		80.4 ± 2.08	62.7 ± 2.48	10.1 ± 0.86	19.9 ± 1.68
	0%	5	CA	70.1 ± 2.99	44.0 ± 13.84	14.8 ± 4.26	22.6 ± 4.41
	5%	5		70.6 ± 1.53	36.0 ± 4.82	17.4 ± 1.46	19.8 ± 1.57

CHX = Chlorhexidine, TA = Thermal aging, CA = Chemical aging, M = Mean, SD = Standard deviation.

Appendix 2 – Figures

Figure 1 – Precision scale (A&D Company, Limited, Tokyo, Japan).

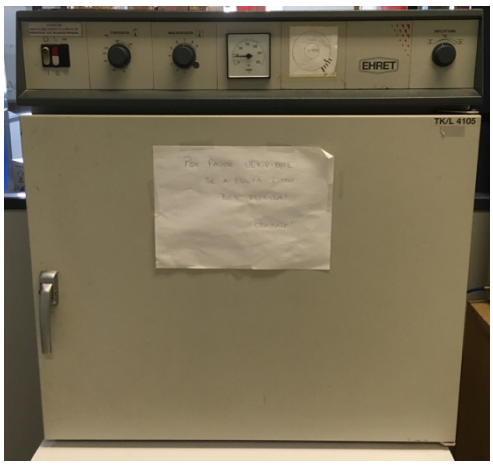


Figure 2 – Stove to maintain specimens at 37±2ºC (Ehret, Mahlberg, Germany).

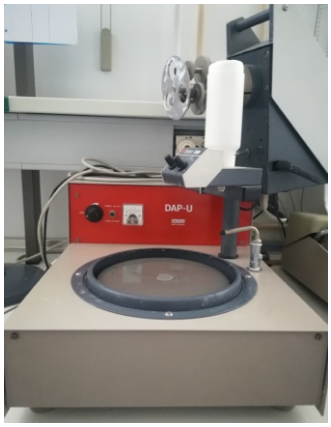


Figure 3 – Rotational polishing machine (DAP-U, Struers, Denmark).

Figure 4 – Spectrophotometer (U-2000, Hitachi).

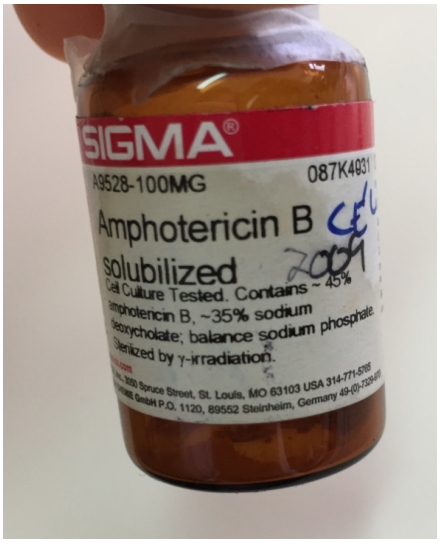


Figure 5 – Amphotericin B solubilized (Sigma Aldrich Co., St Louis, USA).

Figure 6 – Workspace for performing the agar disk diffusion test.

Figure 7 – Microhardness indentation machine (Duramin, Struers DK 2750, Ballerup, Denmark).

Figure 8 – Servo-hydraulic testing machine (Instron model 4502, Instron Ltd, Bucks, England).

Figure 9 – Digital micrometer of ±0.01-mm precision (Mitutoyo Digimatic, MFG.Co, Ltd. Tokyo, Japan).

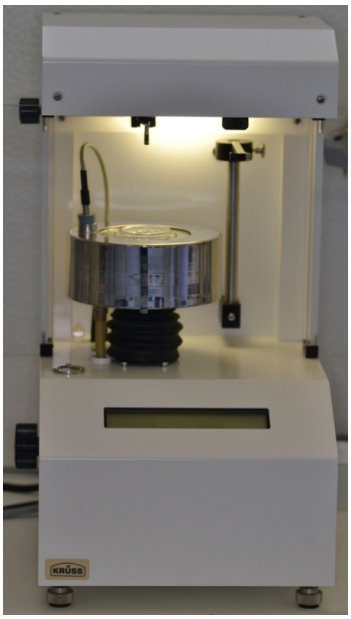


Figure 10 and 11 – Processor Tensiometer K12 (Kruss, Hamburg, Germany): equipment used in Wilhelmy plaque technique.

Figure 12 – 1,2-propanediol (Merck, Germany).

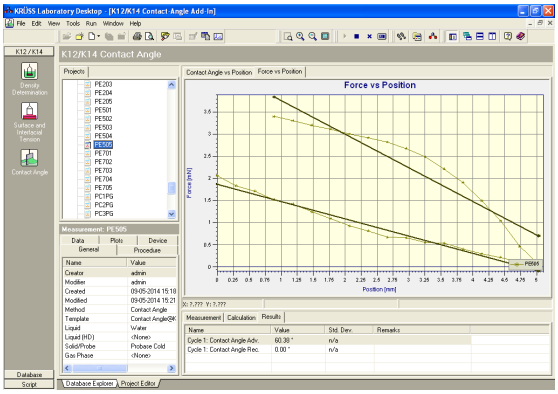


Figure 13 – KRUSS software program: K121contact angle measuring system (version 2.049).

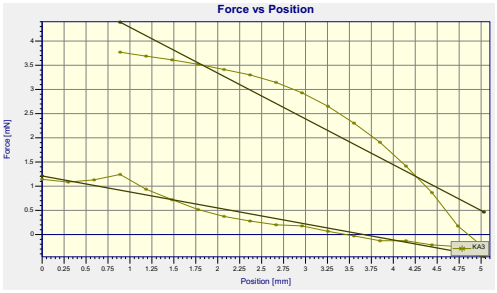


Figure 14 – One example of a graph obtained for the determination of the contact angle of a Kooliner specimen.

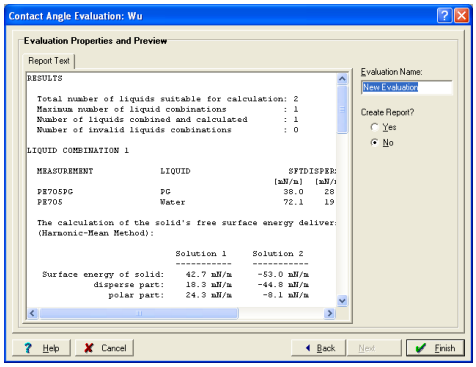


Figure 15 – One example of the determination of the surface free energy of a Probase Cold specimen.

Figure 16 – Probase Hot (Ivoclar Vivadent, Liechtenstein).

Figure 17 – Stereomicroscope (EMZ-8TR, Meiji Techno Co, Saitama, Japan).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
2														
3	User: USER			Path: C:\Program Files\BMG\Omega\User\Data\							Test ID: 8199			
4	Test Name: Clor_abs								Date: 30-10-2020		Time: 7:58:41			
5	ID1: Clor_abs													
6	ID2: Absorbance													
7	ID3: 30-10-2020,7:58:40													
8	Absorbance			Absorbance values are displayed as OD										
9														
10														
11														
12		Raw Data (255)												
13		1	2	3	4	5	6	7	8	9	10	11	12	
14	A	0,1	0,083	0,084	0,104	0,075	0,083	0,072	0,074	0,078	0,078	0,088	0,087	
15	B	2,803	1,523	0,916	0,561	0,377	0,33	0,247	0,222	0,208	0,219	0,18	0,223	
16	C	0,165	0,222	0,166	0,092	0,08	0,09	0,076	0,077	0,078	0,097	0,091	0,076	
17	D	0,766	0,977	0,804	0,426	0,447	0,448	0,417	0,394	0,433	0,476	0,483	0,527	
18	E	0,493	0,487	0,505	0,708	0,759	0,735	0,974	0,591	0,823	0,61	0,619	0,588	
19	F	0,64	0,683	0,668	0,594	0,643	0,621	0,697	0,722	0,695	0,984	1,106	1,144	
20	G	0,395	0,421	0,626	0,592	0,593	0,585	0,55	0,504	0,529	0,597	0,703	0,669	
21	H	0,438	0,451	0,431	0,618	0,62	0,698	0,095	0,079	0,07	0,081	0,079	0,124	
22														
23														
24														
25														

Figure 18 – One example of absorbance values obtained for CHX content using a microplate reader.

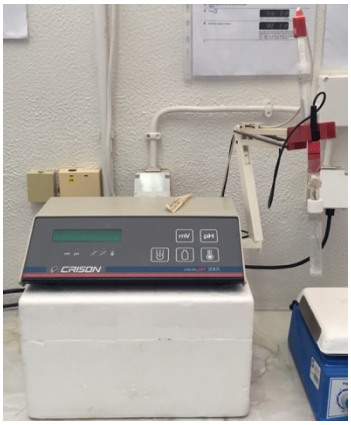


Figure 19 – pH meter (Crison Micro pH 2001, Hach Lange, Barcelona, Spain) used to prepare artificial saliva.

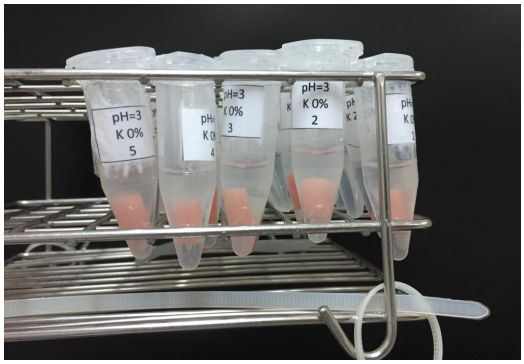


Figure 20 – Immersion of cylinder specimens in an adequate volume of artificial saliva at pH=3.

Appendix 3 – Experimental Data

1. Agar Disk Diffusion Tests

MATERIAL	CHX %	MEASURES (mm)					
		C. albicans			*S. oralis*		
Kooliner	Control	0	0	0	0	0	0
Kooliner	Control	0	0	0	0	0	0
Kooliner	Control	0	0	0	0	0	0
Kooliner	1%	0	0	0	14.03	13.08	14.05
Kooliner	1%	0	0	0	13.06	14.01	13.10
Kooliner	1%	0	0	0	14.13	14.03	13.23
Kooliner	2.5%	8.43	8.25	8.97	17.03	16.46	17.00
Kooliner	2.5%	10.00	9.55	10.63	18.02	18.00	17.01
Kooliner	2.5%	8.05	8.49	8.19	18.03	18.02	17.09
Kooliner	5%	9.15	9.15	9.09	13.04	14.01	13.32
Kooliner	5%	7.86	6.94	7.57	13.00	13.23	13-04
Kooliner	5%	8.10	7.17	7.71	14.05	15.02	15.75
Kooliner	7.5%	9.60	9.98	9.28	18.03	18.02	17.33
Kooliner	7.5%	8.32	8.44	8.44	16.55	16.17	16.20
Kooliner	7.5%	11.59	11.43	11.43	18.11	18.00	18.57
Kooliner	10%	10.17	9.95	10.37	20.02	19.57	20.58
Kooliner	10%	10.78	11.51	11.36	20.03	21.02	18.47
Kooliner	10%	11.25	11.29	10.72	22.48	20.38	20.56
Ufi Gel Hard	Control	0	0	0	0	0	0
Ufi Gel Hard	Control	0	0	0	0	0	0
Ufi Gel Hard	Control	0	0	0	0	0	0
Ufi Gel Hard	1%	0	0	0	12.00	11.04	11.05
Ufi Gel Hard	1%	0	0	0	11.10	11.50	11.48
Ufi Gel Hard	1%	0	0	0	12.43	12.03	11.79
Ufi Gel Hard	2.5%	0	0	0	15.67	14.18	15.69
Ufi Gel Hard	2.5%	0	0	0	12.87	12.87	12.58
Ufi Gel Hard	2.5%	0	0	0	12.59	11.53	13.01
Ufi Gel Hard	5%	10.08	9.83	9.88	16.03	15.98	16.13
Ufi Gel Hard	5%	9.73	9.51	9.95	16.10	16.23	16.02
Ufi Gel Hard	5%	9.98	10.71	10.23	17.45	17.60	17.59
Ufi Gel Hard	7.5%	9.25	8.60	9.07	18.02	17.55	16.82
Ufi Gel Hard	7.5%	10.04	10.58	10.92	13.90	12.56	14.48

Ufi Gel Hard	7.5%	10.10	10.30	10.10	13.81	13.67	13.69
Ufi Gel Hard	10%	8.65	9.06	8.87	16.85	16.58	16.58
Ufi Gel Hard	10%	10.70	10.88	11.12	19.55	14.57	15.58
Ufi Gel Hard	10%	9.71	9.99	9.46	15.67	14.56	20.46
Probase Cold	Control	0	0	0	0	0	0
Probase Cold	Control	0	0	0	0	0	0
Probase Cold	Control	0	0	0	0	0	0
Probase Cold	1%	0	0	0	11.31	10.87	10.94
Probase Cold	1%	0	0	0	7.94	7.68	7.44
Probase Cold	1%	0	0	0	6.80	7.42	7.40
Probase Cold	2.5%	0	0	0	9.03	9.89	10.87
Probase Cold	2.5%	0	0	0	11.59	11.30	10.86
Probase Cold	2.5%	0	0	0	7.87	8.03	8.46
Probase Cold	5%	6.36	6.56	6.11	11.23	10.76	10.98
Probase Cold	5%	6.70	7.20	6.96	10.18	10.59	9.87
Probase Cold	5%	8.25	8.59	9.03	9.59	10.87	8.96
Probase Cold	7.5%	7.72	8.07	8.07	18.78	17.84	18.63
Probase Cold	7.5%	7.85	7.58	7.12	11.30	11.59	10.98
Probase Cold	7.5%	10.29	9.09	9.22	11.43	10.98	11.03
Probase Cold	10%	8.33	8.60	8.13	15.03	13.78	14.28
Probase Cold	10%	8.73	8.96	8.94	12.87	14.87	13.57
Probase Cold	10%	10.17	9.69	10.22	15.10	13.97	13.60

2. Microhardness

MATERIAL	CHX %	INDENTATION (KHN)											
		1	2	3	4	5	6	7	8	9	10	11	12
Kooliner	Control	5.5	5.4	5.8	4.0	5.5	4.7	6.0	5.2	5.6	8.8	6.0	5.0
Kooliner	Control	5.1	5.9	4.8	7.2	5.3	5.4	4.4	6.6	5.3	6.1	5.5	5.9
Kooliner	Control	5.1	4.7	5.3	5.2	5.0	5.7	6.8	4.9	6.6	6.1	6.9	5.0
Kooliner	Control	4.4	4.8	6.8	4.4	3.9	4.3	4.0	3.9	3.7	4.4	3.9	5.9
Kooliner	Control	6.1	4.8	5.6	5.8	5.5	5.2	5.4	5.3	5.0	7.7	6.6	5.2
Kooliner	Control	5.5	7.2	5.9	4.7	7.0	5.1	6.2	4.9	6.1	5.2	4.5	5.4
Kooliner	Control	5.3	5.0	6.4	4.4	6.8	5.7	6.5	5.7	4.5	5.1	4.4	4.5
Kooliner	Control	5.6	5.3	5.1	6.8	4.7	4.3	5.9	6.7	4.8	4.8	5.7	5.6
Kooliner	2.5%	4.8	6.3	7.4	6.1	6.4	5.8	5.8	5.6	5.0	7.6	5.5	5.2
Kooliner	2.5%	7.0	5.2	5.3	6.1	7.5	7.7	6.1	8.1	5.9	6.0	7.5	6.2
Kooliner	2.5%	4.6	5.6	5.0	4.8	5.6	5.4	4.5	4.8	5.5	5.1	4.4	4.5
Kooliner	2.5%	4.0	4.7	4.9	5.2	4.7	4.6	4.0	4.6	4.6	4.0	4.2	4.7
Kooliner	2.5%	4.8	4.7	4.9	6.1	3.9	4.7	4.7	7.5	5.8	4.7	4.4	4.0
Kooliner	2.5%	3.9	3.6	4.3	4.9	5.0	5.4	4.6	5.0	5.6	4.5	6.9	5.5
Kooliner	2.5%	4.1	4.4	4.4	4.0	5.6	4.4	6.1	4.1	6.2	6.8	6.7	4.2
Kooliner	2.5%	7.0	6.2	5.5	5.6	6.2	7.5	6.1	5.0	6.4	5.7	6.8	5.9
Ufi Gel Hard	Control	9.0	9.2	7.7	8.9	8.3	8.4	8.5	8.7	8.2	8.0	9.6	9.1
Ufi Gel Hard	Control	9.1	9.2	8.5	8.6	7.3	8.6	8.7	8.1	6.8	7.0	8.3	8.2
Ufi Gel Hard	Control	7.4	7.7	8.5	10.1	7.7	9.0	8.5	8.4	8.6	8.9	8.2	8.0
Ufi Gel Hard	Control	9.9	9.2	9.0	9.7	9.7	8.6	8.4	9.7	9.3	4.7	10.2	9.2
Ufi Gel Hard	Control	8.9	8.7	9.4	10.2	9.2	9.0	9.9	10.3	8.4	9.8	7.8	8.2
Ufi Gel Hard	Control	8.5	9.8	8.3	11.1	7.9	9.1	7.8	9.2	10.8	7.6	8.1	8.1
Ufi Gel Hard	Control	7.5	7.8	7.7	7.0	8.5	7.8	7.7	8.9	8.4	7.6	8.3	7.8
Ufi Gel Hard	Control	7.7	7.6	9.8	8.4	7.3	8.8	6.9	7.7	8.4	5.1	8.4	8.7
Ufi Gel Hard	5%	9.1	9.4	9.0	10.3	9.4	8.8	9.0	9.9	7.5	9.0	8.8	9.0
Ufi Gel Hard	5%	12.4	10.6	11.8	10.4	12.2	11.4	11.8	11.9	11.4	10.8	9.7	10.9
Ufi Gel Hard	5%	9.3	8.8	8.1	8.8	8.9	9.7	8.8	9.1	9.8	9.2	9.0	7.7
Ufi Gel Hard	5%	9.6	10.3	10.3	10.9	9.5	11.4	11.5	10.8	9.2	9.0	10.1	10.4
Ufi Gel Hard	5%	9.2	11.4	10.4	10.5	9.9	9.6	9.1	10.6	10.5	10.8	9.9	9.1
Ufi Gel Hard	5%	11.0	9.8	11.9	9.1	11.8	11.5	12.0	11.1	11.2	10.9	10.8	11.0
Ufi Gel Hard	5%	9.1	8.5	8.4	8.1	8.4	8.6	9.1	9.4	8.6	10.0	9.7	8.8
Ufi Gel Hard	5%	10.3	7.4	9.2	10.2	9.0	7.9	10.8	12.5	9.1	9.7	10.7	8.5
Probase Cold	Control	10.0	11.2	11.2	11.8	11.1	11.3	13.0	10.4	11.3	11.7	11.8	12.5
Probase Cold	Control	11.6	11.2	11.3	11.0	11.8	11.4	11.7	12.5	12.3	13.2	11.3	10.2
Probase Cold	Control	11.8	11.7	11.5	12.4	11.5	11.8	10.0	11.0	11.5	11.0	12.1	13.2
Probase Cold	Control	11.4	13.6	12.6	11.7	12.5	11.4	13.9	13.2	11.4	13.1	12.7	13.0
Probase Cold	Control	11.4	11.1	11.7	12.4	12.0	10.9	13.6	11.5	11.4	12.1	14.6	13.0
Probase Cold	Control	12.6	12.0	11.5	12.6	11.7	11.6	12.0	9.9	13.3	11.3	11.6	13.2

Probase Cold	Control	12.1	11.5	12.2	11.7	13.1	13.4	12.6	12.1	12.2	12.1	12.1	11.0
Probase Cold	Control	10.8	10.4	10.7	10.8	10.6	10.5	10.3	10.6	10.8	11.4	11.2	11.1
Probase Cold	5%	11.8	14.0	11.5	11.6	10.5	10.1	13.0	10.9	11.7	10.8	12.0	11.1
Probase Cold	5%	11.5	9.9	11.4	10.9	11.1	10.8	12.0	11.2	10.1	11.4	11.5	11.4
Probase Cold	5%	11.0	10.8	10.3	11.4	12.3	11.8	11.4	10.8	11.9	11.6	11.1	12.3
Probase Cold	5%	13.4	12.6	11.7	11.3	12.0	11.3	11.3	10.8	12.4	10.5	11.5	11.4
Probase Cold	5%	11.3	11.5	12.3	13.4	11.5	11.4	12.0	11.1	11.3	12.0	10.8	11.5
Probase Cold	5%	12.4	12.0	11.5	11.0	10.4	11.7	11.4	13.4	11.7	10.9	12.6	11.8
Probase Cold	5%	11.9	12.9	12.3	12.1	12.5	13.4	12.7	11.8	12.2	11.0	12.5	12.3
Probase Cold	5%	13.2	11.5	11.3	10.9	11.4	13.3	12.0	12.3	10.8	11.3	11.7	12.7

3. Flexural Strength

MATERIAL	CHX %	LOAD AT YIELD (KN)	WIDTH (mm)	THICKHNESS (mm)	FLEXURAL STRENGTH (MPa)
Kooliner	Control	0.0539	10.14	3.08	41.94
Kooliner	Control	0.0579	10.08	3. 15	43.50
Kooliner	Control	0.0554	9.93	3.15	42.25
Kooliner	Control	0.0380	9.96	3.11	29.58
Kooliner	Control	0.0553	10.08	3.12	42.19
Kooliner	Control	0.0524	9.95	3.15	39.88
Kooliner	Control	0.0446	10.04	3.13	34.01
Kooliner	Control	0.0784	10.06	3.56	46.04
Kooliner	2.5%	0.0523	10.00	3.08	41.27
Kooliner	2.5%	0.0576	10.06	3.15	43.36
Kooliner	2.5%	0.0542	10.00	3.15	41.05
Kooliner	2.5%	0.0546	10.90	3.11	38.84
Kooliner	2.5%	0.0522	9.98	3.12	40.22
Kooliner	2.5%	0.0460	10.01	3.14	34.80
Kooliner	2.5%	0.0555	10.08	3.13	42.15
Kooliner	2.5%	0.0488	10.11	3.56	28.52
Ufi Gel Hard	Control	0.0439	10.00	3.14	33.38
Ufi Gel Hard	Control	0.0525	10.07	3.22	37.70
Ufi Gel Hard	Control	0.0665	10.00	3.22	48.21
Ufi Gel Hard	Control	0.0475	10.00	3.13	36.37
Ufi Gel Hard	Control	0.0697	10.07	3.29	47.95
Ufi Gel Hard	Control	0.0455	10.03	3.15	34.29
Ufi Gel Hard	Control	0.0499	10.04	3.24	35.52
Ufi Gel Hard	Control	0.0617	10.04	3.28	42.85
Ufi Gel Hard	5%	0.0458	9.97	3.14	34.94
Ufi Gel Hard	5%	0.0340	10.02	3.22	24.54
Ufi Gel Hard	5%	0.0475	10.00	3.22	34.36
Ufi Gel Hard	5%	0.0570	10.09	3.13	43.25
Ufi Gel Hard	5%	0.0483	10.00	3.29	33.47
Ufi Gel Hard	5%	0.0423	10.08	3.15	31.72
Ufi Gel Hard	5%	0.0432	9.97	3.24	30.96
Ufi Gel Hard	5%	0.0588	10.14	3.28	40.43
Probase Cold	Control	0.1351	9.68	3.32	94.97
Probase Cold	Control	0.9087	9.95	3.18	73.57
Probase Cold	Control	0.1149	10.22	3.26	79.34
Probase Cold	Control	0.1073	10.11	3.19	78.22
Probase Cold	Control	0.0911	10.00	3.22	65.90
Probase Cold	Control	0.1382	9.99	3.26	97.63

Probase Cold	Control	0.1082	10.22	3.17	79.02
Probase Cold	Control	0.1326	9.84	3.21	98.08
Probase Cold	5%	0.1125	10.11	3.24	79.50
Probase Cold	5%	0.0900	9.97	3.28	62.93
Probase Cold	5%	0.0776	10.26	3.21	55.05
Probase Cold	5%	0.0881	10.33	3.21	62.08
Probase Cold	5%	0.0828	10.03	3.20	60.46
Probase Cold	5%	0.0953	10.17	3.28	65.33
Probase Cold	5%	0.1050	10.12	3.27	72.77
Probase Cold	5%	0.0868	10.01	3.24	61.95

4. Surface Porosity

MATERIAL	CHX %	W_{dry} (gr)	W_{sus} (gr)	W_{sat} (gr)	POROSITY (%)
Kooliner	Control	0.3573	0.0375	0.3575	0.062
Kooliner	Control	0.3604	0.0371	0.3606	0.062
Kooliner	Control	0.3589	0.0385	0.3590	0.031
Kooliner	Control	0.3613	0.0381	0.3615	0.062
Kooliner	Control	0.3605	0.0388	0.3605	0.000
Kooliner	2.5%	0.3514	0.0459	0.359	2.427
Kooliner	2.5%	0.3638	0.0388	0.3643	0.154
Kooliner	2.5%	0.359	0.0382	0.3601	0.342
Kooliner	2.5%	0.3536	0.0362	0.3544	0.251
Kooliner	2.5%	0.3536	0.0365	0.354	0.126
Ufi Gel Hard	Control	0.3658	0.0415	0.3659	0.031
Ufi Gel Hard	Control	0.3702	0.0400	0.3705	0.091
Ufi Gel Hard	Control	0.3684	0.0383	0.3685	0.030
Ufi Gel Hard	Control	0.3645	0.0383	0.3646	0.031
Ufi Gel Hard	Control	0.3682	0.0374	0.3682	0.000
Ufi Gel Hard	5%	0.3700	0.0368	0.3701	0.030
Ufi Gel Hard	5%	0.3656	0.0319	0.3659	0.090
Ufi Gel Hard	5%	0.3641	0.0271	0.3638	-0.089
Ufi Gel Hard	5%	0.3707	0.044	0.3708	0.031
Ufi Gel Hard	5%	0.3735	0.0406	0.3736	0.030
Probase Cold	Control	0.3796	0.0426	0.3804	0.237
Probase Cold	Control	0.3764	0.0525	0.3765	0.031
Probase Cold	Control	0.3897	0.0567	0.3902	0.150
Probase Cold	Control	0.3873	0.0553	0.3878	0.150
Probase Cold	Control	0.3823	0.0532	0.3824	0.030
Probase Cold	5%	0.3799	0.0478	0.3799	0.000
Probase Cold	5%	0.3826	0.0478	0.3827	0.030
Probase Cold	5%	0.3841	0.051	0.3842	0.030
Probase Cold	5%	0.3804	0.0464	0.3807	0.090
Probase Cold	5%	0.3782	0.0487	0.3807	0.753

5. Surface Free Energy

MATERIAL	CHX %	MEASURES (mm)			ADVANCING CONTACT ANGLE (°)		SURFACE FREE ENERGY (γ) (mN/m)		
		WIDTH	HEIGHT	TICKNESS	WATER	1,2-PROPANEDIOL	TOTAL	DISPERSIVE	POLAR
Kooliner	Control	25.10	17.00	1.00	91.29	65.40	24.20	11.00	13.20
Kooliner	Control	25.60	17.40	1.24	92.14	62.44	24.30	12.40	12.00
Kooliner	Control	25.40	16.60	1.20	84.93	67.40	27.10	9.30	17.80
Kooliner	Control	25.20	16.70	1.20	101.34	62.35	22.00	15.60	6.50
Kooliner	Control	25.30	16.03	1.20	110.74	63.27	24.80	24.10	0.80
Kooliner	2.5%	25.74	16.42	1.15	93.34	46.37	27.90	19.80	8.20
Kooliner	2.5%	25.51	16.46	1.25	91.61	40.56	29.90	21.60	8.30
Kooliner	2.5%	25.68	16.67	1.22	84.61	40.42	21.30	18.90	12.40
Kooliner	2.5%	25.49	15.67	1.32	87.31	42.63	30.10	18.90	11.10
Kooliner	2.5%	25.55	15.61	1.25	86.79	41.94	30.40	19.00	11.30
Ufi Gel Hard	Control	25.18	16.66	1.14	83.35	33.90	33.10	20.90	12.30
Ufi Gel Hard	Control	25.20	15.62	1.12	92.34	41.03	29.70	21.80	7.90
Ufi Gel Hard	Control	24.93	16.70	1.23	91.64	33.23	32.30	24.90	7.30
Ufi Gel Hard	Control	25.12	16.51	1.27	88.43	32.74	32.50	23.40	9.10
Ufi Gel Hard	Control	24.97	16.75	1.19	84.20	27.85	34.30	23.20	11.10
Ufi Gel Hard	5%	24.64	16.54	1.14	71.11	26.32	38.90	19.90	19.00
Ufi Gel Hard	5%	24.79	15.28	1.10	84.36	25.25	34.80	24.10	10.70
Ufi Gel Hard	5%	29	16.16	1.19	74.39	29.54	37.00	19.70	17.20
Ufi Gel Hard	5%	24.84	17.10	1.20	82.65	26.44	34.90	23.10	11.80
Ufi Gel Hard	5%	24.94	16.96	1.32	83.10	22.67	35.60	24.30	11.20
Probase Cold	Control	25.05	16.73	1.06	97.04	61.04	23.10	14.40	8.70
Probase Cold	Control	25.28	16.05	1.21	93.50	57.34	24.90	14.80	10.00
Probase Cold	Control	25.67	15.62	1.22	99.80	59.19	23.20	16.50	6.70
Probase Cold	Control	25.64	16.63	1.25	92.66	60.72	24.40	13.20	11.30
Probase Cold	Control	25.43	16.39	1.23	87.13	54.85	27.50	14.10	13.40
Probase Cold	5%	25.20	15.83	1.05	81.76	47.81	30.90	15.50	15.40
Probase Cold	5%	25.18	16.86	1.07	83.88	42.94	31.00	17.80	13.20
Probase Cold	5%	25.59	16.20	1.01	83.72	48.51	30.00	15.70	14.30
Probase Cold	5%	24.95	16.04	1.04	85.29	47.23	29.60	16.50	13.10
Probase Cold	5%	25.79	15.85	1.01	77.43	48.82	32.70	14.30	18.40

6. Shear Bond Strength

MATERIAL	CHX %	SHEAR BOND STRENGTH (MPa)	FAILURE MODE
Kooliner	Control	12.62	Adhesive
Kooliner	Control	15.72	Adhesive
Kooliner	Control	15.16	Adhesive
Kooliner	Control	7.66	Adhesive
Kooliner	Control	11.71	Adhesive
Kooliner	Control	20.31	Adhesive
Kooliner	Control	11.54	Adhesive
Kooliner	Control	12.72	Adhesive
Kooliner	Control	13.74	Adhesive
Kooliner	Control	10.20	Adhesive
Kooliner	2.5%	16.10	Adhesive
Kooliner	2.5%	17.71	Adhesive
Kooliner	2.5%	16.31	Adhesive
Kooliner	2.5%	20.35	Adhesive
Kooliner	2.5%	17.80	Adhesive
Kooliner	2.5%	16.33	Adhesive
Kooliner	2.5%	16.59	Adhesive
Kooliner	2.5%	22.67	Adhesive
Kooliner	2.5%	29.41	Adhesive
Kooliner	2.5%	14.63	Adhesive
Ufi Gel Hard	Control	23.21	Adhesive
Ufi Gel Hard	Control	24.39	Adhesive
Ufi Gel Hard	Control	23.75	Adhesive
Ufi Gel Hard	Control	23.02	Mixed
Ufi Gel Hard	Control	26.24	Adhesive
Ufi Gel Hard	Control	21.47	Adhesive
Ufi Gel Hard	Control	25.07	Adhesive
Ufi Gel Hard	Control	23.99	Adhesive
Ufi Gel Hard	Control	28.41	Adhesive
Ufi Gel Hard	Control	25.36	Adhesive
Ufi Gel Hard	5%	28.72	Mixed
Ufi Gel Hard	5%	28.58	Mixed
Ufi Gel Hard	5%	22.91	Mixed
Ufi Gel Hard	5%	26.33	Mixed
Ufi Gel Hard	5%	30.99	Mixed
Ufi Gel Hard	5%	31.13	Mixed
Ufi Gel Hard	5%	29.01	Mixed
Ufi Gel Hard	5%	29.35	Mixed

Ufi Gel Hard	5%	26.16	Mixed
Ufi Gel Hard	5%	27.77	Mixed
Probase Cold	Control	40.16	Mixed
Probase Cold	Control	38.95	Adhesive
Probase Cold	Control	43.15	Mixed
Probase Cold	Control	41.34	Mixed
Probase Cold	Control	40.01	Mixed
Probase Cold	Control	42.01	Mixed
Probase Cold	Control	40.95	Adhesive
Probase Cold	Control	33.81	Mixed
Probase Cold	Control	41.10	Mixed
Probase Cold	Control	40.60	Mixed
Probase Cold	5%	25.95	Mixed
Probase Cold	5%	25.98	Adhesive
Probase Cold	5%	26.71	Mixed
Probase Cold	5%	26.38	Adhesive
Probase Cold	5%	25.63	Mixed
Probase Cold	5%	20.86	Mixed
Probase Cold	5%	25.67	Mixed
Probase Cold	5%	18.12	Adhesive
Probase Cold	5%	15.75	Adhesive
Probase Cold	5%	24.33	Adhesive

7. Release Assay

MATERIAL	CHX %	Time (h)	M (cumulative concentration)	SD (cumulative concentration)	M (CHX % released)	SD (CHX % released)
Kooliner	2.5%	0	0	0	0	0
Kooliner	2.5%	1	8.13076615	3.72231364	0.162615323	
Kooliner	2.5%	2	11.2576728	0.711261814	0.225153457	0.014225236
Kooliner	2.5%	4	14.7305514	0.698208591	0.294611028	0.013964172
Kooliner	2.5%	7	16.7940084	0.964196024	0.335880168	0.01928392
Kooliner	2.5%	24	21.0599551	6.511459536	0.421199102	0.130229191
Kooliner	2.5%	48	20.4688187	3.865993205	0.409376374	0.077319864
Kooliner	2.5%	72	20.4351449	4.554676407	0.408702897	0.091093528
Kooliner	2.5%	96	21.3914431	3.70842711	0.427828862	0.074168542
Kooliner	2.5%	168	21.4538428	3.736824767	0.429076856	0.074736495
Kooliner	2.5%	240	21.6221255	4.850920522	0.432442509	0.09701841
Kooliner	2.5%	360	22.5065157	4.617873478	0.450130313	0.09235747
Kooliner	2.5%	578	21.9073453	4.455094196	0.438146905	0.089101884
Kooliner	2.5%	672	25.1885326	6.793366024	0.503770652	0.13586732
Ufi Gel Hard	5%	0	0	0	0	0
Ufi Gel Hard	5%	1	46.6706779	27.5613551	0.466706779	
Ufi Gel Hard	5%	2	69.0993193	33.46841957	0.690993193	0.334684196
Ufi Gel Hard	5%	4	76.8913818	35.14997447	0.768913818	0.351499745
Ufi Gel Hard	5%	7	82.0959635	39.69825009	0.820959635	0.396982501
Ufi Gel Hard	5%	24	98.5851615	49.6689541	0.985851615	0.496689541
Ufi Gel Hard	5%	48	103.919029	52.27018598	1.039190286	0.52270186
Ufi Gel Hard	5%	72	107.284249	51.41195041	1.072842489	0.514119504
Ufi Gel Hard	5%	96	114.333984	52.15194131	1.143339837	0.521519413
Ufi Gel Hard	5%	168	122.39829	53.89011358	1.223982903	0.538901136
Ufi Gel Hard	5%	240	124.691449	55.21417216	1.246914492	0.552141722
Ufi Gel Hard	5%	360	126.053746	55.43299059	1.26053746	0.554329906
Ufi Gel Hard	5%	578	126.341628	54.6638763	1.263416278	0.546638763
Ufi Gel Hard	5%	672	129.017807	55.52292652	1.290178072	0.555229265
Probase Cold	5%	0	0	0	0	0
Probase Cold	5%	1	6.31768953	2.403487575	0.063176895	
Probase Cold	5%	2	8.41237462	1.658677003	0.084123746	0.01658677
Probase Cold	5%	4	8.31092477	2.12248319	0.083109248	0.021224832
Probase Cold	5%	7	8.4702547	3.480465415	0.084702547	0.034804654
Probase Cold	5%	24	17.1917635	4.1077119	0.171917635	0.041077119
Probase Cold	5%	48	19.6216598	4.954768634	0.196216598	0.049547686
Probase Cold	5%	72	19.4611835	5.435107146	0.194611835	0.054351071
Probase Cold	5%	96	23.3760363	4.447190756	0.233760363	0.044471908
Probase Cold	5%	168	26.6388305	4.978043718	0.266388305	0.049780437

Probase Cold	5%	240	27.3376927	6.0319067	0.273376927	0.060319067
Probase Cold	5%	360	30.6432147	7.062490252	0.306432147	0.070624903
Probase Cold	5%	578	34.2074527	7.806724404	0.342074527	0.078067244
Probase Cold	5%	672	35.4735598	8.128105997	0.354735598	0.08128106

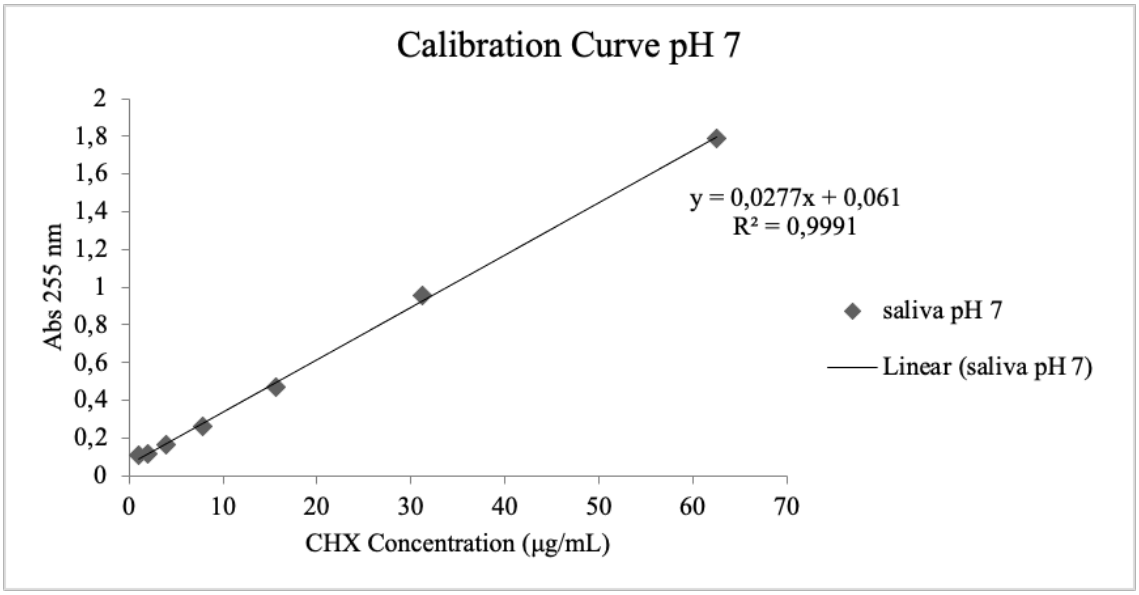


Figure 1 – Linear relationship between absorbance peak areas and the CHX concentration for saliva at pH=7.

8. Cytotoxicity

MATERIAL	CHX %	Absorbance	Cell Viability (%)
Kooliner	Control	1.066	97
Kooliner	Control	1.226	111
Kooliner	Control	1.241	112
Kooliner	Control	1.158	105
Kooliner	Control	1.188	108
Kooliner	Control	1.105	100
Kooliner	Control	1.094	99
Kooliner	Control	1.111	101
Kooliner	Control	1.082	98
Kooliner	Control	1.084	98
Kooliner	Control	1.067	97
Kooliner	Control	1.014	92
Kooliner	2.5%	0.724	66
Kooliner	2.5%	0.719	65
Kooliner	2.5%	0.741	67
Kooliner	2.5%	0.745	67
Kooliner	2.5%	0.708	64
Kooliner	2.5%	0.672	61
Kooliner	2.5%	0.759	69
Kooliner	2.5%	0.762	69
Kooliner	2.5%	0.638	58
Kooliner	2.5%	0.723	65
Kooliner	2.5%	0.714	65
Kooliner	2.5%	0.637	58
Ufi Gel Hard	Control	1.125	102
Ufi Gel Hard	Control	1.193	108
Ufi Gel Hard	Control	1.213	110
Ufi Gel Hard	Control	1.081	98
Ufi Gel Hard	Control	1.121	102
Ufi Gel Hard	Control	1.190	108
Ufi Gel Hard	Control	1.208	109
Ufi Gel Hard	Control	1.150	104
Ufi Gel Hard	Control	1.020	92
Ufi Gel Hard	Control	1.140	103
Ufi Gel Hard	Control	1.109	100

Ufi Gel Hard	Control	0.980	89
Ufi Gel Hard	5%	0.171	15
Ufi Gel Hard	5%	0.254	23
Ufi Gel Hard	5%	0.196	18
Ufi Gel Hard	5%	0.160	14
Ufi Gel Hard	5%	0.230	21
Ufi Gel Hard	5%	0.217	20
Ufi Gel Hard	5%	0.130	12
Ufi Gel Hard	5%	0.258	23
Ufi Gel Hard	5%	0.093	8
Ufi Gel Hard	5%	0.280	25
Ufi Gel Hard	5%	0.146	13
Ufi Gel Hard	5%	0.152	14
Probase Cold	Control	1.065	96
Probase Cold	Control	1.085	98
Probase Cold	Control	1.148	104
Probase Cold	Control	1.134	103
Probase Cold	Control	1.106	100
Probase Cold	Control	1.065	96
Probase Cold	Control	1.030	93
Probase Cold	Control	1.046	95
Probase Cold	Control	0.988	89
Probase Cold	Control	1.091	99
Probase Cold	Control	1.086	98
Probase Cold	Control	0.935	85
Probase Cold	5%	0.726	66
Probase Cold	5%	0.878	80
Probase Cold	5%	0.819	74
Probase Cold	5%	0.778	70
Probase Cold	5%	0.842	76
Probase Cold	5%	0.798	72
Probase Cold	5%	0.751	68
Probase Cold	5%	0.809	73
Probase Cold	5%	0.697	63
Probase Cold	5%	0.780	71
Probase Cold	5%	0.739	67
Probase Cold	5%	0.626	57

9. Microhardness (Thermal Aging)

MATERIAL	CHX %	INDENTATION (KHN)											
		1	2	3	4	5	6	7	8	9	10	11	12
Kooliner	Control	18.6	30.6	4.0	8.0	4.6	4.7	17.1	4.5	6.5	12.0	15.8	6.1
Kooliner	Control	13.6	22.8	4.0	6.1	3.4	6.1	4.1	5.8	7.9	11.5	6.8	7.2
Kooliner	Control	28.3	12.6	6.4	3.1	4.1	4.6	3.8	3.4	3.9	4.0	12.5	13.4
Kooliner	Control	7.7	11.3	4.0	6.0	5.5	22.5	9.3	4.6	4.0	8.4	3.9	4.0
Kooliner	Control	3.0	3.9	4.1	15.4	5.0	3.3	3.5	6.2	3.6	5.1	5.2	9.1
Kooliner	Control	5.2	4.4	5.5	6.3	8.0	5.0	5.1	4.3	4.6	5.9	3.5	4.3
Kooliner	Control	10.2	10.2	6.0	6.9	6.9	5.1	6.8	5.6	7.0	8.6	12.4	15.2
Kooliner	Control	4.3	4.6	5.9	3.5	4.3	10.2	6.9	5.1	5.5	6.3	8.0	5.0
Kooliner	2.5%	5.5	7.4	3.8	4.0	3.4	3.9	4.9	4.1	4.4	3.7	3.7	3.3
Kooliner	2.5%	4.5	5.2	6.2	4.9	10.0	8.8	5.9	8.5	11.2	8.4	11.3	8.5
Kooliner	2.5%	9.4	12.3	3.4	9.4	10.9	13.1	6.4	12.9	3.3	5.9	3.9	6.4
Kooliner	2.5%	4.3	5.8	6.0	4.9	5.2	4.7	7.6	5.0	6.4	6.3	19.5	25.0
Kooliner	2.5%	13.5	15.4	3.8	7.2	4.5	3.5	4.0	3.4	4.5	4.0	3.2	11.9
Kooliner	2.5%	6.6	12.3	8.5	8.0	7.0	7.0	13.2	7.0	7.1	7.7	10.9	9.7
Kooliner	2.5%	11.7	4.0	4.4	5.3	6.1	5.8	3.8	9.1	3.4	6.1	22.0	18.6
Kooliner	2.5%	7.2	8.3	6.9	7.8	6.1	9.1	5.2	9.4	7.6	8.8	7.4	5.3
Ufi Gel Hard	Control	6.0	9.0	9.6	8.0	9.2	9.2	8.3	8.7	6.6	7.9	7.7	7.5
Ufi Gel Hard	Control	7.6	8.9	8.9	8.33	8.3	7.8	7.5	9.1	7.9	7.9	6.8	10.2
Ufi Gel Hard	Control	7.8	7.0	7.0	7.4	7.4	7.4	8.0	7.1	7.2	7.4	7.4	6.8
Ufi Gel Hard	Control	8.0	7.7	7.3	6.9	7.5	7.6	6.2	7.9	7.5	6.7	7.1	7.9
Ufi Gel Hard	Control	8.3	6.2	8.1	6.8	8.3	6.9	6.4	7.3	5.8	7.0	8.0	6.4
Ufi Gel Hard	Control	7.7	8.4	12.2	9.0	12.2	9.9	12.4	10.6	6.3	6.1	9.0	7.2
Ufi Gel Hard	Control	7.7	10.4	9.0	7.4	12.5	7.3	6.7	13.2	9.4	10.5	9.7	9.8
Ufi Gel Hard	Control	9.1	8.8	7.4	9.0	9.5	9.1	9.1	8.9	8.7	9.4	9.1	9.8
Ufi Gel Hard	5%	7.8	8.7	9.9	12.7	8.6	8.6	10.0	8.0	9.5	9.7	5.7	6.0
Ufi Gel Hard	5%	7.6	7.6	9.3	8.5	8.7	7.6	9.7	7.9	6.3	9.0	9.4	9.4
Ufi Gel Hard	5%	6.9	9.7	8.6	5.4	5.5	7.1	6.1	7.6	5.8	7.2	9.1	13.1
Ufi Gel Hard	5%	7.4	6.1	8.8	9.1	11.0	8.6	8.6	7.0	4.0	9.6	9.6	7.6
Ufi Gel Hard	5%	11.2	9.4	15.8	7.8	9.2	10.0	5.6	3.5	4.4	4.4	7.4	6.6
Ufi Gel Hard	5%	8.2	9.4	8.4	7.7	7.9	9.5	8.6	7.9	9.6	6.5	8.5	9.0
Ufi Gel Hard	5%	9.3	8.8	7.5	10.9	8.3	7.9	8.6	9.3	7.6	7.5	8.6	8.4
Ufi Gel Hard	5%	8.0	8.3	8.3	7.9	7.9	9.0	8.7	8.9	8.3	8.5	8.8	8.2
Probase Cold	Control	13.6	13.0	13.6	12.5	13.6	12.6	13.3	15.3	15.1	14.2	9.3	12.8
Probase Cold	Control	12.8	13.6	13.1	12.5	15.1	15.6	13.0	11.2	14.8	15.9	14.2	12.1
Probase Cold	Control	10.9	11.8	11.8	15.0	13.4	15.0	14.6	13.6	15.2	9.7	14.3	14.3
Probase Cold	Control	19.5	15.4	16.9	13.4	16.4	14.4	12.9	10.2	13.6	17.9	17.9	14.6
Probase Cold	Control	12.6	11.6	9.21	13.2	12.6	14.5	13.0	11.4	11.5	16.3	14.0	14.4
Probase Cold	Control	12.9	11.5	15.6	11.9	12.3	12.5	13.6	13.2	14.3	10.9	20.5	17.5

Probase Cold	Control	14.2	13.3	12.6	8.4	11.3	12.6	13.9	13.9	15.9	15.1	14.1	15.0
Probase Cold	Control	15.0	14.3	11.8	11.8	10.7	12.3	16.8	13.2	13.0	13.3	14.7	16.7
Probase Cold	5%	14.5	15.2	9.5	10.5	10.8	12.1	14.5	11.0	11.3	13.7	11.8	15.1
Probase Cold	5%	13.3	14.7	13.9	12.1	13.3	13.3	14.4	14.5	13.6	13.6	14.2	15.2
Probase Cold	5%	8.5	12.3	15.9	12.6	13.0	13.9	12.6	13.3	11.4	14.2	11.1	14.2
Probase Cold	5%	14.2	15.3	12.3	11.6	11.6	13.3	12.8	13.3	14.4	11.4	12.1	14.1
Probase Cold	5%	13.3	12.2	12.6	16.8	11.5	11.7	11.9	13.6	11.4	13.2	13.0	12.3
Probase Cold	5%	12.9	12.7	15.2	12.1	12.9	12.9	9.7	11.5	13.0	11.8	13.7	14.7
Probase Cold	5%	11.9	9.6	13.1	14.0	10.1	17.1	11.5	13.3	12.5	15.2	13.3	15.9
Probase Cold	5%	10.2	16.2	14.9	12.5	10.6	11.9	11.2	10.8	13.5	13.3	10.2	14.8

10. Flexural Strength (Thermal Aging)

MATERIAL	CHX %	LOAD AT YIELD (KN)	WIDTH (mm)	THICKHNESS (mm)	FLEXURAL STRENGTH (MPa)
Kooliner	Control	0.0964	10.34	3.4	60.49
Kooliner	Control	0.1223	10.34	3.34	79.52
Kooliner	Control	0.1351	10.28	3.35	87.83
Kooliner	Control	0.109	10.5	3.38	68.15
Kooliner	Control	0.1171	10.19	3.38	75.44
Kooliner	Control	0.1386	10.18	3.17	101.62
Kooliner	Control	0.1331	10.26	3.14	98.68
Kooliner	Control	0.124	10.64	3.34	78.35
Kooliner	2.5%	0.1443	10.18	3.25	100.65
Kooliner	2.5%	0.132	10.55	3.38	82.14
Kooliner	2.5%	0.14	10.5	3.46	83.53
Kooliner	2.5%	0.1552	10.01	3.36	103.00
Kooliner	2.5%	0.145	10.01	3.48	89.71
Kooliner	2.5%	0.1286	10.19	3.2	92.43
Kooliner	2.5%	0.1248	10.26	3.21	88.54
Kooliner	2.5%	0.1612	10.41	3.35	103.49
Ufi Gel Hard	Control	0.0895	10.19	3.12	67.67
Ufi Gel Hard	Control	0.0915	9.99	3.26	64.64
Ufi Gel Hard	Control	0.0710	10.19	3.18	51.68
Ufi Gel Hard	Control	0.1074	10.05	3.11	82.87
Ufi Gel Hard	Control	0.1003	10.18	3.18	73.07
Ufi Gel Hard	Control	0.0751	10.16	3.28	51.53
Ufi Gel Hard	Control	0.1020	9.94	3.19	75.63
Ufi Gel Hard	Control	0.0913	9.97	3.22	66.24
Ufi Gel Hard	5%	0.1148	10.26	3.53	67.35
Ufi Gel Hard	5%	0.1397	10.08	3.48	85.83
Ufi Gel Hard	5%	0.1047	10.25	3.2	74.81
Ufi Gel Hard	5%	0.1404	10.27	3.51	83.22
Ufi Gel Hard	5%	0.0941	10.13	3.52	56.23
Ufi Gel Hard	5%	0.1122	10.08	3.27	78.07
Ufi Gel Hard	5%	0.1097	9.94	3.29	76.47
Ufi Gel Hard	5%	0.0588	10.14	3.28	71.32
Probase Cold	Control	0.2341	10.19	3.41	148.18
Probase Cold	Control	0.123	10.13	3.24	86.75
Probase Cold	Control	0.2627	10.17	3.08	204.22
Probase Cold	Control	0.2517	10.24	3.14	186.98
Probase Cold	Control	0.276	10.27	3.23	193.19
Probase Cold	Control	0.2544	10.37	3.18	181.95

Probase Cold	Control	0.2712	10.3	3.33	178.08
Probase Cold	Control	0.1867	10.22	3.25	129.71
Probase Cold	5%	0.1516	10.35	3.25	104.00
Probase Cold	5%	0.1592	10.29	3.13	118.44
Probase Cold	5%	0.1976	10.11	3.15	147.73
Probase Cold	5%	0.2071	10.3	3.2	147.27
Probase Cold	5%	0.1557	10.93	3.2	104.33
Probase Cold	5%	0.1574	10.73	3.16	110.18
Probase Cold	5%	0.1828	10.26	3.13	136.40
Probase Cold	5%	0.1937	10.14	3.31	130.77

11. Microhardness (Chemical Aging)

MATERIAL	CHX %	INDENTATION (KHN)											
		1	2	3	4	5	6	7	8	9	10	11	12
Kooliner	Control	8.0	9.5	8.0	9.2	8.2	8.7	8.6	8.7	8.6	7.7	8.0	8.8
Kooliner	Control	5.4	9.3	9.0	7.7	9.5	7.1	9.2	7.9	9.0	9.8	9.2	10.5
Kooliner	Control	9.4	10.0	8.3	8.9	6.9	8.4	9.8	7.7	8.6	9.8	10.1	8.7
Kooliner	Control	8.4	8.5	7.8	7.7	9.9	8.9	8.3	7.6	7.5	9.4	7.3	8.4
Kooliner	Control	5.5	4.9	5.3	6.3	5.2	6.6	4.5	6.7	3.7	7.2	5.5	5.3
Kooliner	Control	4.0	3.6	3.8	3.8	6.1	4.0	4.1	4.5	4.1	4.5	3.4	5.3
Kooliner	Control	6.0	4.7	6.3	6.1	5.8	5.3	6.2	6.2	5.8	6.2	6.2	7.3
Kooliner	Control	4.7	6.2	6.0	6.7	4.7	5.4	5.7	7.6	4.8	7.5	5.5	5.1
Kooliner	2.5%	8.7	9.7	9.9	8.3	8.7	8.4	8.8	8.3	6.9	8.7	8.3	9.8
Kooliner	2.5%	9.4	8.3	8.8	7.9	7.9	8.1	10.0	7.8	6.0	9.5	7.3	8.0
Kooliner	2.5%	8.1	6.3	7.8	9.3	8.5	8.6	6.8	7.8	9.1	8.9	8.8	8.2
Kooliner	2.5%	9.1	8.4	8.2	9.4	9.9	9.6	9.5	8.9	8.5	9.1	7.5	9.1
Kooliner	2.5%	3.9	4.2	4.5	4.6	5.6	4.2	6.1	5.5	5.8	4.4	3.7	3.5
Kooliner	2.5%	4.7	6.4	6.6	6.7	6.1	5.7	5.2	6.6	5.1	5.5	5.8	5.4
Kooliner	2.5%	5.4	6.2	5.6	5.4	5.7	6.2	4.8	5.9	4.9	5.0	5.5	7.1
Kooliner	2.5%	5.3	4.4	4.9	4.9	5.5	6.1	5.5	4.1	3.5	4.6	6.0	4.8
Ufi Gel Hard	Control	9.0	7.7	9.9	8.9	8.8	8.7	9.4	9.2	9.5	8.5	9.9	9.2
Ufi Gel Hard	Control	8.7	9.1	8.5	8.0	8.3	8.4	10.0	7.4	9.3	8.5	7.3	8.3
Ufi Gel Hard	Control	7.4	7.9	8.8	8.4	7.6	9.7	8.0	7.9	9.3	9.2	8.2	9.3
Ufi Gel Hard	Control	7.9	9.2	8.6	9.8	10.0	9.2	9.4	8.2	9.4	8.0	8.3	7.8
Ufi Gel Hard	Control	6.7	5.5	5.6	6.8	6.6	7.4	5.7	6.3	7.6	6.4	6.3	5.9
Ufi Gel Hard	Control	7.3	7.6	6.1	6.6	6.8	7.5	5.9	6.9	8.4	5.8	5.5	6.3
Ufi Gel Hard	Control	7.5	7.1	5.0	7.2	7.8	7.0	7.2	7.5	7.6	5.5	5.3	6.6
Ufi Gel Hard	Control	6.6	6.5	5.2	5.2	6.0	7.2	6.3	5.9	6.6	6.6	6.3	7.1
Ufi Gel Hard	5%	7.2	9.4	8.3	9.7	8.7	8.7	8.6	9.1	9.2	7.4	8.3	8.1
Ufi Gel Hard	5%	7.1	8.4	8.2	8.3	8.3	8.1	8.4	8.8	8.4	8.9	7.9	7.9
Ufi Gel Hard	5%	8.8	8.0	8.1	9.3	8.6	7.0	8.7	9.8	8.1	8.3	8.9	8.3
Ufi Gel Hard	5%	7.6	8.8	8.3	9.6	8.5	8.0	8.4	8.8	8.1	9.2	8.7	8.3
Ufi Gel Hard	5%	5.9	6.7	6.8	6.8	6.6	7.1	6.8	5.5	6.6	6.1	6.8	6.3
Ufi Gel Hard	5%	6.3	6.5	7.2	5.8	6.8	6.0	6.7	6.9	6.5	6.1	6.2	7.5
Ufi Gel Hard	5%	7.0	7.0	6.8	6.2	6.2	7.2	7.7	6.7	6.9	6.7	5.5	6.0
Ufi Gel Hard	5%	6.9	5.5	7.8	7.0	8.3	6.9	8.7	8.3	7.7	7.2	7.5	8.4
Probase Cold	Control	15.2	15.9	16.3	15.6	14.4	14.9	16.1	15.8	15.3	15.2	14.4	13.9
Probase Cold	Control	15.9	15.8	16.1	15.4	16.6	16.1	15.8	15.5	15.7	15.6	15.7	15.8
Probase Cold	Control	13.4	14.2	15.2	14.9	14.1	15.2	14.2	13.4	15.4	15.1	14.8	14.6
Probase Cold	Control	17.4	18.0	15.0	17.4	15.6	15.8	16.9	17.9	17.2	16.6	16.8	17.2
Probase Cold	Control	11.5	11.8	11.5	11.5	11.1	11.4	12.2	11.3	11.7	11.2	11.2	10.8
Probase Cold	Control	12.1	12.0	11.8	11.6	11.5	10.7	11.5	12.0	11.6	11.8	10.9	10.5

Probase Cold	Control	11.5	11.7	11.4	11.9	11.1	11.2	11.6	11.8	11.9	11.7	11.3	11.5
Probase Cold	Control	11.4	11.7	12.2	11.5	11.6	11.7	11.3	11.6	11.8	11.8	11.5	11.3
Probase Cold	5%	14.1	16.1	17.8	16.8	16.1	15.6	15.2	16.7	15.3	15.8	15.7	11.0
Probase Cold	5%	14.8	13.5	14.9	14.5	17.1	12.5	13.7	15.3	14.9	12.9	14.6	14.0
Probase Cold	5%	14.6	13.7	15.6	15.4	16.0	14.0	15.0	15.2	15.8	15.3	14.6	13.8
Probase Cold	5%	13.3	12.2	13.8	12.5	13.7	14.3	12.5	14.2	15.1	14.5	14.5	15.2
Probase Cold	5%	9.8	11.2	10.9	11.0	11.0	11.3	11.2	10.8	11.8	11.1	11.1	10.5
Probase Cold	5%	11.0	11.0	10.8	11.4	10.3	10.5	11.3	11.0	11.5	10.4	10.3	10.5
Probase Cold	5%	10.4	10.4	10.5	10.6	10.6	9.4	10.5	10.2	10.7	10.9	10.7	11.5
Probase Cold	5%	11.2	11.0	11.0	11.1	11.1	11.0	11.2	10.9	8.6	10.4	11.8	11.5

12. Flexural Strength (Chemical Aging)

MATERIAL	CHX %	LOAD AT YIELD (KN)	WIDTH (mm)	THICKHNESS (mm)	FLEXURAL STRENGTH (MPa)
Kooliner	Control	0.0596	10.03	3.04	48.22
Kooliner	Control	0.0622	10.00	3.00	51.83
Kooliner	Control	0.0598	10.12	3.17	44.10
Kooliner	Control	0.0669	10.01	3.11	51.82
Kooliner	Control	0.0455	10.03	3.11	35.18
Kooliner	Control	0.0351	9.92	2.95	30.49
Kooliner	Control	0.0514	10.05	3.15	38.66
Kooliner	Control	0.0505	10.18	3.05	39.99
Kooliner	2.5%	0.0609	10.28	3.10	46.23
Kooliner	2.5%	0.0685	10.17	3.08	53.25
Kooliner	2.5%	0.0668	10.33	3.10	50.47
Kooliner	2.5%	0.0683	10.29	3.22	48.01
Kooliner	2.5%	0.0458	10.07	3.22	32.90
Kooliner	2.5%	0.0503	10.14	3.13	37.98
Kooliner	2.5%	0.0504	10.18	3.16	37.19
Kooliner	2.5%	0.0494	10.25	3.13	36.90
Ufi Gel Hard	Control	0.0611	10.02	3.16	45.80
Ufi Gel Hard	Control	0.0486	10.15	3.22	34.64
Ufi Gel Hard	Control	0.0492	10.26	3.25	34.05
Ufi Gel Hard	Control	0.0500	10.20	3.22	35.46
Ufi Gel Hard	Control	0.0534	10.27	3.22	37.61
Ufi Gel Hard	Control	0.0563	10.04	3.18	41.59
Ufi Gel Hard	Control	0.0440	10.19	3.16	32.43
Ufi Gel Hard	Control	0.0505	10.16	3.13	38.05
Ufi Gel Hard	5%	0.0554	10.12	3.15	41.38
Ufi Gel Hard	5%	0.0371	10.22	3.14	27.61
Ufi Gel Hard	5%	0.0531	10.30	3.25	36.61
Ufi Gel Hard	5%	0.0532	10.31	3.20	37.79
Ufi Gel Hard	5%	0.0581	10.17	3.19	42.11
Ufi Gel Hard	5%	0.0502	10.15	3.20	36.22
Ufi Gel Hard	5%	0.0508	10.10	3.19	37.07
Ufi Gel Hard	5%	0.0569	10.05	3.14	43.07
Probase Cold	Control	0.1365	10.21	3.28	93.20
Probase Cold	Control	0.1044	10.57	3.25	70.13
Probase Cold	Control	0.0834	10.23	2.57	92.57
Probase Cold	Control	0.0987	10.45	2.83	88.45
Probase Cold	Control	0.1142	10.06	3.27	79.62
Probase Cold	Control	0.1273	10.12	3.29	87.16

Probase Cold	Control	0.0925	10.11	2.80	87.53
Probase Cold	Control	0.0665	10.01	2.92	58.44
Probase Cold	5%	0.1063	10.18	3.49	64.30
Probase Cold	5%	0.0907	10.57	3.23	61.69
Probase Cold	5%	0.1014	10.02	3.22	73.20
Probase Cold	5%	0.1014	10.15	3.31	68.39
Probase Cold	5%	0.1117	10.13	3.41	71.12
Probase Cold	5%	0.0675	10.16	2.73	66.86
Probase Cold	5%	0.0820	10.31	3.15	60.12
Probase Cold	5%	0.0964	10.24	3.42	60.37

13. Surface Free Energy (Thermal aging)

MATERIAL	CHX %	MEASURES (mm)			ADVANCING CONTACT ANGLE (°)		SURFACE FREE ENERGY (γ) (mN/m)		
		WIDTH	HEIGHT	TICKNESS	WATER	1,2-PROPANEDIOL	TOTAL	DISPERSIVE	POLAR
Kooliner	Control	24.23	16.34	1.05	84.07	69.92	27.90	8.30	19.60
Kooliner	Control	24.27	16.81	1.09	87.37	54.11	26.90	14.90	12.00
Kooliner	Control	24.03	16.88	1.07	79.08	59.25	30.80	11.10	19.70
Kooliner	Control	23.77	16.42	1.02	85.98	62.03	26.70	11.30	15.40
Kooliner	Control	24.27	16.60	1.07	77.63	59.62	31.70	10.70	21.00
Kooliner	2.5%	25.18	16.64	1.07	86.89	55.05	27.00	14.30	12.60
Kooliner	2.5%	24.42	17.15	1.09	85.66	75.24	27.20	6.80	20.40
Kooliner	2.5%	24.40	17.12	1.08	87.77	61.08	25.90	12.10	13.80
Kooliner	2.5%	24.24	16.69	1.10	84.51	63.27	27.50	10.60	16.90
Kooliner	2.5%	24.20	16.96	1.09	88.84	63.69	25.10	11.20	13.90
Ufi Gel Hard	Control	24.60	16.95	1.10	92.90	75.58	22.70	7.80	14.90
Ufi Gel Hard	Control	24.71	17.28	1.03	90.88	66.70	24.00	10.60	13.30
Ufi Gel Hard	Control	24.94	16.05	1.01	93.05	44.76	28.50	22.90	5.70
Ufi Gel Hard	Control	24.29	16.92	1.07	82.84	57.59	28.70	12.40	16.30
Ufi Gel Hard	Control	25.05	16.66	1.03	92.55	82.22	23.20	5.60	17.60
Ufi Gel Hard	5%	24.18	17.27	1.06	75.25	51.40	33.30	13.20	20.20
Ufi Gel Hard	5%	24.95	16.47	1.11	88.93	50.84	27.00	16.90	10.10
Ufi Gel Hard	5%	24.70	16.72	1.09	86.91	78.36	26.60	6.00	20.60
Ufi Gel Hard	5%	24.19	16.66	1.07	92.12	54.89	33.30	12.00	21.40
Ufi Gel Hard	5%	24.18	16.31	1.06	75.13	59.37	32.00	10.80	21.20
Probase Cold	Control	24.74	17.08	1.03	87.16	78.37	26.40	6.00	20.40
Probase Cold	Control	24.90	16.36	1.03	86.21	62.80	26.60	11.10	15.50
Probase Cold	Control	25.09	16.98	1.05	90.64	93.73	26.30	2.30	24.10
Probase Cold	Control	25.43	16.44	1.07	87.43	77.07	26.10	6.50	19.70
Probase Cold	Control	24.70	17.06	1.04	88.75	56.12	26.10	14.40	11.60
Probase Cold	5%	24.58	16.43	1.01	79.66	64.31	30.50	9.50	21.10
Probase Cold	5%	24.18	17.24	1.02	78.60	61.13	31.10	10.40	20.80
Probase Cold	5%	24.99	16.61	1.01	81.95	66.35	29.10	9.10	20.00
Probase Cold	5%	24.66	16.98	1.04	78.73	61.21	31.10	10.40	20.70
Probase Cold	5%	24.83	17.15	1.08	83.30	60.72	28.30	11.30	17.00

14. Surface Free Energy (Chemical Aging)

MATERIAL	CHX %	MEASURES (mm)			ADVANCING CONTACT ANGLE (°)		SURFACE FREE ENERGY (γ) (mN/m)		
		WIDTH	HEIGHT	TICKNESS	WATER	1,2-PROPANE DIOL	TOTAL	DISPERSIVE	POLAR
Kooliner	Control	24.91	15.87	1.08	88.93	35.24	31.80	25.20	6.50
Kooliner	Control	24.87	15.92	1.05	84.51	44.56	29.70	18.10	11.60
Kooliner	Control	24.97	16.60	1.20	84.93	67.40	32.40	13.80	18.60
Kooliner	Control	25.06	16.08	1.24	76.80	41.53	33.90	16.60	17.30
Kooliner	Control	24.64	15.95	1.19	81.34	45.36	30.70	16.70	14.00
Kooliner	2.5%	24.79	15.96	1.16	73.38	43.04	34.90	15.70	19.20
Kooliner	2.5%	24.93	15.85	1.18	76.52	48.86	32.70	14.30	18.40
Kooliner	2.5%	24.92	15.98	1.13	86.98	38.97	30.50	21.90	8.60
Kooliner	2.5%	24.88	15.78	1.13	75.31	49.58	33.40	13.80	19.60
Kooliner	2.5%	24.80	16.04	1.06	76.01	35.89	34.40	18.80	15.60
Ufi Gel Hard	Control	24.50	15.95	1.13	61.59	39.37	42.60	15.00	27.60
Ufi Gel Hard	Control	24.36	15.88	1.04	61.35	25.05	43.10	18.70	24.40
Ufi Gel Hard	Control	24.55	16.00	1.10	64.76	20.61	41.50	20.30	21.10
Ufi Gel Hard	Control	23.16	16.03	1.17	64.54	28.14	41.80	18.30	23.50
Ufi Gel Hard	Control	24.33	15.90	1.09	67.92	32.48	38.80	18.00	20.80
Ufi Gel Hard	5%	25.04	15.88	1.02	61.83	43.96	42.50	13.70	28.80
Ufi Gel Hard	5%	24.40	16.09	1.07	64.59	22.29	41.40	19.90	21.50
Ufi Gel Hard	5%	24.50	15.26	1.14	61.95	26.64	42.70	18.50	24.20
Ufi Gel Hard	5%	24.77	15.88	1.15	62.22	22.39	43.60	19.20	24.30
Ufi Gel Hard	5%	24.67	16.06	1.06	63.15	25.41	42.00	19.00	23.10
Probase Cold	Control	24.97	15.97	1.06	67.25	40.70	38.70	15.40	23.30
Probase Cold	Control	24.50	15.76	1.12	70.86	32.40	37.20	18.60	18.60
Probase Cold	Control	24.19	15.99	1.14	99.80	59.19	36.50	7.50	29.00
Probase Cold	Control	24.15	16.04	1.07	66.78	39.25	39.50	15.60	23.90
Probase Cold	Control	24.47	15.71	1.12	73.47	39.78	35.10	16.80	18.30
Probase Cold	5%	24.42	15.88	1.12	71.31	36.26	36.60	17.50	19.10
Probase Cold	5%	24.22	15.95	1.12	70.57	43.68	36.50	15.00	21.50
Probase Cold	5%	24.52	15.86	1.12	69.05	34.32	38.70	17.40	21.30
Probase Cold	5%	24.33	15.77	1.01	72.85	35.50	35.90	18.10	17.80
Probase Cold	5%	24.48	15.91	1.13	69.41	30.45	38.20	18.90	19.30

15. Color Stability (Thermal Aging)

MATERIAL	CHX %	*VITA Easyshade (ES)*					
		L_i	C_i	h_i	L_f	C_f	h_f
Kooliner	Control	51.72	23.93	43.90	56.20	24.93	45.70
Kooliner	Control	53.18	23.40	45.72	56.23	23.73	47.45
Kooliner	Control	52.70	19.75	45.72	55.83	22.35	46.95
Kooliner	Control	52.57	24.93	45.40	55.13	24.92	44.50
Kooliner	Control	52.97	25.95	44.55	57.48	26.08	46.77
Kooliner	2.5%	62.38	27.23	48.50	60.82	36.65	55.38
Kooliner	2.5%	63.30	26.78	47.87	61.20	36.20	55.40
Kooliner	2.5%	64.03	28.92	48.87	62.18	37.32	57.53
Kooliner	2.5%	63.28	27.45	48.47	61.05	37.20	53.18
Kooliner	2.5%	61.10	25.02	44.87	63.58	29.05	47.60
Ufi Gel Hard	Control	44.15	31.02	43.95	46.60	30.57	44.60
Ufi Gel Hard	Control	44.23	31.40	44.58	46.85	31.68	45.37
Ufi Gel Hard	Control	42.42	33.13	43.67	44.03	33.58	44.25
Ufi Gel Hard	Control	43.53	32.17	44.78	45.20	32.62	43.90
Ufi Gel Hard	Control	43.28	32.35	44.97	46.35	32.97	44.98
Ufi Gel Hard	5%	56.12	32.70	46.62	55.97	33.88	49.10
Ufi Gel Hard	5%	54.72	32.83	46.55	53.98	34.53	43.97
Ufi Gel Hard	5%	47.83	32.67	45.95	56.22	31.32	48.57
Ufi Gel Hard	5%	56.62	31.85	45.82	57.33	32.65	49.28
Ufi Gel Hard	5%	55.63	34.08	45.17	57.07	35.62	48.18
Probase Cold	Control	55.52	27.80	43.03	56.80	27.92	43.48
Probase Cold	Control	52.63	26.48	40.97	52.30	26.35	35.77
Probase Cold	Control	55.18	28.20	42.83	55.55	25.90	43.30
Probase Cold	Control	53.13	25.75	41.37	53.35	26.25	41.27
Probase Cold	Control	55.05	29.85	43.00	57.22	28.43	42.40
Probase Cold	5%	63.97	27.00	40.08	62.80	26.33	41.07
Probase Cold	5%	62.82	27.32	39.47	62.13	27.05	41.68
Probase Cold	5%	62.88	28.45	42.30	64.13	28.88	42.07
Probase Cold	5%	63.47	27.42	40.27	62.27	26.40	41.28
Probase Cold	5%	62.00	27.98	41.05	65.92	29.48	41.12

MATERIAL	CHX %	*Spectroshade Micro (SS)*					
		L_i	C_i	h_i	L_f	C_f	h_f
Kooliner	Control	55.62	24.17	38.95	57.88	22.92	39.63
Kooliner	Control	56.27	23.25	39.38	58.10	22.17	39.92
Kooliner	Control	56.45	24.05	38.67	57.85	22.73	39.83
Kooliner	Control	55.83	23.92	39.57	58.25	23.23	39.92
Kooliner	Control	55.47	24.55	38.52	57.93	23.63	39.23
Kooliner	2.5%	59.40	23.05	40.53	60.67	25.07	47.43
Kooliner	2.5%	59.25	23.12	40.48	61.12	24.83	46.85
Kooliner	2.5%	59.98	23.23	40.77	61.27	26.05	48.42
Kooliner	2.5%	56.83	23.38	39.43	61.20	25.38	45.68
Kooliner	2.5%	58.42	22.13	37.82	60.82	22.78	41.30
Ufi Gel Hard	Control	49.92	31.92	43.37	51.32	29.48	42.97
Ufi Gel Hard	Control	49.57	32.27	43.83	52.02	28.20	42.25
Ufi Gel Hard	Control	48.70	34.32	42.65	49.38	32.52	42.62
Ufi Gel Hard	Control	48.35	33.50	43.08	50.35	30.23	41.47
Ufi Gel Hard	Control	49.30	32.57	43.63	50.98	30.02	42.68
Ufi Gel Hard	5%	54.90	29.62	44.18	56.62	26.80	44.08
Ufi Gel Hard	5%	54.47	29.75	44.32	56.58	26.62	46.03
Ufi Gel Hard	5%	55.38	29.50	43.57	57.90	25.10	43.35
Ufi Gel Hard	5%	55.18	28.05	43.30	45.88	27.73	41.35
Ufi Gel Hard	5%	54.58	29.45	42.92	55.65	27.68	42.97
Probase Cold	Control	56.48	25.65	36.50	56.90	25.43	37.13
Probase Cold	Control	55.42	25.98	37.02	55.58	24.93	37.08
Probase Cold	Control	57.13	26.27	36.53	56.55	25.72	37.43
Probase Cold	Control	56.00	25.78	36.80	55.87	25.13	36.33
Probase Cold	Control	55.70	26.20	36.03	56.27	25.62	36.33
Probase Cold	5%	60.57	23.12	36.12	60.38	22.68	36.33
Probase Cold	5%	55.58	24.93	35.65	60.63	22.58	36.68
Probase Cold	5%	62.15	23.87	34.45	61.10	24.12	36.53
Probase Cold	5%	60.10	22.90	35.72	60.38	22.60	36.22
Probase Cold	5%	60.90	23.70	33.33	60.95	23.52	34.63

16. Color Stability (Chemical Aging)

MATERIAL	CHX %	*VITA Easyshade (ES)*					
		L_i	C_i	h_i	L_f	C_f	h_f
Kooliner	Control	52.72	25.05	46.97	51.30	28.15	47.53
Kooliner	Control	51.70	22.22	46.65	52.68	26.00	49.57
Kooliner	Control	52.62	26.20	44.27	51.50	30.40	47.62
Kooliner	Control	53.93	25.22	45.64	52.65	28.45	47.08
Kooliner	Control	52.63	24.07	45.70	52.12	28.45	47.88
Kooliner	2.5%	53.83	30.25	40.22	50.37	45.18	41.47
Kooliner	2.5%	51.88	30.07	40.18	49.03	45.82	41.62
Kooliner	2.5%	51.87	31.15	40.08	49.12	45.53	41.32
Kooliner	2.5%	52.50	28.22	39.73	50.33	43.00	40.57
Kooliner	2.5%	50.65	30.70	40.13	49.25	45.05	41.07
Ufi Gel Hard	Control	43.65	31.35	45.93	44.92	34.03	47.35
Ufi Gel Hard	Control	43.08	30.15	45.37	44.48	33.92	47.57
Ufi Gel Hard	Control	42.93	32.47	46.33	43.60	34.82	47.77
Ufi Gel Hard	Control	43.65	30.80	46.45	44.02	35.02	47.77
Ufi Gel Hard	Control	42.95	30.07	45.55	44.72	33.33	47.53
Ufi Gel Hard	5%	46.70	30.80	47.13	49.17	36.68	51.02
Ufi Gel Hard	5%	47.55	30.63	48.15	49.45	36.13	52.10
Ufi Gel Hard	5%	47.27	27.52	47.63	49.98	34.17	50.95
Ufi Gel Hard	5%	46.78	32.45	46.62	48.67	36.02	50.73
Ufi Gel Hard	5%	49.32	31.28	47.73	50.93	35.22	52.10
Probase Cold	Control	53.00	27.32	40.48	54.68	29.88	40.82
Probase Cold	Control	53.80	26.53	40.95	53.97	27.13	40.20
Probase Cold	Control	53.68	27.92	41.37	55.17	28.88	41.22
Probase Cold	Control	53.58	26.92	43.17	56.12	30.48	40.98
Probase Cold	Control	53.40	28.40	41.25	54.40	29.83	40.60
Probase Cold	5%	57.55	27.27	39.28	52.92	41.18	36.40
Probase Cold	5%	57.83	28.13	39.27	51.95	43.33	36.57
Probase Cold	5%	58.97	30.00	39.13	53.18	43.78	36.53
Probase Cold	5%	57.30	26.90	38.67	51.95	42.27	36.20
Probase Cold	5%	55.72	26.52	39.13	52.62	43.82	36.23

MATERIAL	CHX %	*Spectroshade Micro (SS)*					
		L_i	C_i	h_i	L_f	C_f	h_f
Kooliner	Control	56.62	24.50	40.65	55.62	25.55	43.22
Kooliner	Control	57.08	23.93	40.80	56.30	24.95	43.15
Kooliner	Control	56.20	24.30	40.57	55.53	25.87	42.75
Kooliner	Control	56.87	24.75	40.23	56.40	25.97	42.85
Kooliner	Control	57.30	23.77	40.63	56.80	25.68	43.58
Kooliner	2.5%	55.85	28.28	35.65	52.32	36.28	35.93
Kooliner	2.5%	55.63	28.50	36.42	52.48	36.07	36.80
Kooliner	2.5%	56.10	28.43	36.02	52.40	36.68	36.15
Kooliner	2.5%	56.02	27.30	35.85	52.15	35.67	35.50
Kooliner	2.5%	55.62	28.28	35.47	52.13	36.33	36.05
Ufi Gel Hard	Control	51.60	30.83	45.07	51.45	31.63	46.27
Ufi Gel Hard	Control	51.58	30.83	45.03	51.02	31.75	47.23
Ufi Gel Hard	Control	51.10	32.08	44.83	51.35	32.58	47.25
Ufi Gel Hard	Control	51.18	31.88	45.62	51.12	32.50	46.98
Ufi Gel Hard	Control	51.65	31.13	45.22	51.42	31.62	47.08
Ufi Gel Hard	5%	53.43	30.43	46.08	54.73	29.88	48.02
Ufi Gel Hard	5%	53.62	29.78	46.08	54.27	30.75	48.65
Ufi Gel Hard	5%	53.22	29.87	45.82	53.78	30.75	48.48
Ufi Gel Hard	5%	53.90	30.05	46.00	53.92	31.35	48.73
Ufi Gel Hard	5%	54.15	29.50	46.32	53.80	31.70	49.48
Probase Cold	Control	57.12	27.15	38.08	57.35	26.98	38.47
Probase Cold	Control	57.70	26.05	38.40	58.20	25.97	38.50
Probase Cold	Control	57.55	26.82	38.37	58.25	25.92	37.67
Probase Cold	Control	57.42	27.18	38.07	58.00	27.27	37.60
Probase Cold	Control	57.35	26.77	38.32	58.47	28.02	38.08
Probase Cold	5%	59.72	25.33	36.75	56.07	34.42	34.28
Probase Cold	5%	59.52	25.18	37.17	55.18	35.27	34.35
Probase Cold	5%	59.42	25.23	37.22	55.85	34.98	34.25
Probase Cold	5%	59.63	25.58	37.20	55.80	35.15	34.20
Probase Cold	5%	59.42	25.42	37.00	55.47	35.97	34.28

Appendix 4 – Preparation of Artificial Saliva

Artificial saliva used in the present investigation was prepared according to a formula by the Faculty of Pharmacy of the University of Lisbon, courtesy of PhD student Joana Marto:

1. Boiling 50mL (F12 – ED Refrigerated/Heating Circulator) of phosphate buffer at pH=7.0 (anhydride disodium phosphate, monosodium phosphate anhydride and deionized water) at 60ºC;

2. Sprinkling 0.05g of xanthan gum into boiling buffer and stirring until the xanthan gum was completely dissolved;

3. Dissolving 0.04g of calcium chloride dihydrate (EW – N/EG – N balance), 0.08g of sodium chloride, and 0.08g of potassium chloride in solution 2 and stirring until materials were completely dissolved;

4. Dissolving 15g of propylene glycol in solution 3 and stirring until propylene glycol was completely dissolved;

5. Pouring the solution 4 into a graduated beaker and complete the solution with phosphate buffer at pH=7.0 to 100mL;

Solutions were kept out of light at room temperature.

For the chemical aging (Chapter 5), we needed artificial saliva at pH=3, so step 6 was added:

6. Adjusting the pH of artificial saliva to pH=3 with HCl 1M.

Appendix 5 – Manufacturer's Instructions

1. Kooliner

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

KOOLINER™

HARD CHAIRSIDE DENTURE RELINE

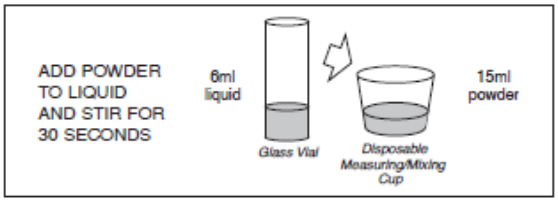
For use only by a dental professional in the recommended indications.

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

GC

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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
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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