UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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Synthesis of silver nanoparticles associated with denture adhesive: an antimicrobial approach against *Candida albicans* biofilms

Síntese de nanopartículas de prata associadas ao adesivo protético: uma abordagem antimicrobiana contra biofilmes de *Candida albicans*

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Orientador: Prof. Dr.Vinicius Carvalho Porto.

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"Ensinar não é transferir conhecimento, mas criar as possibilidades para sua produção. Quem ensina aprende ao ensinar e quem aprende ensina a aprender".

Paulo Freire

RESUMO

A associação de compostos antimicrobianos aos adesivos protéticos pode ser considerada uma alternativa promissora para usuários de prótese total, pois, além de auxiliar na retenção da prótese durante as forças mastigatórias, pode colaborar no tratamento e/ou prevenção da estomatite protética, infecção na mucosa de suporte causada principalmente pela colonização do fungo *Candida albicans* na superfície interna da dentadura em forma de biofilme, proporcionando melhores condições de saúde bucal e de qualidade de vida para a população edêntula. Neste contexto, a nanotecnologia tem ganhado espaço na Odontologia, uma vez que nanopartículas, especialmente as de prata, apresentam potencial antimicrobiano e demostram ser biocompatíveis podendo ser utilizadas no tratamento de infecções orais. Adicionalmente, a nanotecnologia oferece inúmeras vantagens para o estudo laboratorial de compostos com aplicação na saúde e pode tornar-se uma grande aliada na confecção ou no aperfeiçoamento de materiais odontológicos. Assim, o objetivo deste estudo foi avaliar a atividade antimicrobiana de nanopartículas de prata, sintetizadas por três rotas distintas (método de luz ultravioleta, método de Turkevich e método de química verde utilizando extrato de *Glycine max*), associadas ao adesivo protético COREGA® em pó frente ao biofilme de *C. albicans*. Para tanto, corpos de prova de resina acrílica termopolimerizável foram confeccionados como substrato (n=3 por grupo). Após, a superfície dos espécimes foi tratada com a associação do adesivo com as nanopartículas de prata, sintetizadas pelo método de luz ultravioleta (grupo **AD + Ag UV**), método de Turkevich (**AD + Ag Turk**) e o método de química verde utilizando extrato de *Glycine max* (**AD + Ag Gm**). Como controles, espécimes foram tratados com nistatina associada ao adesivo (grupo **AD + Nist**), apenas o adesivo (grupo **AD**) ou submersos em PBS (grupo **PBS**). Após os tratamentos, o biofilme de *C. albicans* foi desenvolvido durante 3, 6 e 12 horas sobre a superfície dos corpos de prova. Na sequência, foram realizadas análises quanto à atividade antimicrobiana e viabilidade celular fúngica, por meio da concentração inibitória e fungicida mínima, pela quantificação das unidades formadoras de colônias por mililitro, pelo ensaio colorimétrico de sais de tetrazólio e por microscopia confocal de varredura a lazer. Foram realizados três experimentos independentes e os resultados apresentados pela média ± desvio padrão, considerando valores significativos quando $p<0.05$. Após o período de 3, 6, 12 horas de desenvolvimento do biofilme, os grupos tratados com as associações do adesivo e as nanopartículas, independentemente da rota, apresentaram uma carga fúngica reduzida e um percentual de redução da atividade metabólica do biofilme ≥50% em comparação com os grupos

controle, sendo estes resultados similares e/ou melhores do que os obtidos com o grupo controle de tratamento AD + Nist. Conclui-se que as nanopartículas de prata sintetizadas pelo método de luz UV, Turkevich e pela química verde por meio do extrato de *Glycine max* têm excelente atividade antimicrobiana contra biofilmes de *Candida albicans.* A associação de nanopartículas de prata com o adesivo COREGA® é uma alternativa inovadora que pode ter um efeito potencial preventivo sobre a estomatite protética.

Palavras chave*: Nanopartículas metálicas. Estomatite sob prótese. *Candida albicans*. Adesivos teciduais.

*Em acordo com os Descritores em Ciências da Saúde (DeCS), disponíveis no domínio http://decs.bvr.br/

ABSTRACT

Synthesis of silver nanoparticles associated with denture adhesive: an antimicrobial approach against *Candida albicans* biofilms

The association of antimicrobial compounds with prosthetic adhesives can be considered a promising alternative for users of complete dentures, because, in addition of helping to retain the denture during masticatory forces, it can collaborate in the treatment and/or prevention of prosthetic stomatitis, also in infections in the supporting mucosa caused mainly by the colonization of the fungus *Candida albicans* on the inner surface of the denture in the form of a biofilm, providing better oral health and quality of life conditions for the edentulous population. In this context, nanotechnology has gained ground in Dentistry, since nanoparticles, especially silver, have antimicrobial potential and prove to be biocompatible and can be used in the treatment of oral infections. Additionally, nanotechnology offers numerous advantages for laboratory studies of compounds with application in health and can become a great ally in the manufacture or improvement of dental materials. Thus, the aim of this study was to evaluate the antimicrobial activity of silver nanoparticles, synthesized by three distinct routes (Ultraviolet light method, Turkevich method and green chemistry method using *Glycine max* extract), associated with the COREGA® powder prosthetic adhesive against the biofilm of *C. albicans*. For this purpose, thermopolymerizable acrylic resin specimens were made as a substrate (n=3 per group). Afterwards, the surface of the specimens were treated with the association of the denture adhesive and silver nanoparticles, synthesized by the ultraviolet light method (**AD + Ag UV** group), the Turkevich method (**AD + Ag Turk**) and the green chemistry method using *Glycine max* extract (**AD + Ag Gm**). As controls, specimens were treated with nystatin associated with the patch (**AD + Nist** group), only the patch (**AD** group) or were submerged in PBS (**PBS** group). After the treatments, the biofilm of *C. albicans* were developed for 3, 6 and 12 hours on the surface of the specimens. Subsequently, analyzes were carried out for antimicrobial activity and fungal cell viability, through the minimum inhibitory and fungicidal concentration, by quantifying the colony-forming units per milliliter, by the colorimetric assay of salts of tetrazolium and confocal laser scanning microscopy. Three independent experiments were carried out and the results were presented as mean ± standard deviation, considering significant values when $p<0.05$. After 3,

6 and 12 hours of biofilm development, the groups treated with adhesive and nanoparticles association, regardless of the route, presented a reduced fungal load and a percentage of reduction of biofilm metabolic activity ≥50% compared to the control groups, and these results were similar and/or better than those obtained with the treatment control group AD + Nist. It was concluded that silver nanoparticles synthesized by UV light, Turkevich method and by the green synthesis through *Glycine max* extract have excellent antimicrobial activity against *Candida albicans* biofilms. The combination of silver nanoparticles with COREGA® adhesive is an innovative alternative that can have a potential preventive effect for denture stomatitis.

Keywords*: Metallic nanoparticles. Stomatitis under prosthesis. *Candida albicans*. Tissue adhesives.

*According to the Health Sciences Descriptors (DeCS), available in the domahttp://decs.bvr.br/

LISTA DE ILUSTRAÇÕES

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LISTA DE ABREVIATURA E SIGLAS

EP Estomatite protética
DS Denture stomatitis
PTs Próteses totais
Ag Prata/Silver
AD Adesivo
NPs Nanopartículas
Gm *Glycine max* extract
UV Ultraviolet light
Turk Turkevich route of synthesis
XTT Tetrazolium salt solution
PBS Phosphate buffered saline solution
MIC Minimum inhibitory concentration
MFC Minimum fungicidal concentration
CFU Colony forming units
CLSM Confocal laser scanning microscopy
XDR X-ray diffraction analysis
TEM Transmission electronic microscopy analysis
SAED Selected area electron diffraction
EDX Energy Dispersive X-Ray Analysis
HRTEM Transmission electron microscopy and high-resolution transmission electron microscopy
SMF Supplementary files

SUMÁRIO

Introdução

1 INTRODUÇÃO

As próteses totais (PTs) removíveis são o principal tratamento reabilitador de base para o edentulismo completo (ALVES et al., 2018; SUN et al., 2018). Ainda uma grande parte da população mundial continuará tendo este tratamento como a primeira escolha para devolver a função e estética dos pacientes totalmente desdentados, pelas características desta modalidade restauradora e os resultados satisfatórios que podem ser conseguidos com ela (CARLSSON; OMAR, 2010; LEE; SAPONARO, 2019; SLADE, AKINKUGBE, SANDERS, 2014; CARDOSO et al. 2016).

A Estomatite Protética (EP) tem sido relatada como uma das mais frequentes lesões de tecidos moles decorrentes do uso de PTs, principalmente dos maxilares, apresentando-se em mais de 75% dos usuários (PURYER, 2016). Este tipo de lesão é um processo inflamatório crônico iniciado pelo desenvolvimento de um biofilme microbiano complexo na superfície interna da prótese, que depois é observado como placa bacteriana (HANNAH et al., 2017; DE SOUZA et al., 2017). Os principais fatores de risco da EP estão relacionados a fatores locais específicos, como o tabagismo, a má higiene oral e a xerostomia além de fatores sistêmicos, como a imunossupressão, as desordens endócrinas e o próprio uso prolongado das próteses que cria condições favoráveis para o desenvolvimento e permanência do biofilme, principalmente de *Candida albicans*, na superfície interna da prótese, em contato com os tecidos de suporte (SHARON; FAZEL, 2010; HANNAH et al., 2017; BRANTES et al., 2019; CONTALDO et al., 2019).

Segundo as suas manifestações clínicas existem três tipos de EP ou estágios: hiperemia localizada (tipo I), eritema geral difuso no palato (tipo II) ou hiperplasia papilar inflamatória (tipo III) (NEWTON, 1962).

A formação do biofilme de *C. albicans* começa com a adesão ou união da levedura do fungo, presente na microbiota oral de forma comensal, à superfície acrílica da prótese. Este processo de desenvolvimento ocorre em três tempos diferentes: o inicial, de 0 a 11 horas, o intermediário, de 12 a 30 horas e o maduro, de 38 a 72 horas. (CHANDRA, 2001). No primeiro tempo o fungo, em forma de levedura, se adere à superfície acrílica e forma pequenas colônias nas rugosidades do material. Na fase intermediária as microcolônias aparecem com uma cobertura de material não celular que se desenvolve sobre as colônias. Ao alcançar a maturação, este biofilme se constitui em uma comunidade de

microrganismos protegida na sua totalidade por uma camada de matriz extracelular densa responsável pelas diversas infecções fúngicas no organismo como a EP (CHANDRA, 2001; BHATTACHRYA; SAE-TIA; FRIES, 2020). O desenvolvimento do biofilme é estimulado também por condições inerentes da resina acrílica termopolimerizável na qual as PTs são confeccionadas, como sua rugosidade, porosidade e hidrofobicidade (PERO et al., 2010; DE FOGGI et al., 2014), que favorecem a adesão de fungos do gênero *Candida* spp. podendo ser isoladas principalmente espécies de *C. albicans*, e em menor ordem, *C. famata* e *C. tropicallis* (GLEIZNYS; ZDANAVIčIENÉ; ŽILINSKAS, 2015; GAUCH et al., 2018).

O gênero *Candida* spp. caracteriza-se por desenvolver resistência aos fármacos, possuindo a capacidade de mudar a sua morfologia desde levedura até sua forma filamentosa ou hifa, que é a forma patogênica presente na maturação do biofilme (AL-FATTANI; DOUGLAS, 2006).

A abordagem convencional da EP associada a *C. albicans*, inclui duas fases: a primeira contempla a troca ou ajuste da base da prótese, o controle da predisposição sistêmica do paciente (PURYER, 2016), o aumento da higiene protética (YARBOUROUGH et al., 2016), e a eliminação do uso noturno da prótese (BRANTES et al.,2019). Em um segundo enfoque é feita a indicação de medicamentos, que podem ser administrados de forma tópica e sistêmica ou pela combinação de ambas as vias, utilizando antifúngicos do tipo azoles (SAKAGUCHI, 2017; DI STASIO et al.,2018), e poliênicos (WEBB et al., 1998; MILLSOP; FAZEL, 2016).

O antifúngico tópico considerado padrão ouro no tratamento da EP é a nistatina (SCHEIBLER et al., 2017). Seu mecanismo de ação acontece por meio da união ao ergosterol da célula fúngica, criando porosidades na membrana celular, aumentando a permeabilidade da célula, ocasionando extravasamento de conteúdo citoplasmático, e consequentemente, levando a morte (KINOSHITA, 2016). Atualmente, estudos *in vitro* demonstram que a nistatina pode ser utilizada como excelente controle na avaliação da ação antifúngica de novos compostos (GAVANJI; LARKI, 2017; NOSRATZEHI et al., 2019; POMA-CASTILLO et al., 2019; LOYAGA-CASTILLO et al., 2020). Embora apresente eficácia na erradicação fúngica, efeitos adversos foram relatados em relação ao seu uso, tais como gosto ruim, náuseas, vômitos, diarreia e dores abdominais (PANKHURTS, 2013).

Sendo assim, os efeitos adversos quanto à utilização dos fármacos sintéticos, associada à baixa solubilidade e sua biodisponibilidade, bem como a reincidência da EP gerada pelo desenvolvimento da resistência dos microrganismos aos fármacos convencionais de amplo

espectro utilizados para tratamento, despertaram o interesse pela busca de novas alternativas terapêuticas (ZHANG, 2010; RAUTEMA; RAMAGE, 2011; PRASAD; NAIR; BARNERJEE, 2019).

A má adaptação das próteses, bem como o aumento da porosidade e rugosidade da superfície interna, assim como o uso prolongado facilita o acúmulo de alimentos na interface mucosa subjacente e prótese, funcionando como um reservatório de microrganismos, auxiliando no crescimento de biofilmes (SALERNO et al., 2011). Sendo assim, como uma possível medida profilática, o uso de adesivos protéticos, como COREGA®, FIXODENT® dentre outros, pode contribuir para a redução deste fator de risco para o desenvolvimento da EP (SAMPAIO-MAIA et al., 2012; RAJARAM; MANOJ, 2017).

Os adesivos protéticos são materiais muito indicados para os pacientes usuários de PT, visto que servem para conferir maior retenção e estabilidade à prótese, melhorar a realização de atividades funcionais com a prótese, fornecendo assim conforto e segurança ao paciente (KUMAR, 2015; MATALON et al., 2017; SHAMSOLKETABI; NILL, 2018). O uso destes produtos é também recomendado para casos de pacientes que apresentam xerostomia, que usarão prótese imediata despois de uma cirurgia, quando existem desordens hormonais e neuromusculares, que possam afetar a coordenação motora e as condições bucais, e para prevenção de ulcerações e traumas na mucosa, além de serem usados na instalação de próteses novas (GRASSO, 2004). Assim, eles estão contraindicados em casos de alergia aos componentes, perda da dimensão vertical por reabsorção óssea, para retenção de PTs fraturadas e em casos de dificuldade de higienização pelo paciente. (KUMAR, et al., 2015).

Os adesivos, por si só, possuem uma ação antimicrobiana básica por conta de alguns elementos da sua composição como o hexaclorofeno, borato sódio e o metil salicilato (GRASSO, 2004; PRADÍES et al., 2009; ILAKKIYA, 2016). Além disso, na composição estão presentes polímeros sintéticos e outras substâncias como o sódio carboximetil celulosa e o polivinil acetato responsáveis pelas propriedades adesivas do produto, o dióxido de silicone que previne a aglutinação do pó, além de outros componentes estabilizantes (RAJARAM; MANOJ, 2017).

Ao longo do tempo esses produtos têm sido aperfeiçoados com a adição de antimicrobianos na busca da prevenção e terapêutica contra biofilmes de *C. albicans* (PARK et al., 2015; ALMEIDA et al., 2018; GARAICOA et al., 2018). Estudos prévios têm enriquecido a composição de material odontológico por meio da adição de substâncias

sintéticas, como os sais de clorexidina e nistatina (MATALON et al., 2017; GARAICOA et al., 2018). Outros trabalhos avaliaram *in vitro* a incorporação de fitoterápicos, como *Punica granatum* e *Equisetum giganteum*, ao adesivo, observando diminuição/inibição do crescimento de *C. albicans* (ALMEIDA et.al, 2018).

Neste contexto, a Nanotecnologia surge como uma importante ferramenta para a Odontologia oferecendo benefícios e múltiplas vantagens como: atividade farmacológica otimizada (DILNAWAZ; ACHARYA; SAHOO, 2018), maior biodisponibilidade (IQBAL et al. 2017), carregamento de substâncias terapêuticas (SOUSA et al., 2020), e a redução dos efeitos secundários provocados pelos fármacos (ONOUE; YAMADA; CHAN, 2014; LIU; YANG; XIONG; GU, 2016; WANG; HU; SHAO, 2017; RAZA et al., 2019). A nanotecnologia objetiva à síntese de substâncias inorgânicas em escala nanométrica com um diâmetro de 1-100nm, fornecendo resultados promissores na abordagem terapêutica contra biofilmes de fungos e bactérias, além de conferir resultados com menores reações adversas quanto ao seu uso em relação aos fármacos comumente utilizados (MBA; NWEZE, 2020; GAD; FOUDA, 2020, LIU et al., 2019).

As nanopartículas (NPs) metálicas apresentam maior potencialidade antimicrobiana, podendo constituir uma nova geração de agentes antifúngicos ou estarem associadas a outros medicamentos, e um caminho alternativo no combate da crescente resistência microbiana, por meio de mecanismos de ação diferenciados quando comparados com os compostos convencionalmente utilizados para terapêutica (SLAVIN et al., 2017). Os metais que podem ser utilizados na terapêutica antimicrobiana combinada com a nanotecnologia são a Prata (Ag), Ouro (Au), Zircônia (Zr), Cobre (Cu), Zinco (Zn), Titânio (Ti) (GAD et al., 2017; GAD; FOUDA, 2020; SÁNCHEZ- LOPES et al., 2020), sendo a prata o metal mais utilizado e descrito na literatura em relação ao seu potencial antimicrobiano, abrangendo diversos campos da área biomédica e odontológica (SINGH et al., 2015; ZHANG et al., 2016; BAPAT et al., 2018; AGNIHOTRI; GAUR; ALBIN, 2020; ALMATROUDI, 2020)

As nanopartículas de prata (Ag NPs) possuem diversas propriedades as quais guardam íntima relação com a sua funcionalidade, a especificidade da ação antimicrobiana e o seu comportamento biológico (DOS SANTOS et al., 2014; SHANMUGANATHAN et al., 2019; XU et al., 2020). As principais propriedades das Ag NPs são ópticas, físicas e catalíticas e são conferidas pelos resultados obtidos dos diversos métodos de síntese e caracterização que conferem a sua morfologia, o tamanho, o tipo de carga da superfície, a área de superfície, a agregação e a dissolução entre outras (WEI et al., 2015; HOSEINZADEH et al., 2017;

TANG; ZHENG, 2018). As características mais importantes como a morfologia, o tamanho e a carga de superfície influenciam no efeito antimicrobiano e, em relação à forma, destacam-se com esse efeito antimicrobiano basicamente três formas de NPs: haste, triangular e esférica, sendo que as triangulares e esféricas têm mostrado os melhores resultados (PAL; TAK; SONG, 2007). O tamanho das partículas de prata utilizadas na literatura está no intervalo de 1 até 100nm, salientando-se que partículas de menor tamanho (1-10nm) apresentam uma maior toxicidade contra as células fúngicas e bacterianas, sendo isso o suficiente só para agredir ou danificar a membrana do microrganismo (MORONES et al., 2005; SIDDIQI; HUSEN; RAO, 2018; ALMATROUDI, 2020; YIN et al., 2020). Partículas menores possuem maiores áreas de superfície e isso se traduz em um mecanismo antimicrobiano onde existe uma captação maior de íons de prata naquela superfície e estresse oxidativo para a célula fúngica (TANG; ZHENG, 2018). O tipo de carga de superfície das Ag NPs é positivo o que facilita o fenômeno de atração pela carga negativa que possui o microrganismo, permitindo-se a ação contra essas células microbianas (ABBASZADEGAN et al., 2015).

Dentre os supostos mecanismos relatados sobre estes compostos nanoparticularizados estão os danos provocados à membrana celular, interrompendo a funcionalidade e a homeostasia das células fúngicas ou bacterianas (MBA; NWEZE, 2020). Estudos recentes têm incluído nanopartículas de alguns metais, como Ag, Zr e Zn no material de base das PTs como o polimetilmetacrilato (PMMA) e condicionadores de tecidos ou resinas macias, modificando as suas características físicas com o objetivo de prevenir a adesão do biofilme na superfície das PTs (GAD; FOUDA, 2020; AHMAD; KHAN, 2020; TAKAMIYA et al., 2021; MOUSAVI et al., 2020).

Partindo destes pressupostos e aplicabilidade das Ag Nps, vem sendo considerado também o efeito do método de obtenção utilizado para a sua síntese (físico, químico e biológico) (ABASSI et al., 2016) nas características do material nanométrico, como o tamanho e a forma, visando a melhoria das propriedades das nanopartículas que possam repercutir no efeito biológico e antimicrobiano (PATIL; KIM, 2017; RÓNAVARÍ et al., 2021).

O desenvolvimento de materiais em escala nanométrica é um caminho inovador e promissor para a criação de terapias farmacológicas mais eficientes em relação à resistência desenvolvida pelos microrganismos, além da possibilidade de diminuição de efeitos citotóxicos gerados ao nível celular com as terapêuticas fúngicas convencionais (RIVERA; RAMOS, 2014; SOUSA et al., 2020).

Tendo em vista a relevância do papel antimicrobiano no que diz respeito à nanotecnologia, o aperfeiçoamento de materiais odontológicos, através da incorporação de Ag NPs pode ser considerado uma alterativa inovadora no contexto de saúde bucal, especialmente para pacientes usuários de PTs, visando o tratamento e/ou prevenção da EP. Portanto, este estudo avaliou a atividade antimicrobiana de Ag NPs, associadas ao adesivo protético COREGA® contra biofilmes de *C. albicans*, sintetizadas por diferentes rotas.

2 Artigo

2 ARTICLE

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Silver nanoparticles in denture adhesive:

an antimicrobial approach against *Candida albicans*

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Silver nanoparticles in denture adhesive: an antimicrobial approach against *Candida albicans*

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CONFLICT OF INTEREST

The authors declare disclosure of non-conflict of interests of authorship and publication of this article.

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AUTHOR CONTRIBUTIONS

Laura Ferreira Peralta: Conceptualization; Formal analysis; Investigation; Writing original draft preparation. **Nara Almeida Martins:** Investigation; Project administration, Methodology; Writing- review and editing. **Fenelon Martinho Pontes:** Methodology; Investigation; Resources. **Daniel Rinaldo:** Resources. **Camila Alves:** Writing original draft preparation. **Vanessa Soares Lara:** Methodology; Resources; review and editing. **Vinicius Carvalho Porto:** Funding acquisition; supervision; writing- review and editing.

Silver nanoparticles in denture adhesive: an antimicrobial approach against *Candida albicans*

ABSTRACT

Objective: To evaluate the antimicrobial activity of silver nanoparticles, synthesized by three different routes and associated with COREGA® prosthetic adhesive powder against *C. albicans* biofilm. **Methods:** Specimens of thermopolymerizable acrylic resin were made as substrate, their surface was treated with silver nanoparticles, synthesized by the ultraviolet light method, Turkevich method or green chemistry method using *Glycine max* extract, associated with the adhesive (AD + Ag UV, AD + Ag Turk and AD groups + Ag Gm, respectively). As controls, specimens were treated with nystatin plus adhesive (AD + Nist group), adhesive alone (AD group) or submerged in PBS (PBS group). After the treatments, *C. albicans* biofilm of was developed for 3, 6 and 12 hours on the surface of the specimens. Subsequently, the biofilm was quantified using colony forming units per milliliter, by colorimetric assay and by confocal laser scanning microscopy. **Results:** Regardless of the period, a significant reduction ($p<0.05$) of fungal load and viable cells was observed in the groups treated with the combinations of the adhesive and the nanoparticles, in addition to the inhibition of metabolic activity compared to the AD and PBS groups, mainly in the AD + Ag Gm group, these results being similar to those obtained in AD + Nist. **Conclusion:** Silver nanoparticles exerted excellent antimicrobial activity against *C. albicans* biofilms mainly by the green chemistry methods using the *Glycine max* extract. The proposal of the association of silver nanoparticles with the COREGA® adhesive can become an innovative preventive alternative with potential on denture stomatitis.

Clinical significance: Innovation in dentistry materials is a crucial matter for studying dental new preventive approaches for oral lesions. This study brought an interesting association of nanotechnology and dentistry sciences, proportionating novel antimicrobial properties to a frequently used product in denture users, denture adhesives, for the prevention of denture related stomatitis.

1. INTRODUCTION

The *Candida albicans* biofilm causes several opportunistic human infections, including oral candidiasis, and have developed resistance to conventional treatments [1-3]. The prevalence rates of oral candidiasis associated with oral lesions have been related to 80% of isolated *Candida albicans* fungi [4]. The proportion of denture wearers with oral candidiasis lesions represents 75% of this edentulous population [5]. This fungus is characterized by the ability to change its morphology from yeast to its filamentous or hyphae pathogenic form [6].

The biofilms are form by an adhered community of microorganisms protected by an extracellular matrix, in oral cavity the development of *C. albicans* biofilms occurs in different stages through time, this fact is stimulated by poor hygiene, prolonged use of artificial denture and deficient denture fitting facilitating the accumulation of food on the underlying mucosal interface and the denture [7]. In addition, the inherent characteristics of the acrylic resin of the base of an artificial denture, such as roughness, porosity and hydrophobicity, becomes this material a reservoir for microorganisms like *Candida* spp., related to a common inflammatory infection called denture stomatitis (DS) [8- 10].

In order to improve the misfit of dentures the use of adhesives, such as Corega® and Fixodent® are options that can contribute to the reduction of the risks factors and effects of DS [5, 11]. Denture adhesives are suitable materials for patients using complete denture, improving retention and stability of prosthesis, performance of functional activities with the denture, and consequently comfort and safety to the patient [12-14]. These products by themselves have a basic antimicrobial activity arranged by some of its components like hexaclorophene, sodium borate, ethanol and methyl salycilate [15-17]. Thus became interesting the association of antimicrobial substances into the prosthetic material formula, including synthetic drugs and phytotherapeutic extracts [13,18,19]. The gold standard treatment for oral candidiasis is nystatin in suspension and the systemic approach includes azoles-derived drugs. Despite the great pharmacological relevance of these drugs, they have adverse effects such as nausea and vomiting for those in topical use, *versus* hepatotoxicity and gastrointestinal disorders for systemic ones [4, 20]. This has stimulated the search for new therapeutic methods for the prevention and treatment of fungal diseases [21-23].

In this context, nanotechnology has emerged as an important tool for dentistry, offering multiple benefits. The improved physical, chemical, and biological characteristics compared to classical treatments are similar or better than conventional treatment. They provide optimized pharmacological activity [24], greater bioavailability [25], loading of therapeutic substances [26], and reduced side effects [27-30].

The development of materials on a nanometric scale is an innovative and promising method to create more efficient pharmacological therapies causing less resistance, in addition to reducing the cytotoxic effects generated at the cellular level with conventional antifungal therapies [31,32].

Thus, metallic nanoparticles, such as silver nanoparticles (Ag NPs), have great antimicrobial potential, varying based on their synthesis method (physical, chemical, or green synthesis) [33]. Ag NPs may constitute a new generation of antifungal agents or be associated with other drugs, providing an alternative way to combat the microbial colonization through different mechanisms of action compared to conventionally used compounds for therapy, for example interacting with cell membrane, nuclei, mitochondrial and other ways [34-39]. For these reason different dentistry materials like nanocomposites, implants, endodontic solutions and others have been associated with Ag NPs [40, 41].

Based on the possibilities of the nanotechnology role, this study aimed to assess the antimicrobial activity of Ag NPs synthesized by different routes and associated with Corega® denture adhesive against biofilms of *C. albicans.*

2. MATERIALS AND METHODS

2.1 Synthesis and characterization of silver nanoparticles (Ag NPs)

Here, we used a biological and two chemical synthesis methods of silver nanoparticles (Ag NPs); cost-effective and eco-friendly.

2.1.1 ***UV light method (Called Route 1):*** This route was an adapted form of other studies with some modifications [42,43] Ag NPs were obtained from the mixture of the reducing agent sodium citrate (6 mL) in 10-mM of concentration and the silver nitrate solution $AgNO_3$ (50 mL) in 4-mM of concentration, at constant temperature (23°C) under magnetic stirring.

The resulting solution was exposed to UV radiation for 30 min until the formation of Ag NPs.

2.1.2 ***Turkevich method (Called Route 2):*** For this route was follow other studies with some modifications [44- 46] 50 mL of $AgNO_3$ solution (4-mM of concentration) was initially heated to 80°C; then, 6 mL of the reducing agent sodium citrate solution at 10-mM of concentration was added dropwise under magnetic stirring. The solution hue changed from colorless to light golden, indicating the formation of Ag NPs. The heating was stopped, and the solution was allowed to cool naturally to room temperature.

2.1.3 ***Green chemical method using Glycine max Extract (Called Route 3)***: This route was an adapted form of other studies with some modifications [47, 48]. Initially, 30 mg of the reducing agent *G. max* extract (soybean) was dissolved in 10 mL of distilled water. After complete dissolution of the extract, 50 mL of $AgNO_3$ solution at 4-mM of concentration was added at constant temperature (23°C) under magnetic stirring. The resulting solution was then exposed to radiation from a 367-nm LED lamp for 60 min to accelerate the formation of Ag NPs.

The formation of all Ag NPs was monitored using (Agilent/HP 8453) UV-visible spectrophotometer at a wavelength varying from 300 to 900 nm using quartz cuvette (optical path length 10 mm).

Silver nanoparticles were characterized by different techniques. The crystalline nature and phase identification of the synthesized Ag NPs was analyzed by an X-ray diffractometer (XRD; Miniflex 600, Rigaku) using a Cu Kα radiation ($\lambda = 1.5406$ Å) operating at a voltage of 40 kV and a current of 15 mA. The samples were prepared by dropping on a glass slide and air-dried before transferring to XRD analysis. Transmission electron microscopy and high-resolution transmission electron microscopy (TEM/HRTEM) connected to Energy Dispersive X-Ray Analysis (EDX) was used for morphological characterization, size distributions and elemental composition of the NPs'. The samples were deposited on a carbon-coated copper holder and dried at room temperature before observing under TEM operating at 200 kV. The particle size was analyzed from TEM images using the Image J software.

2.2 Microorganism

C. albicans strain (SC5314) was used, and the inoculum was prepared and standardized according to a previously described protocol [18]. The strain was grown in YEPD broth (Difco®, Sparks, MD, USA), and cells were inoculated into Sabouraud dextrose broth (Difco®) to obtain only the yeast form.

2.3 Determination of minimum inhibitory concentration and minimum fungicidal concentration of Ag NPs against planktonic cells of *C. albicans*

For an initial screening the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) were determined.

MIC was identify as recommended by the EUCAST protocol (European Committee on Antimicrobial Susceptibility Testing [49], and was considered the lowest concentration of nanoparticles that inhibited 50% of the growth of *C. albicans*. The inoculum of *C. albicans* was standardized to a concentration of 1×10^6 cells/mL. Serial dilutions of the nanoparticles were performed in 96-well plates (TPP® 91015 - Techno Plastic Products, Trasadingen, SH,

Switzerland) containing RPMI-1640 culture medium + 2% glucose (Dulbecco's Modified Eagle Medium, Gibco®).

The initial concentrations of nanoparticles were 4-mM/mL synthesized by the different routes (Ag UV, Ag Turk and Ag Gm) (according to item 2.1), for the Ag Gm group (was used 26 mg of *Glycine max* extract (Gm) only and 1,3 mg of the same extract for its association with Ag NPs) **Figure 1**. Starting from this low values is a choice to obtain smaller particles. High concentrations run the risk of larger nuclear particles, and we need other reagents in the synthesis to prevent particle growth. Therefore, was choose lower concentrations and less use of reagents for a clean synthesis. In addition, the results were quite satisfactory with this concentration. In each well, 100 µL of the suspension of *C. albicans* was added, and the plate was incubated in an oven at 35ºC under agitation (75 rpm). After 24 hours, the reading was performed at 540 nm. As controls, wells containing only the culture medium (BLANK), and wells containing the inoculum + RPMI-1640 (growth control group) were added. For comparison, serial nystatin dilutions (32 mg/mL) were used as a fungal death control. Three independent experiments were performed in triplicate and data were transported to the percentage of death by optical density (OD) obtained in the growth control group.

The minimum fungicidal concentration (MFC) was determined based on a previous methodology [50], with some modifications. An aliquot of 50 µL of these concentrations characterized by MIC ≥ 50% was seeded on the surface of Agar Sabouraud in Petri dishes with the aid of the Drigalsky loop, in duplicate. The plates were incubated at 37°C and, after 48 hours, the colony-forming units (CFU/mL) were quantified and the MFC was defined as the lowest concentration capable of causing the death of the inoculum, that is, the Petri dish must be free of microbial growth (absence of CFUs).

2.4 Fabrication of specimens

The specimens were fabricated as described previously in other studie [18]. A total of 240 heat-cured acrylic resin specimens (Lucitone 550; Dentsply International Inc., Chicago, MI, USA) were made (10 mm × 10 mm × 5 mm), according to the manufacturer's instructions. Both sides of all the specimens were ground in a horizontal polisher (ER 27000; Erios, São Paulo, Brazil) and were randomly selected to simulate the inner portion of the denture base with a mean surface roughness (Ra) of 3 µm (Surftest SJ-301; Mitutoyo Corporation, Kanagawa, Japan). After polishing, the specimens were then subjected to ultrasonic cleaning (Arotec Odontobrás, Ribeirão Preto, SP, Brazil) for 20 min in distilled water, maintained at 37°C for 48 h to allow the release of the residual monomers [51] and sent for sterilization using ethylene oxide (Acecil® - Central Sterilization of Commerce and Industry Ltda, Campinas, Brazil).

2.5 Surface treatment of specimens

The surface treatment of specimens followed the protocol of Almeida et al.[18] with some modifications. Previously, 1 mL of the pre-defined concentration of Ag NPs (4Mm) for all routes (as described in section 2.1) was mechanically homogenized mixed with the aid of a microbrushes, in 10 mg of denture adhesive powder (AD) (Corega® Powder, GlaxoSmithKline Brasil Ltda. Rio de Janeiro, RJ, Brazil) in 2 mL microtubes for centrifugation (Safe-lock – 2.0 mL, Eppendorf® do Brasil Ltda., São Paulo, Brazil). Thus, the combinations consisted of a suspension containing 1% of denture adhesive (10 mg/mL). The entire volume of the suspension was brushed onto the acrylic resin specimen surfaces which

were then placed in a 24-well cell culture plate for 50 min, to allow for the complete adhesion of all the components on the surfaces.

Then, the specimens were dried at room temperature for 24 hours, resulting in three different experimental groups: AD + Ag UV, AD + Ag Turk and AD + Ag Gm groups according to the three Ag NP synthesis method: UV light, Turkevich or Green chemical, respectively (n = 3 per group in each experiment).

As controls, some specimens were untreated and immersed in 1 mL of PBS (PBS group), allowing fungal growth, while others were treated only with 10 mg/mL of COREGA® to evaluate the possible antimicrobial action of the vehicle (AD group) or with an adhesive/nystatin combination (10 mg/mL of adhesive and 32 mg/mL of nystatin – Pharmacia Specifica Ltda, Bauru, Brazil) [52] to authenticate antimicrobial action (AD + Nist group).

2.6 *C. albicans* biofilm growth on the specimen surface

After treatment, the specimens were placed in 24-well culture plates (TPP® - Techno Plastic Products, Trasadingen, Switzerland) and immersed in 1 mL of *C. albicans* inoculum (at a concentration of 1×10^7 cells/mL). The adhesion time was 90 min at 37°C and 75-rpm agitation [53, 54]. Subsequently, the non-adherent fungal cells were removed by washing the specimens with PBS, and then transferring them to another 24-well cell culture plate. Next, the specimens were immersed in 1 mL of RPMI-1640 [55] and incubated at 37°C under 75-rpm agitation for 3, 6, or 12 hours [53, 56]. After, the specimens were carefully washed three times with 1 mL of PBS. The acrylic specimens should be positioned vertically in the wells of the 24-well plates, favoring active microbial adhesion. This would avoid the influence of gravity on biofilm development.

2.7 Biofilm quantification by counting colony forming units

After the biofilm growth (for 3, 6, or 12 hours), the specimens were previously accommodated in the 24-well cell culture plate (TPP® - Techno Plastic Products) with 1 mL of PBS. Subsequently, the fungal cells were detached using a cell scraper (Costar® 3010 Cell Scrapers, Corning Inc., Corning, NY, USA) [57]. After scraping, the suspension containing the fungal cells was recovered and serially diluted. Then, 50-mL of each dilution, in duplicate, were seeded in Sabouraud dextrose agar (Difco®, Thermo Fisher Scientific,Waltham, MA, USA) containing chloramphenicol and incubated in an oven at 37°C for 24 hours [58]. Then, viable colonies of *Candida* were visually quantified by counting the colony forming units (CFU/mL). Three independent experiments were performed for each biofilm induction period.

2.8 Biofilm quantification by the colorimetric tetrazolium salt reduction assay

The susceptibility of *C. albicans* to the association of adhesive and Ag NPs was evaluated as the percentage of reduction in metabolic activity of the fungal biofilm formed. The biofilm quantification was performed using the colorimetric test of reduction of tetrazolium salt XTT (2,3 Bis (2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-carboxanilide – Sigma Aldrich® Inc., St Louis, MO, USA) [59-61].

The XTT solution was prepared according to a previously described protocol [18, 62]. Briefly, after biofilm growth for 3, 6 and 12 hours in a 24-well cell culture plate, 2 mL of XTT solution at 0.5 mg ml−1 menadione, 0.1 mM (Sigma-Aldrich® Inc.) were added to each

well containing the previously treated or untreated specimens. The plates were covered with aluminum foil and incubated at 37°C for 3 hours.

Then, a volume of 1.6 ml from each well was centrifuged at 10°C for 2 min at 10,000 rpm to decant the fungal cells present in the supernatant [1, 59]. Aliquots of 200 µl were plated in triplicate in a 96-well plate (TPP® – Techno Plastic Products, Trasadingen, Switzerland) and analyzed by absorbance at 550 nm using a spectrophotometer (Biotek Synergy MX based Monochromator®, Winoosky, VT, USA). The reduction in metabolic activity of viable *C. albicans* biofilm cells was calculated as percentages of optical density (OD) in the wells containing the specimens without treatment (PBS group, representing 100% of metabolic activity/viability). Three independent experiments were performed for each time interval.

2.9 *C. albicans* biofilm mass analysis by confocal laser scanning microscopy

After biofilm growth for 3, 6, and 12 hours, on the surface of the previously treated (AD, AD + Ag UV, AD + Ag Turk, AD + Ag Gm and AD + Nist) or not (PBS), the samples were transferred to another 24-well plate (TPP® - Techno Plastic Products) and delicately washed three times in 1 mL of PBS. The specimens were then stained with 200 µL of the LIVE/DEAD® BacLight™ L7007 kit (Molecular Probes, Invitrogen Ltd., Thermo Fisher), according to a previously described protocol [18] and incubated at room temperature in the dark for 15 min. Subsequently, the samples were examined by confocal laser scanning microscopy (CLSM) (Leica Microsystems GmbH, Mannheim, Germany) from which digital images were obtained using 20.0 µm of magnification). The cells were labeled in fluorescent green by the dye SYTO 9® or red by the propidium iodide, and through this, was possible to identify viable and dead cells, respectively. The biofilm volume was quantified and subdivided into viable and non-viable cells using LAS X software (Leica Microsystems); the results were expressed in μm^3. A greater decrease in biofilm volume and a greater number of cells stained red (non-viable) corresponded to a more effective anti-biofilm activity. Three independent experiments were performed for each evaluation period in duplicate per group.

2.10 Statistical Analysis

The Kolmogorov–Smirnov test was used to examine the normality of the data distribution, with $p<0.05$ considered significant for all data. A parametric one-way analysis of variance with Tukey's test was applied for multiple comparisons between groups. The data not following normal distribution were analyzed using the Kruskal-Wallis and Dunn's multiple comparison tests. For this statistical analysis was used the Prism GraphPad software version 5.00.288 (Graphad®, Inc., San Diego, CA, USA).

3. RESULTS

3.1 X-ray diffraction analysis

X-ray diffraction (XRD) analysis has been done to examine the crystalline phase of the prepared Ag NPs. **SMF 1** shows the XRD pattern of the Ag NPs obtained by three different chemical synthesis methods. To all methods, four diffraction peaks in the 2θ range of 30°–80° were detected, corresponding to the (111), (200), (220) and (311) planes of fcc structure of metallic silver [63].

3.2 UV-Visible analysis

The UV–Vis spectra of the formed Ag NPs in the reactions under different routes (1, 2 and 3) were also monitored. The formation of Ag NPs was firstly detected by surface plasmon resonance (SPR) peaks observed at 398, 407 and 432 nm (**SMF 2**) for route 1, route 2 and route 3, respectively [64]. The SPR peaks were slightly right shifted to the longer wavelength, which is a tendency for increase in the size of synthesized Ag NPs. These results confirmed the effect of different routes on the Ag NPs formation. Further, in the UV-Visible spectra widening of the SPR peak indicated that the Ag NPS were polydisperse.

3.3 Transmission Electron Microscopy analysis

Transmission electron microscopy (TEM) analysis was carried out for Ag samples to visualize the size, shape, and particles size distribution. These parameters play a significant role on physical, chemical, and biological properties of nanoparticles. **SMF 3** showed the TEM images of Ag NPs synthesized using route 1, 2 and 3. The prepared particles were nearly spherical in shape and polydisperse in size distribution. The mean particle size obtained using route 1, route 2 and route 3 were 11, 21 and 32 nm, respectively. In addition, the HRTEM image of an individual nanoparticle shows the well crystallized structure (**SMF 4a**). The lattice fringes space of the Ag NPs was about 0.28 and 0.23 nm corresponding to the (110) and (111) planes cubic Ag phase respectively. **SMF 4b** shows selected area electron diffraction (SAED) pattern demonstrating diffraction spots, which were indexed as (111), (200), (220), and (311) planes which agreed to the Bragg's planes of Ag NPs cubic structures [65]. All the results confirm the successful growth of Ag NPs of the polycrystalline nature.

3.4 Minimum inhibitory concentration and minimum fungicidal concentration

After 24 hours of incubation for planktonic cells growth, in relation to nystatin the MIC and MFC values, all evaluated concentrations resulted in a percentage of death above 50% (up to 0.12 mg/mL). At the same time, the concentration capable of inhibiting all fungal growth was 0.5 mg/mL (MFC). As for the different synthesis routes, Ag UV showed similar MIC and MFC values, with 50% of death and absence of colonies being observed at a concentration of 0.12 mM/mL. Similarly for Ag Turk the results were also concomitant and determined at 0.25 mM/mL (**Figure 1**).

The *Glycine max* extract was able to inhibit the fungal growth of planktonic cells only at the initial concentration of 26 mg/mL. On the other hand, when we used the green synthesis route with *Glycine max* at an initial concentration of 1.3 mg/mL, it was possible to reach MFC values of 0.16 mg/mL and MIC values of 0.32 mg (49.7% of death percentage) (**Figure 1**).

3.5 Quantification of *Candida albicans* biofilm

In the present study, the adhesive did not interfere with the adhesion and growth of the *C. albicans* biofilm on the surface of specimens (AD group *versus* PBS group). The biofilm of the AD group showed similar values of CFU/mL in relation to PBS (growth control) and did not reach values greater than 50% of reduction in metabolic activity in all evaluated periods (3, 6 and 12 hours) (**Figures 2a, 2b, 3a, 3b, 4a 4b**). However, the CLSM analysis revealed lower biomass values compared to the PBS group at 3 and 6 hours (mean values of 7336.7 and 27225.0 μm^3 for AD; 25892.8 and 66804.0 μm^3 for PBS, respectively 3, 6 hours)

(**Figures 2c, 2d, 3c, 3d**) and higher compared to the PBS group at 12 hours (mean values of 30292.477 μm³ for AD; 9730.6 μm^3 for PBS) (**Figures 4c, 4d**).

Our results revealed the anti-*Candida* potential of silver nanoparticles, regardless of the route in which they were synthesized, when the resin surface was treated with nanomaterial associated with the adhesive. Statistically significant differences in CFU/mL values were found when AD + Ag Turk and AD + Ag Gm were compared with the AD group in all periods (AD + Ag Turk vs AD and AD + Ag Gm *vs* AD: $p < 0.05$ at 3, 6 and 12 hours). The AD + Ag UV group differed statistically only in the initial periods (AD + Ag UV vs AD: $p>0.05$ at 12 hours) (**Figures 2a**, **3a**, **4a**).

The treatment with silver nanoparticles, synthesized by the Turkevich and Gm route (AD + Ag Turk and AD + Ag Gm), was associated with a significant increase ($p<0.05$) in the percentage of reduction in the metabolic activity of the *C. albicans* biofilm adhered to the resin in the initial period (3 hours), approximately 70% compared to the AD group, which achieved only 28% reduction. When comparing the AD + Ag UV *versus* AD group, no significant differences ($p>0.05$) were observed on the percentage (%) of reduction of *C. albicans* metabolic activity, in all evaluated periods (3, 6 and 12 hours), while for AD + Ag Gm *versus* AD was observed only in the 6 hour period ($p>0.05$) (**Figures 2b**, **3b**, **4b**).

For complementing the CFU/mL analysis and the XTT assay results, CLSM quantification showed that biofilms on specimens treated with the nanoparticles (AD + Ag UV, AD + Ag Turk and AD + Ag Gm) exhibited lower mean values of biomass (μm^3) compared to with the resin treated only with the adhesive (AD), at 3, 6 and 12 hours of incubation (859.3, 216.3 and 797.1 μm^3 for AD + Ag UV, AD + Ag Turk and AD + Ag Gm *versus* 7336.7 μm^3 for AD in 3 hours; 540.8, 359.2 and 242.2 μm^3 for AD + UV, AD + Ag Turk and AD + Ag Gm *versus* 27225.0 μm^3 for AD in 6 hours; 5018.425, 136.719 and 3582.256 μm^3 for AD + Ag UV, AD + Ag Turk and AD + Ag Gm *versus* 30292.477 μm^3 for AD in 12 hours). Among the different synthesis routes, the highest percentages of non-viable cells were found in those resin specimens treated with AD + Ag Gm at 3 and 12 hours of *C. albicans* biofilm development (58.3% and 68.2%, respectively) (**Figures 2c, 2d, 3c, 3d, 4c, 4d**).

As expected, the AD + Nist group presented the lowest values of CFU mL^{-1}, regardless of the period (**Figures 2a**, **3a**, **4a**). However, after 3 hours of biofilm growth induction, it was possible to observe that nystatin reduced the metabolism of *C. albicans* adhered to the acrylic resin, with mean values above 50% and, concomitantly, exhibited the lowest mean values of biomass (μm^3) in relation to the other groups (**Figures 2c, 3b, 3c, 4c**). Although the values found in the analysis performed on the specimens treated with the nanoparticles synthesized by the UV light and Turkevich route (AD + Ag UV and AD + Ag Turk) have shown positive results in relation to the reduction of biofilm compared to treatment with the adhesive alone (group AD), surprisingly, those treated with the nanoparticles synthesized by the chemical route *versus* with the *G. max* extract associated with the adhesive (AD + Ag Gm), in all analysis (UFC/mL, XTT and CLSM) and all periods (3, 6 and 12 hours), showed similar or even lower values compared to AD + Nist, authenticating its antimicrobial potential when associated with COREGA®.

4. DISCUSSION

In this experimental study, the association of Ag NPs, synthesized by different routes, with Corega® denture adhesive powder had a considerable antimicrobial effect against *Candida albicans* biofilms, maintained during all periods evaluated (3, 6 and 12 hours).

The antifungal effects of Ag NPs added to Corega® are comparable to those obtained by incorporating these nanoparticles into other specific materials in dentistry, such as polymethylmethacrylate (PMMA) denture base and tissue conditioners for the treatment of DS. Ag NPs reduce fungal cell viability and adhesion to the surface of the prosthesis material in all of the aforementioned cases [66-73]. These promising results showed an antimicrobial effect when used with other dental materials indicated for short periods or temporary use.

In prosthetic use materials, such as DA, the synergistic effect of the adhesive and other substances with antimicrobial action has been evaluated, considering phytotherapeutic compounds, such as *Equisetum Giganteum* and *Punica Granatum* [18], synthetic drugs, peptides [19], miconazole microparticles [74], and pre-reacted glass ionomer [75]. Our results are comparable with these previous studies, showing that the adhesive materials can be improved by adding antimicrobial compounds. This finding is an innovation in dental materials for prosthetic use [76, 77]. The first part of this work was dedicated to the synthesis of Ag NPs; the average size was confirmed through the XRD, UV Vis spectra and TEM techniques. In the spectra UV Vis analysis was observed absorbance peaks at range of 398, 407 and 432 nm, matching with the typical range obtained in the synthesis results of these NPs by Abbasi et al. [33], ranging into 400 to 530 nm. Through these characterization techniques it was possible to verify that the Ag NPs formed were homogenous in relation to their shape and mean size.

The physical characteristics of nanometric materials, such as size and shape, influence their biological and antimicrobial effects [78-80]. These characteristics are influenced by the synthesis route used; this study included three different routes, resulting in spherical shape NPs with different mean average sizes (11, 21 and 32 nm for route 1, 2, 3 respectively). The differences in antifungal activity between Ag NPs groups could be explained by the size and shape-dependent effect in relation to the total surface area and the ion release in every form [81, 82]. Some studies obtained effective results with the spherical NPs shape, matching with this *in vitro* study results [83, 84] promising a better action than well-known drugs [85], in contrast when compared with other shapes spherical had lower comparative antimicrobial effect than different shapes like triangular [86].

The second part of the work was directed to evaluate the antifungal effect of the different synthesis routes of Ag NPs in different concentrations for the inhibition of planktonic fungus growth. The minimum inhibitory concentration is defined as the lowest concentration of the antimicrobial agent capable of inhibiting the growth of the microorganism under standardized conditions [87].

As expected, the concentrations used of nystatin dilutions showed the best results in the inhibition of fungal growth. It was observed in these tests that the MIC and MFC of Ag NPs were similar and also showed optimal inhibition of *Candida* development. However, the Ag UV NPs at concentration of 0.12 mg/mL, the Ag Turk NPs at 0.25 mg/mL and the Ag Gm NPs at 0.16 mg/mL did not allow the development and growth of the planktonic form of

Candida albicans, that is a reduction of approximately 50% of the metabolic activity of the evaluated fungi. This is comparable with the effect of Ag NPs when associated with the tissue conditioner having satisfactory antimicrobial action at the lowest concentration [87, 88] (above 0.1% and at 2.0%) no viable cells were grown, being maintained after 24 and 72 hours [89]. When associated with silver, *Glycine max* reaches significantly lower concentration values for fungal growth inhibition and death, in relation to those observed only in the presence of the crude extract at a concentration of 26 mg.

These concentrations mentioned above suggest that there is a concentration-dependent effect, that is, with increasing concentration of NPs, inhibition was pronounced and growth was reduced. The use of high concentrations of Ag NPs is not necessary, as they have a better interaction with microorganisms due to their high surface area. In this way, they attack the cell surface and penetrate the interior of microorganisms, requiring significantly lower doses to eliminate the fungus [67, 90, 91].

Thus, the antifungal action of Ag NPs can be compared to the results of the gold standard treatment with nystatin. In this study, nystatin was used in its pure powder form, like its conventional administer for topical treatment of stomatitis, and was not nanoparticularized like silver. Thus, this Ag NP effect may be related to its optical and physical properties acquired by nanotechnology [74, 92- 95].

Nystatin works by binding with fungal ergosterol, creating pores in the cell membrane, increasing permeability and causing leakage of cytoplasmic content, with consequent death [96, 97]. In contrast, some of the putative effects of Ag NPs in cells are their interference in metabolic processes, such as interruption of DNA replication by the release of silver ions, easy adhesion to the microorganism membrane due to its large surface area, leading to oxidative stress and alteration of the mitochondrial respiratory chain ending with cell death [91, 98, 99- 102]. In this case, the structural aspects of the cell would not be affected. These different interactions may explain the microscopic features shown in the images obtained by the CLSM in this study; where non-viable cells (in red) appeared well circumscribed and delimited in the groups treated with Ag NPs, while in the AD + Nist group, it is possible to observe diffuse areas around the cells suggesting the disruption and/or disarray of the cell wall/cell membrane (**SMF 5**).

In addition, the results of this study reflect general similarities between the antimicrobial effect in the groups treated with AD + Ag NPs and AD + Nist, whereas nystatin is already a studied, effective and long-term product on the market for the treatment of oral candidiasis infections [103, 104].

The experimental incubation periods of 3, 6, and 12 hours of this study mimic two clinical occurrences: the denture adhesive recommendations until 12 hours or more of use per day [105-107], and biofilm development under realistic clinical conditions [5, 108]. *C. albicans* biofilm formation on the acrylic resin surface occurs in three phases, beginning with the adhesion or union of the fungus yeast (present in the oral microbiota in a commensal form) to the acrylic surface of the prosthesis. The three phases occur at different times: initial, from 0 to 11 hours; intermediate, from 12 to 30 hours; and mature, that commonly begins from 24 hours until 38 to 72 hours period [7, 109]. The *Candida albicans* strain used in this study was SC5314; a wild standard reference type of the fungi originally obtained of candidiasis spread lesions, for these reason this is the principal strain broadly applied in laboratory studies all

over the world [110, 111]. This justifies the not using of different clinic isolated *Candida* strains for this *in vitro* study.

After 3 hours of *C. albicans* development, the treatment with AD and any Ag NP showed metabolic activity inhibition (≥ 50%) than in the control group with AD + Nist, which arrived at similar levels only after six hours of biofilm induction (**Figure 2b, 3b**). The cell membrane wall and nanoparticles interaction due to their morphological characteristics, accelerating the antifungal action could justify these results [95, 112, 113]. Until 12 hours of adhesive treatment over the acrylic surface, the AD group showed a greater quantity of viable cells than after the 3 and 6 hours periods (**Figure 2c, 2d, 3c, 3d, 4c, 4d**). According to the propriety of material, this occurred by the enhanced material dilution over time, leading to more favorable conditions for fungal replication. An *in vitro* study showed that some adhesives provide optimal retention action for 3 to 5 hours, and a decrease in material viscosity started after 6 hours of using [106, 114, 115]. The association of these different methodologies (XTT and CLSM) authenticates the antimicrobial potential of the studied compounds.

In general, all Ag NPs achieved satisfactory therapeutic effects but AD + Turk and AD + Gm groups showed more effective and prolonged antifungal results in all periods than AD + UV and AD + Nist groups. Despite these two synthesis methods provided similar results, specimens treated with Ag NPs by green synthesis route showed higher nonviable cells in all periods, placing AD + Turk group in second level of efficacy. Thus, green chemical synthesis, using natural compounds such as soybeans (*Glycine max*), have been very used lately for being an eco-friendly route that does not leaves toxic residues and can promise better interesting aspects that other methods by reducing nanoparticle toxicity, side effects, synthesis costs and offering optimal antioxidant [116-118].

Previous studies related positive biologic results of NPs synthesized through these routes, by UV light and with Turkevich, generally obtaining spherical NPs with controllable sizes, associated with better antimicrobial activities than other forms and low toxicity dose-dependent, and being suitable for the treatment of skin pathologies and with antifungal action [119-122]. Thus, green synthesis routes have resulted in biocompatible NPs with effects against many microorganisms such as *Candida*, thanks to the natural components participating in Ag NPs reduction process [123, 124].

Despite the favorable antifungal results obtained with AgNPs, the methodologies applied in this study have limitations related to possible factors of laboratory influence and microbial growth that may affect the experimental processes. Accurately establishing the viability of a fungal cell is considered particularly difficult when evaluating the effectiveness of antifungal agents. We cannot confirm a linear relationship between the number of viable microorganisms and the colorimetric signal provided during the XTT assay and, although the viability of the fungal cell counted in CFU/mL is an unquestionable fact, it also has its limitations, since *C. albicans* biofilms have different morphologies (blastospores, pseudohyphae and hyphae) [125], thus resulting in variability in quantification between samples from the same group. In addition, to perform the count using this methodology, it was necessary to perform mechanical scraping of the biofilm adhered to the surface of the specimens. We must count on the possibility that this maneuver can damage the fungal cells; in this case, different results can be obtained in the same group, whether experimental or control.

Therefore, regarding the methodologies used in the present work, we believe in the importance of using more than one method, when the objective is to investigate the antifungal action of nanoparticles and nystatin against *C. albicans* biofilms. We consider in the present

study that both complement each other for such investigation, providing a better understanding of the action of nanoparticles on microorganisms. This idea of complementarity can be strengthened by looking at our results regarding the CLSM, which in fact points to viable and non-viable cells. We believe that the investigation methods, applied by us, have different sensitivities, for the same purpose, and were, therefore, complementary during the analysis and authentication of the results obtained in this work.

Finally, this study was only restricted to know the antimicrobial affect with the association of Ag NPs to denture adhesive, but it was not specifically evaluated the adhesives properties of the material after the association. It will still be necessary the clinical application of this laboratorial results for approaching the antimicrobial effect *in vivo* and knowing the possible adverse effects of its application, although future studies would be considered for evaluating some new tests and focuses of Ag NPs antimicrobial activity in denture adhesive, and also evaluate toxicity, the characteristics of resin and the adhesive after applying and mixture this association elements.

In conclusion, Ag NPs, synthesized by chemical and green methods, presented excellent antimicrobial action against *C. albicans* biofilms, especially the green synthesis routes because of their better results and advantages. Ag NPs could represent an effective alternative approach for preventing oral *C. albicans* infections. Associating Ag NPs with the Corega® denture adhesive can aggregate preventive effects to this prosthetic material that could contribute avoiding DS development.

REFERENCES

[1] E. Abbasi, M. Milani, S. Fekri Aval, M. Kouhi, A. Akbarzadeh, H. Tayefi Nasrabadi, P. Nikasa, S. W. Joo, Y. Hanifehpour, K. Nejati-Koshki, M. Samiei, Silver nanoparticles: Synthesis methods, bio-applications and properties, **Crit Rev Microbiol**. 42(2) (2016), pp.173–180. https://doi.org/10.3109/1040841X.2014.912200

[2] I. K. Adisman, The use of denture adhesives as an aid to denture treatment, **J Prosthet Dent**, 62(6) (1989), pp.711–715. https://doi.org/10.1016/0022-3913(89)90598-2

[3] R. Agnihotri, S. Gaur, S. Albin, Nanometals in Dentistry: Applications and Toxicological Implications-a Systematic Review, **Biol Trace Elem Res**. 197(1) (2020), 70–88. https://doi.org/10.1007/s12011-019-01986-y

[4] M. A. Al-Fattani, L. J. Douglas, Biofilm matrix of *Candida albicans* and *Candida tropicalis*: chemical composition and role in drug resistance, **J Med Microbiol**. 55(Pt 8), (2006), pp. 999–1008. https://doi.org/10.1099/jmm.0.46569-0

[5] A. Almatroudi, Silver nanoparticles: synthesis, characterization and biomedical applications, **Open Life Sci**. 15(1) (2020), pp.819–839. https://doi.org/10.1515/biol-2020-0094

[6] N. Almeida, L. L. Saldanha, R. A. da Silva, K. H. Pinke, E. F. da Costa, V.C. Porto, A.L. Dokkedal, V. S. Lara, Antimicrobial activity of denture adhesive associated with *Equisetum giganteum*- and *Punica granatum*-enriched fractions against *Candida albicans* biofilms on acrylic resin surfaces, **Biofouling**. 34(1) (2018), pp. 62–73. https://doi.org/10.1080/08927014.2017.1407408

[7] M. F. Anwar, D. Yadav, S. Jain, S. Kapoor, S. Rastogi, I. Arora, M. Samim, Size- and shape-dependent clinical and mycological efficacy of silver nanoparticles on dandruff, **Int J Nanomedicine**. 11 (2016), pp. 147–161. https://doi.org/10.2147/IJN.S86828

[8] S. G. Aziz, S.G. Aziz, A. Akbarzadeh, Advances in Silver Nanotechnology: An Update on Biomedical Applications and Future Perspectives, **Drug Res**. 67(4) (2017), pp. 198–203. https://doi.org/10.1055/s-0042-112810

[9] C. Bacali, I. Baldea, M. Moldovan, R. Carpa, D. E. Olteanu, G. A. Filip, … F. Badea, Flexural strength, biocompatibility, and antimicrobial activity of a polymethyl methacrylate denture resin enhanced with graphene and silver nanoparticles, **Clin Oral Investig**. 24(8) (2020), pp. 2713–2725. https://doi.org/10.1007/s00784-019-03133-2

[10] R. A. Bapat, T. V. Chaubal, C. P. Joshi, P. R. Bapat, H. Choudhury, M. Pandey, … P. Kesharwani, An overview of application of silver nanoparticles for biomaterials in dentistry, **Mater Sci Eng C Mater Biol Appl**. 91(2018), pp.881–898. https://doi.org/10.1016/j.msec.2018.05.069

[11] F. C. Bizerra, C. V. Nakamura, C. de Poersch, T. I. Estivalet Svidzinski, R. M. Borsato Quesada, S. F. Yamada-Ogatta, Characteristics of biofilm formation by *Candida tropicalis* and antifungal resistance, **FEMS Yeast Res**. 8(3) (2008), pp. 442–450. https://doi.org/10.1111/j.1567-1364.2007.00347.x

[1] J. Chandra, P. K. Mukherjee, S. D. Leidich, F. F. Faddoul, L. L. Hoyer, L. J. Douglas, M. A. Ghannoum, Antifungal resistance of *candida* biofilms formed on denture acrylic *in vitro*, **J Dent Res**. 80(3) (2001b), pp. 903–908. https://doi.org/ 10.1177/00220345010800031101

[2] R. A. da Silva, B. Ishikiriama, M. M. Ribeiro Lopes, R. D. de Castro, C. R.Garcia, V. C. Porto, C. F. Santos, K. H. Neppelenbroek, V. S. Lara, Antifungal activity of Punicalagin-nystatin combinations against *Candida albicans*, **Oral Dis**. 26(8) (2020), pp.1810–1819. https://doi.org/10.1111/odi.13507

[3] U. G. Spizzirri, F. Aiello, G. Carullo, A. Facente, D. Restuccia, Nanotechnologies: An Innovative Tool to Release Natural Extracts with Antimicrobial Properties, **Pharmaceutics**. 13(2) (2021), pp. 230. https://doi.org/10.3390/pharmaceutics13020230

[4] J.W. Millsop, N. Fazel, Oral candidiasis, **Clin Dermatol**. 34 (4) (2016), pp. 487-94, https://doi.org/10.1016/j.clindermatol.2016.02.022

[5] B. Sampaio-Maia, M. H. Figueiral, P. Sousa-Rodrigues, M. H. Fernandes, C. Scully, The effect of denture adhesives on *Candida albicans* growth *in vitro*. **Gerodontology**. 29(2) (2012), pp. 348–356. https://doi.org/10.1111/j.1741-2358.2011.00478.x

[6] M. A. Al-Fattani, L. J. Douglas, Biofilm matrix of *Candida albicans* and *Candida tropicalis*: chemical composition and role in drug resistance, **J Med Microbiol**. 55(Pt 8), (2006), pp. 999–1008. https://doi.org/10.1099/jmm.0.46569-0

[7] J. Chandra, D. M. Kuhn, P. K. Mukherjee, L. L. Hoyer, T. McCormick, M. A. Ghannoum, Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance, **J Bacteriol**. 183(18) (2001a), pp. 5385–5394. https://doi.org/10.1128/JB.183.18.5385-5394.2001

[8] C. C. De Foggi, A. L. Machado, C. A. Zamperini, D. Fernandes, A. F. Wady, C. E. Vergani, Effect of surface roughness on the hydrophobicity of a denture-base acrylic resin and *Candida albicans* colonization, **J Investig Clin Dent**. 7(2) (2016), pp.141–148. https://doi.org/10.1111/jicd.12125

[9] C. Salerno, M. Pascale, M. Contaldo, V. Esposito, M. Busciolano, L. Milillo, A. Guida, M. Petruzzi, R. Serpico, *Candida*-associated denture stomatitis. **Med Oral Patol Oral Cir Bucal**. 16(2) (2011), pp. 139–143. https://doi.org/10.4317/medoral.16.e139

[10] A. C. Pero, J. Marra, A. G. Paleari, W. R. Pereira, D. B., Barbosa, M. A. Compagnoni, Measurement of interfacial porosity at the acrylic resin/denture tooth interface, **J Prosthodont**. 19(1) (2010), pp. 42–46. https:// doi.org/10.1111/j.1532-849X.2009.00511.x

[11] A. Rajaram, S. S. Manoj, Influence of 3 different forms of a commercially available denture adhesive material on the growth of *Candida* species: An *in vitro* study, **J Prosthet Dent.** 118(3) (2017), pp. 379–385.
https:// doi.org/10.1016/j.prosdent.2016.11.015

[12] P. R. Kumar, P. A. Shajahan, J. Mathew, A. Koruthu, P. Aravind, M. F. Ahammed, Denture Adhesives in Prosthodontics: An Overview, **J Int Oral Health**. 7(Suppl 1) (2015), pp. 93–95. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4516076/

[13] S. Matalon, G. Frydman, D. Lauritano, H. Slutzky, E. Shlomo, S. Levartovsky, F., Carinci, Z. Ormianer, The effect of enriching denture adhesives with chlorhexidine diacetate on the proliferation of *Candida albicans*: an *in vitro* analysis, **J Biol Regul Homeos Agents**. 31(2 Suppl 1) (2017), pp. 45–52.
https://europepmc.org/article/MED/28691453

[14] S. Shamsolketabi, M. Nill, The effect of denture adhesive on the efficiency of complete denture in patients with different alveolar ridges, **Dent Res J**. 15(4) (2018), pp. 271–275. https://doi.org/10.4103/1735-3327.237243

[15] J.E. Grasso, Denture adhesives, **Dent Clin North Am**. 48 (2004), pp.721-733, vii. http://doi.org/ 10.1016/j.cden.2004.04.002

[16] G. Pradíes, I. Sanz, O. Evans, F. Martnez, M. Sanz, Clinical study comparing the efficacy of two denture adhesives in complete denture patients, **Int J Prosthodont**. 22(4) (2009), 361–367

[17] E. Ilakkiya, Denture Adhesives - A Review, **Int J Sci Res**. 5(8) (2016), pp. 272 – 273. https://www.ijsr.net/get_abstract.php?paper_id=NOV164502

[18] N. Almeida, L. L. Saldanha, R. A. da Silva, K. H. Pinke, E. F. da Costa, V.C. Porto, A.L. Dokkedal, V. S. Lara, Antimicrobial activity of denture adhesive associated with *Equisetum giganteum*- and *Punica granatum*-enriched fractions against *Candida albicans* biofilms on acrylic resin surfaces, **Biofouling**. 34(1) (2018), pp. 62–73. https://doi.org/10.1080/08927014.2017.1407408

[19] J. L. Garaicoa, C. L. Fischer, A. M. Bates, J. Holloway, G. Avila-Ortiz, J. M. Guthmiller, G. K. Johnson, C. Stanford, K. A. Brogden, Promise of Combining Antifungal Agents in Denture Adhesives to Fight *Candida* Species Infections, **J Prosthodont.** 27(8) (2018), pp.755–762. https://doi.org/10.1111/jopr.12565

[20] V. Sharon, N. Fazel, Oral candidiasis and angular cheilitis, **Dermatol Ther**. 23(3) (2010), pp.230–242. https://doi.org/10.1111/j.1529-8019.2010.01320.x

[21] R. Rautemaa, G. Ramage, Oral candidosis--clinical challenges of a biofilm disease. **Crit Rev Microbiol**, 37(4) (2011), pp.328–336. https://doi.org/10.3109/1040841X.2011.585606

[22] R. Prasad, R. Nair, A. Banerjee, Emerging Mechanisms of Drug Resistance in *Candida albicans*, **Prog Mol Subcell Biol**. 58(2019), pp.135–153. https:// doi.org/10.1007/978-3-030-13035-0_6

[23] L. Zhang, D. Pornpattananangku, C. M. Hu, C. M. Huang, Development of nanoparticles for antimicrobial drug delivery, **Curr Med Chem**. 17(6) (2010), pp.585–594. https://doi.org/10.2174/0929867107904162

[24] F. Dilnawaz, S., Acharya, S. K. Sahoo, Recent trends of nanomedicinal approaches in clinics, **Int J Pharm**. 538(1-2) (2018), pp. 263–278. https://doi.org/10.1016/j.ijpharm.2018.01.016

[25] Z. Iqbal, M. S. Zafar, Role of antifungal medicaments added to tissue conditioners: A systematic review, **J Prosthodont Res.** 60(4) (2016), pp. 231–239. https://doi.org/10.1016/j.jpor.2016.03.006

[26] R. F. De Souza, M. F. Khiyani, C. Chaves, J. Feine, J. Barbeau, R. Fuentes, E. Borie, L. C. Crizostomo, C. H. Silva-Lovato, P. Rompre, E. Emami, Improving practice guidelines for the treatment of denture-related erythematous stomatitis: a study protocol for a randomized controlled trial, **Trials**. 18(1) (2017), pp.211. https://doi.org/10.1186/s13063-017-1947-y

[27] D. Liu, F. Yang, F. Xiong, N. Gu, The Smart Drug Delivery System and Its Clinical Potential, **Theranostics**. 6(9) (2016), pp. 1306–1323. https:// doi.org/10.7150/thno.14858

[28] S. Onoue, S. Yamada, H. K. Chan, Nanodrugs: pharmacokinetics and safety. **Int J Nanomedicine**. 9 (2014), pp.1025–1037. https:// doi.org/10.2147/IJN.S38378

[29] A. Raza, F. B. Sime, P. J. Cabot, F. Maqbool, J. A. Roberts, J. R. Falconer, Solid nanoparticles for oral antimicrobial drug delivery: a review, **Drug Discov Today**. 24(3) (2019), pp. 858–866. https://doi.org/10.1016/j.drudis.2019.01.004

[30] L.Wang, C. Hu, L. Shao, The antimicrobial activity of nanoparticles: present situation and prospects for the future, **Int J Nanomedicine**. 12(2017), pp.1227–1249. https://doi.org/10.2147/IJN.S121956

[31] L. Rivera, A. Ramos, Actividad antimicótica de nanopartículas, **Mundo Nano. Rev Interdisciplinaria en Nanociencias y Nanotecnologia**. 7(12) (2014). https://doi.org/10.22201/ceiich.24485691e.2014.12.49099

[32] M. Sousa, M. R. Maximiano, R. A. Costa, T. Rezende, O. L. Franco, Nanofibers as drug-delivery systems for infection control in dentistry. **Expert Opin Drug Deliv**, 17(7) (2020), pp. 919–930. https://doi.org/10.1080/17425247.2020.1762564

[33] E. Abbasi, M. Milani, S. Fekri Aval, M. Kouhi, A. Akbarzadeh, H. Tayefi Nasrabadi, P. Nikasa, S. W. Joo, Y. Hanifehpour, K. Nejati-Koshki, M. Samiei, Silver nanoparticles: Synthesis methods, bio-applications and properties, **Crit Rev Microbiol**. 42(2) (2016), pp.173–180. https://doi.org/10.3109/1040841X.2014.912200

[34] R. Agnihotri, S. Gaur, S. Albin, Nanometals in Dentistry: Applications and Toxicological Implications-a Systematic Review, **Biol Trace Elem Res**. 197(1) (2020), 70–88. https://doi.org/10.1007/s12011-019-01986-y

[35] A. Almatroudi, Silver nanoparticles: synthesis, characterization and biomedical applications, **Open Life Sci**. 15(1) (2020), pp.819–839. https://doi.org/10.1515/biol-2020-0094

[36] R. A. Bapat, T. V. Chaubal, C. P. Joshi, P. R. Bapat, H. Choudhury, M. Pandey, … P. Kesharwani, An overview of application of silver nanoparticles for biomaterials in dentistry, **Mater Sci Eng C Mater Biol Appl**. 91(2018), pp.881–898. https://doi.org/10.1016/j.msec.2018.05.069

[37] R. Singh, U.U. Shedbalkar, S.A. Wadhwani, B. Chopade, Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications, **Appl Microbiol Biotechnol**. [s. l.], 99(11) (2015), pp. 4579–4593. https://doi.org/10.1007/s00253-015-6622-1.

[38] Y. N. Slavin, J. Asnis, U. O. Häfeli, H. Bach, Metal nanoparticles: understanding the mechanisms behind antibacterial activity, **J Nanobiotechnology**.15(1) (2017), pp. 65. https://doi.org/10.1186/s12951-017-0308-z

[39] X. F. Zhang, Z. G. Liu, W. Shen, S. Gurunathan, Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches, **Int J Mol Sci**. 17(9) (2016), pp. 1534. https://doi.org/10.3390/ijms1709153

[40] V. T. Noronha, A. J. Paula, G. Durán, A. Galembeck, K. Cogo-Müller, M. Franz-Montan, N. Durán, Silver nanoparticles in dentistry, **Dent Mater**. 33(10) (2017), pp. 1110–1126. https://doi.org/10.1016/j.dental.2017.07.002

[41] J. Talapko, T. Matijević, M. Juzbašić, A. Antolović-Požgain, I. Škrlec, Antibacterial Activity of Silver and Its Application in Dentistry, Cardiology and Dermatology, **Microorganisms**. *8*(9) (2020), pp.1400. https://doi.org/10.3390/microorganisms8091400

[42] A. Maity, S. K. Panda, Colloidal silver nanoparticles prepared by UV-light induced citrate reduction technique for the quantitative detection of uric acid, **AIP Conf Proc**. v. 1942 (2018). http://doi.org/10.1063/1.5028688

[43] X. Wang, H. Tang, X. Tian, R. Zeng, Z. Jia, X. Huang, Sunlight and UV driven synthesis of Ag nanoparticles for fluorometric and colorimetric dual-mode sensing of ClO, **Spectrochim Acta A Mol Biomol Spectrosc**. 229(117996) (2020), pp. 1386-1425. https://doi.org/10.1016/j.saa.2019.117996

[44] N. Blommaerts, H. Vanrompay, S. Nuti, S. Lenaerts, S. Bals, S. W. Verbruggen, Unraveling Structural Information of Turkevich Synthesized Plasmonic Gold-Silver Bimetallic Nanoparticles, **Small**. 15(42) (2019), pp.e1902791. https://doi.org/10.1002/smll.201902791

[45] J. Dong, P. L. Carpinone, G. Pyrgiotakis, P. Demokritou, B. M. Moudgil, Synthesis of Precision Gold Nanoparticles Using Turkevich Method, **Kona**. 37 (2020), pp. 224–232. https://doi.org/10.14356/kona.2020011

[46] M. Wuithschick, A. Birnbaum, S. Witte, M. Sztucki, U. Vainio, N. Pinna, K. Rademann, F. Emmerling, R. Kraehnert, J. Polte, Turkevich in New Robes: Key Questions Answered for the Most Common Gold Nanoparticle Synthesis, **ACS Nano**. 9(7) (2015), pp. 7052–7071.

[47] S. Vivekanandhan, M. Misra A.K. Mohanty, Biological synthesis of silver nanoparticles using *Glycine max* (soybean) leaf extract: an investigation on different soybean varieties, **J Nanosci Nanotechnol**. 9(12) (2009), pp. 6828-6833. doi:10.1166/jnn.2009.2201

[48] J. Flieger, W. Franus, R. Panek, M. Szymańska-Chargot, W. Flieger, M. Flieger, P. Kołodziej, Green Synthesis of Silver Nanoparticles Using Natural Extracts with Proven Antioxidant Activity, **Molecules**. 26(16) (2021), pp.4986. https://doi.org/10.3390/molecules26164986

[49] European Commite for Antimicrobial Susceptibility Testing of the European Society of Clinical, M; Infectious, D. Eucast. Definitive document. E. DEF. 31, June 2000. Determination of minimum inhibitory concentrations (MICs) of antimicrobial agents by agar dilution, **Clin Microbial Infect**. 6(9) (2000), pp. 509-15.

[50] E.A. Bona, F.P. da Silva, T.K. Fruet, T.M. Jorge, A.C. Moura, Comparação de métodos para avaliação da atividade antimicrobiana e determinação da concentração inibitória mínima (cim) de extratos vegetais aquosos e etanólicos, **Arq Inst Biol**. 81(3) (2014), pp.218-225. https://doi.org/10.1590/1808-1657001192012

[51] K. H. Neppelenbroek, A. C. Pavarina, C. E. Vergani, E. T. Giampaolo, Hardness of heat-polymerized acrylic resins after disinfection and long-term water immersion, **J Prosthet Dent**. 93(2) (2005), pp. 171–176.

https://doi.org/10.1016/j.prosdent.2004.10.020

[52] M. G. Bueno, V. M. Urban, G. S. Barbério, W. J. da Silva, V. C. Porto, L. Pinto, K. H. Neppelenbroek, Effect of antimicrobial agents incorporated into resilient denture relines on the *Candida albicans* biofilm, **Oral Dis**. 21(1) (2015), pp.57–65.
https://doi.org/10.1111/odi.12207

[53] Chandra, D. M. Kuhn, P. K. Mukherjee, L. L. Hoyer, T. McCormick, M. A. Ghannoum, Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance, **J Bacteriol**. 183(18) (2001a), pp. 5385–5394. https://doi.org/10.1128/JB.183.18.5385-5394.2001

[54] W. J. da Silva, J. Seneviratne, L. P. Samaranayake, A. A. Del Bel Cury, Bioactivity and architecture of *Candida albicans* biofilms developed on poly(methyl methacrylate) resin surface, **J Biomed Mater Res** B Appl Biomater. 94(1) (2010), pp. 149–156. https://doi.org/10.1002/jbm.b.31635

[55] S. Kucharíková, H. Tournu, K. Lagrou, P.Van Dijck, H. Bujdáková, Detailed comparison of *Candida albicans* and *Candida glabrata* biofilms under different conditions and their susceptibility to caspofungin and anidulafungin, **J Med** Microbiol. 60(Pt 9) (2011), pp. 1261–1269. https://doi.org/10.1099/jmm.0.032037-0

[56] F. C. Bizerra, C. V. Nakamura, C. de Poersch, T. I. Estivalet Svidzinski, R. M. Borsato Quesada, S. F. Yamada-Ogatta, Characteristics of biofilm formation by *Candida tropicalis* and antifungal resistance, **FEMS Yeast Res**. 8(3) (2008), pp. 442–450. https://doi.org/10.1111/j.1567-1364.2007.00347.x\

[57] S. Tobudic, A. Lassnigg, C. Kratzer, W. Graninger, E. Presterl, Antifungal activity of amphotericin B, caspofungin and posaconazole on *Candida albicans* biofilms in intermediate and mature development phases, **Mycoses**. 53(3) (2010), pp.208–214. https://doi.org/10.1111/j.1439-0507.2009.01690.x

[58] D. Estivill, A. Arias, A. Torres-Lana, A. J. Carrillo-Muñoz, M. P. Arévalo, Biofilm formation by five species of *Candida* on three clinical materials, **J Microbiol Methods**, 86(2), (2011) pp. 238–242. https://doi.org/10.1016/j.mimet.2011.05.019

[59] D. M. Kuhn, M. Balkis, J. Chandra, P. K. Mukherjee, M. A. Ghannoum, Uses and limitations of the XTT assay in studies of *Candida* growth and metabolism, **J Clin Microbiol**. 41(1) (2003), pp.506–508.
https:// doi.org/10.1128/JCM.41.1.506-508.2003

[60] G. Ramage, K. Vandewalle, B. L. Wickes, J. L. López-Ribot, Characteristics of biofilm formation by *Candida albicans*. **Rev Iberoam Micol**. 18(4) (2001), pp. 163–170.http://www.reviberoammicol.com/pubmed_linkout.php?18p163

[61] S. Redding, B. Bhatt, H. R. Rawls, G. Siegel, K. Scott, J. Lopez-Ribot, Inhibition of *Candida albicans* biofilm formation on denture material, **Oral Surg Oral Med Oral Pathol Oral Radiol Endod**. 107(5) (2009), pp. 669–672.
https://doi.org/10.1016/j.tripleo.2009.01.021

[62] W. J. da Silva, J. Seneviratne, N. Parahitiyawa, E. A. Rosa, L. P. Samaranayake, A. A. Del Bel Cury, Improvement of XTT assay performance for studies involving *Candida albicans* biofilms, **Braz Dent J**. 19(4) (2008), pp. 364–369. https://doi.org/10.1590/s0103-64402008000400014

[63] B. Paul, B. Bhuyan, D. D. Purkayastha, S. S. Dhar, Photocatalytic and antibacterial activities of gold and silver nanoparticles synthesized using biomass of Parkia roxburghii leaf, **J Photochem Photobiol B**. 154(2016), pp. 1–7.
https://doi.org/10.1016/j.jphotobiol.2015.11.004

[64] P. Cardoso-Avila, E. Cardoso-Avila, R. Patakfalvi, C. Rodríguez-Pedroza, X. Aparicio-Fernández, S. Loza-Cornejo, V. Villa-Cruz, E. Martínez-Cano, One-pot green synthesis of gold and silver nanoparticles using Rosa canina L. extract, **RSC Adv**. 11 (2021), pp.14624-14631. https://doi.org/10.1039/d1ra01448j

[65] J. Saha, A. Begum, A. Mukherjee, S. Kumar, A novel green synthesis of silver nanoparticles and their catalytic action in reduction of Methylene Blue dye, **Sustain Environ Res**. 27(5) (2017), pp.245-250.
https://doi.org/10.1016/j.serj.2017.04.003

[66] C. Bacali, I. Baldea, M. Moldovan, R. Carpa, D. E. Olteanu, G. A. Filip, … F. Badea, Flexural strength, biocompatibility, and antimicrobial activity of a polymethyl methacrylate denture resin enhanced with graphene and silver nanoparticles, **Clin Oral Investig**. 24(8) (2020), pp. 2713–2725.
https://doi.org/10.1007/s00784-019-03133-2

[67] V. De Matteis, M. Cascione, C. C. Toma, G. Albanese, M. L. De Giorgi, M. Corsalini, R. Rinaldi, Silver Nanoparticles Addition in Poly(Methyl Methacrylate) Dental Matrix: Topographic and Antimycotic Studies, **Int J Mol Sci**. 20(19) (2019), pp. 4691. https://doi.org/10.3390/ijms20194691

[68] J. Deng, L. Ren, Y. Pan, H. Gao, X. Meng, Antifungal property of acrylic denture soft liner containing silver nanoparticles synthesized in situ, **J Dentistry**. 106 (2021), pp. 103589. https://doi.org/10.1016/j.jdent.2021.103589

[69] M. M. Gad, R. Abualsaud, A. Rahoma, A. M. Al-Thobity, S. Akhtar, S. M. Fouda, Double-layered acrylic resin denture base with nanoparticle additions: An *in vitro* study, **J Prosthet Den**. 127(1) (2020), pp.174–183.
https://doi.org/10.1016/j.prosdent.2020.08.021

[70] A. Garcia, C. Sugio, L. J. de Azevedo-Silva, A. Gomes, A. Batista, V. C. Porto, S.

Soares, K. H Neppelenbroek, Nanoparticle-modified PMMA to prevent denture stomatitis: a systematic review, **Archiv Microbiol**. 204(1) (2021), pp.75. https://doi.org/10.1007/s00203-021-02653-4

[71] Z. Iqbal, M. S. Zafar, Role of antifungal medicaments added to tissue conditioners: A systematic review, **J Prosthodont Res.** 60(4) (2016), pp. 231–239. https://doi.org/10.1016/j.jpor.2016.03.006

[72] S. A. Mousavi, R. Ghotaslou, A. Khorramdel, A. Akbarzadeh, A. Aeinfar, Antibacterial and antifungal impacts of combined silver, zinc oxide, and chitosan nanoparticles within tissue conditioners of complete dentures *in vitro*, **I J Med Sci**. 189(4) (2020), pp. 1343–1350. https:// doi.org/10.1007/s11845-020-02243-1

[73] A. Shenava, 48.Redifining precision with methods of synthesis of silver nanoparticles and the uses in prosthodontics-a review. **J Indian Prosthodont Soc**. 18(Suppl 2) (2018), S89. https://doi.org/10.4103/0972-4052.246552

[74] A. F. Cartagena, L. A. Esmerino, R. Polak-Junior, S. Olivieri Parreiras, M. Domingos Michél, P. V. Farago, N. H. Campanha, New denture adhesive containing miconazole nitrate polymeric microparticles: Antifungal, adhesive force and toxicity properties, **Dent Mater**. 33(2) (2017), pp. e53–e61.
https://doi.org/10.1016/j.dental.2016.09.039

[75] K. Hatano, M. Inokoshi, M. Tamura, M. Uo, M. Shimizubata, W. Tonprasong, T. Wada, R. Takahashi, K. Imai, S. Minakuchi, Novel antimicrobial denture adhesive containing S-PRG filler, **Dent Mater J**. 40(6) (2021), pp.1365–1372. https://doi.org/10.4012/dmj.2020-443

[76] J. M. Corrêa, M. Mori, H. L. Sanches, A. D. da Cruz, E. Jr, Poiate, I. A. Poiate, Silver nanoparticles in dental biomaterials, **Int J Biomater**. 2015, pp. 485275. https://doi.org/10.1155/2015/485275

[77] C. C. Fernandez, A. R. Sokolonski, M. S. Fonseca, D. Stanisic, D. B. Araújo, V. Azevedo, R. D. Portela, L. Tasic, Applications of Silver Nanoparticles in Dentistry: Advances and Technological Innovation. **Int J Mol Sci**, 22(5) (2021), pp. 2485. https://doi.org/10.3390/ijms22052485

[78] N. Patill, R. Bhaskar, V. Vyavhare, R. Dhadge, V. Khaire Y. Patil, Overview on methods of synthesis of nanoparticles. **Int J Curr Pharm Res**.13 (2) (2021), pp.11-16.https:// doi.org/ 10.22159/ijcpr.2021v13i2.41556

[79] A. Rónavári, N. Igaz, D. I. Adamecz, B. Szerencsés, C. Molnar, Z. Kónya, I. Pfeiffer, M. Kiricsi, Green Silver and Gold Nanoparticles: Biological Synthesis Approaches and Potentials for Biomedical Applications, **Molecules (Basel)**. 26(4) (2021), pp. 844. https://doi.org/10.3390/molecules26040844

[80] S. Tang, J. Zheng, Antibacterial Activity of Silver Nanoparticles: Structural Effects, **Adv Healthc Mater**. 7(13) (2018), pp.1701503.
https://doi.org/10.1002/adhm.201701503

[81] O. Pryshchepa, P. Pomastowski, B. Buszewski, Silver nanoparticles: Synthesis, investigation techniques, and properties, **Adv Colloid Interface Sci**. 284(2020), pp. 102246. https://doi.org/10.1016/j.cis.2020.102246

[82] M. A. Raza, Z. Kanwal, A. Rauf, A. N. Sabri, S. Riaz, S. Naseem, Size- and Shape-Dependent Antibacterial Studies of Silver Nanoparticles Synthesized by Wet Chemical Routes, **Nanomaterials (Basel)**. 6(4) (2016), pp.74. https://doi.org/10.3390/nano6040074

[83] J. Y. Cheon, S. J. Kim, Y. H. Rhee, O. H. Kwon, W. H. Park, Shape-dependent antimicrobial activities of silver nanoparticles, **Int J Nanomedicine**. 14 (2019), pp. 2773–2780. https://doi.org/10.2147/IJN.S196472

[84] F. J. Osonga, A. Akgul, I. Yazgan, A. Akgul, G. B. Eshun, L. Sakhaee, O. Sadik, A. Size and Shape-Dependent Antimicrobial Activities of Silver and Gold Nanoparticles: A Model Study as Potential Fungicides, **Molecules**. 25(11) (2020), pp. 2682. http:// doi.org/10.3390/molecules25112682

[85] M. F. Anwar, D. Yadav, S. Jain, S. Kapoor, S. Rastogi, I. Arora, M. Samim, Size- and shape-dependent clinical and mycological efficacy of silver nanoparticles on dandruff, **Int J Nanomedicine**. 11 (2016), pp. 147–161.
https://doi.org/10.2147/IJN.S86828

[86] Pal, Y. K. Tak, J. M. Song, Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli, **App Environ Microbiol**. 73(6) (2007), pp. 1712–1720. https:// doi.org/10.1128/AEM.02218-06

[87] D. M. Gomes, N. Durán, A. Seabra, L. Silva, F. Prado, T. Silva, M. F. Teixeira, Síntese verde de nanopartículas de prata intermediada por fungo anamórfico e eficácia antibacteriana e antifúngica, **Bol Mus Paraense Emílio Goeldi**.15(2) (2020), pp. 433-443. https://doi.org/10.46357/bcnaturais.v15i2.332

[88] L. Gabrielyan, A. Trchounian, Antibacterial activities of transient metals nanoparticles and membranous mechanisms of action. **World J Microbiol Biotechnol**, 35(10) (2019), pp.162. https://doi.org/10.1007/s11274-019-2742-6

[89] K. Y. Nam, *In vitro* antimicrobial effect of the tissue conditioner containing silver nanoparticles, **J Adv Prosthodont**. 3(1) (2011), pp. 20–24.
https:// doi.org/10.4047/jap.2011.3.1.20

[90] A. Fouda, S. E. Hassan, A. M. Abdo, M. S. El-Gamal, Antimicrobial, Antioxidant and Larvicidal Activities of Spherical Silver Nanoparticles Synthesized by *Endophytic Streptomyces* spp., **Biol Trace Elem Res**. 195(2) (2020), pp.707–724. https://doi.org/10.1007/s12011-019-01883-4

[91] K.K. Wong, X. Liu, Silver nanoparticles—the real “silver bullet” in clinical medicine?, **Med Chem Comm**. 1(2010), pp.125-131.
https://doi.org/10.1039/C0MD00069H

[92] S. G. Aziz, S.G. Aziz, A. Akbarzadeh, Advances in Silver Nanotechnology: An Update on Biomedical Applications and Future Perspectives, **Drug Res**. 67(4) (2017), pp. 198–203. https://doi.org/10.1055/s-0042-112810

[93] E. D. Cavassin, L. F. de Figueiredo, J. P. Otoch, M. M. Seckler, R. A. de Oliveira, F. F.Franco, V. S. Marangoni, V. Zucolotto, A. S. Levin, S. F. Costa, Comparison of methods to detect the *in vitro* activity of silver nanoparticles (AgNP) against multidrug resistant bacteria, **J Nanobiotechnology**. 13 (2015), p.64.
https://doi.org/10.1186/s12951-015-0120-6

[94] S. H. Lee, B. H. Jun, Silver Nanoparticles: Synthesis and Application for Nanomedicine, **Int J Mol Sci**. 20(4) (2019), pp. 865.
https:// doi.org/10.3390/ijms20040865

[95] I. X. Yin, J. Zhang, I. S. Zhao, M. L. Mei, Q. Li, C. H. Chu, The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry, **Int J Nanomedicine**. 15(2020), pp. 2555–2562.
https://doi.org/10.2147/IJN.S246764

[96] A. G. Dos Santos, J. T. Marquês, A. C. Carreira, I. R. Castro, A. S. Viana, M. P. Mingeot-Leclercq, R. de Almeida, L. C. Silva, The molecular mechanism of Nystatin action is dependent on the membrane biophysical properties and lipid composition, **Phys Chem Chem Phys**. 19(44) (2017), pp. 30078–30088.
https://doi.org/10.1039/c7cp05353c

[97] H. Kinoshita, M. Yoshioka, F. Ihara, T. Nihira, Cryptic antifungal compounds active by synergism with polyene antibiotics, **J Biosci Bioeng.** 121(4) (2016), pp.394–398. https://doi.org/10.1016/j.jbiosc.2015.08.003

[98] N. Durán, M. Durán, M. B. de Jesus, A. B. Seabra, W. J. Fávaro, G. Nakazato, Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity, **Nanomedicine**. 12(3) (2016), pp. 789–799. https://doi.org/10.1016/j.nano.2015.11.016

[99] C. Marambio-Jones, E.M.V. Hoek, A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment, **J Nanopart Res**. 12(2010), pp. 1531–1551. https:// doi.org/10.1007/s11051-010-9900-y

[100] Y. Matsumura, K. Yoshikata, S. Kunisaki, T. Tsuchido, Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate, **Appl Environ Microbiol**.69(7) (2003), pp. 4278–4281.
https://doi.org/10.1128/AEM.69.7.4278-4281.2003

[101] I. Sondi, B. Salopek-Sondi, Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. **J Colloid Interface Sci**, 275(1) (2004), pp.177–182. https://doi.org/10.1016/j.jcis.2004.02.012

[102] L. Xu, Y. Y. Wang, J. Huang, C. Y. Chen, Z. X. Wang, H. Xie, Silver nanoparticles: Synthesis, medical applications and biosafety, **Theranostics**. 10(20) (2020), pp. 8996–9031. https://doi.org/10.7150/thno.45413

[103] E. Scheibler, M. Garcia, R. Medina da Silva, M. A. Figueiredo, F. G. Salum, K. Cherubini, Use of nystatin and chlorhexidine in oral medicine: Properties, indications and pitfalls with focus on geriatric patients, **Gerodontology**. 34(3) (2017), pp.291–298. https://doi.org/10.1111/ger.12278

[104] E. H. Taudorf, G. Jemec, R. J. Hay, D. Saunte, Cutaneous candidiasis - an evidence-based review of topical and systemic treatments to inform clinical practice, **J Eur Acad Dermatol Venereol**. 33(10) (2019), pp. 1863–1873. https://doi.org/10.1111/jdv.15782

[105] I. K. Adisman, The use of denture adhesives as an aid to denture treatment, **J Prosthet Dent**, 62(6) (1989), pp.711–715. https://doi.org/10.1016/0022-3913(89)90598-2

[106] Z. A. Bogucki, Denture adhesives' effect on retention of prostheses in patients with xerostomia, **Adv Clin Exp Med**. 27(9) (2018), pp. 1247–1252.
https://doi.org/10.17219/acem/69078
\
[107] Y. Kulak, M. Ozcan, A. Arikan, Subjective assessment by patients of the efficiency of two denture adhesive pastes, **J Prosthodont.** 14(4) (2005), pp. 248–252. https:// doi.org/10.1111/j.1532-849X.2005.00049.x

[108] T. Nomura, T. Murakami, Y. Shimoyama, T. Kobayashi, J. Furuya, M. Sasaki, H. Kondo, Effects of denture adhesives on growth and morphological transformation of *Candida albicans*, **J Prosthodont Res**, 64(1) (2020), pp. 78–84. https:// doi.org/10.1016/j.jpor.2019.04.006

[109] H. Tournu, P. Van Dijck, *Candida* biofilms and the host: models and new concepts for eradication. **Int J Microbiol**, *2012*(2012), pp. 845352. https://doi.org/10.1155/2012/845352

[110] M. Bazzi, K. Nawrocki, C. Brown, N. Falkowski, K. Stark, G. Huffnagle, The microbial ecology of *Candida albicans* strains CHN1 and SC5314 in mice, **Access Microbiol**. 3(12) (2021). http://doi.org/10.1099/acmi.cc2021.po0097

[111] S. K. Carvajal, M. Alvarado, Y. M. Rodríguez, C. M. Parra-Giraldo, C. Varón, S. E. Morales-López, J. Y. Rodríguez, B. L. Gómez, P. Escandón, Pathogenicity Assessment of Colombian Strains of *Candida auris* in the *Galleria mellonella* invertebrate model, **J Fungi (Basel)**. *7*(6) (2021), pp. 401. https://doi.org/10.3390/jof7060401

[112] G. V. Vimbela, S. M. Ngo, C. Fraze, L. Yang, D. A. Stout, Antibacterial properties and toxicity from metallic nanomaterials, **Int J Nanomedicine**. 12(2017), pp. 3941–3965. https://doi.org/10.2147/IJN.S134526

[113] B. Żarowska, T. Koźlecki, M.Piegza, K. Jaros-Koźlecka, M. Robak, New Look on Antifungal Activity of Silver Nanoparticles (AgNPs), **P J Microbiol**. 68(4) (2019), pp. 515–525. https://doi.org/10.33073/pjm-2019-051

[114] F. Fløystrand, R. Koppang, V. D. Williams, J. Orstavik, A method for testing denture adhesives, **J Prosthet Dent**. 66(4) (1991), pp. 501–504. https://doi.org/10.1016/0022-3913(91)90512-u

[115] N. Okita, D. Orstavik, J. Orstavik, K. Ostby, *In vivo* and *in vitro* studies on soft denture materials: microbial adhesion and tests for antibacterial activity. **Dent Mater**. 7(3) (1991), pp. 155–160. https:// doi.org/10.1016/0109-5641(91)90035-w

[116] M. Mousavi-Khattat, M. Keyhanfar, A. Razmjou, A comparative study of stability, antioxidant, DNA cleavage and antibacterial activities of green and chemically synthesized silver nanoparticles, **Artif Cells Nanomed Biotechnol**. 46(sup3) (2018), S1022–S1031. https:// doi.org/10.1080/21691401.2018.1527346

[117] S. M. Mousavi, S. A. Hashemi, Y. Ghasemi, A. Atapour, A. M. Amani, A. Savar Dashtaki, A. Babapoor, O. Arjmand, Green synthesis of silver nanoparticles toward bio and medical applications: review study, **Artif Cells Nanomed Biotechnol**. 46(sup3) (2018), S855–S872. https://doi.org/10.1080/21691401.2018.1517769

[118] K.S. Siddiqi, A. Husen, R.A.K. Rao A review on biosynthesis of silver nanoparticles and their biocidal properties, **J Nanobiotechnology**. 16(1) (2018), pp.14. https://doi.org/10.1186/s12951-018-0334-5

[119] X. Hong, J. Wen, X. Xiong, Y. Hu, Shape effect on the antibacterial activity of silver nanoparticles synthesized via a microwave-assisted method, **Environ Sci Pollut Res Int**. 23(5) (2016), pp. 4489–4497. https://doi.org/10.1007/s11356-015-5668-z

[120] A. Maity, S. K. Panda, Colloidal silver nanoparticles prepared by UV-light induced citrate reduction technique for the quantitative detection of uric acid, **AIP Conf Proc**. v. 1942 (2018). http://doi.org/10.1063/1.5028688

[121] I. Pinzaru, D. Coricovac, C. Dehelean, E. A. Moacă, M. Mioc, F. Baderca, I. Sizemore, S. Brittle, D. Marti, C. D. Calina, A. M. Tsatsakis, C. Şoica, Stable PEG-coated silver nanoparticles - A comprehensive toxicological profile. **Food Chem Toxicol**. 111 (2018), pp.546–556. https://doi.org/10.1016/j.fct.2017.11.051

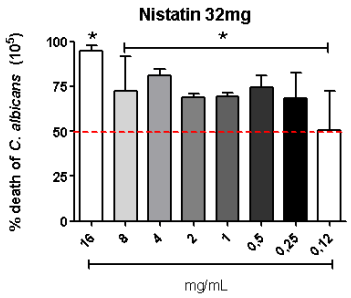
[122] V. S. Radhakrishnan, M. K. Reddy Mudiam, M. Kumar, S. P. Dwivedi, S. P. Singh, T. Prasad, Silver nanoparticles induced alterations in multiple cellular targets, which are critical for drug susceptibilities and pathogenicity in fungal pathogen (*Candida albicans*), **Int J Nanomedicine**. 13 (2018), pp. 2647–2663.
https://doi.org/10.2147/IJN.S150648

[123] S. Donga, S. Chanda, Facile green synthesis of silver nanoparticles using Mangifera indica seed aqueous extract and its antimicrobial, antioxidant and cytotoxic potential (3-in-1 system), **Artif Cells Nanomed Biotechnol**. 49(1) (2021), pp. 292–302. https://doi.org/10.1080/21691401.2021.1899193

[124] N. T. Khan, M. Mushtaq, Determination of antifungal activity of silver nanoparticles produced from Aspergillus Niger, **Biol Med.** 9(1) (2017), pp.1. http://doi.org/10.4172/0974-8369.1000363

[125] A. Srinivasan, P. Uppuluri, J. Lopez-Ribot, A.K. Ramasubramanian, Development of a high-throughput *Candida albicans* biofilm chip, **PLoS One**. 6(4) (2011), pp. e19036. https://doi.org/10.1371/journal.pone.0019036

FIGURES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% death of C. albicans (10^5)
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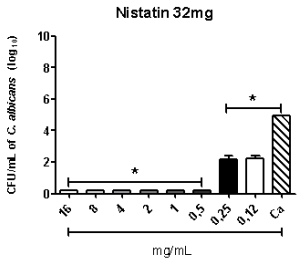
mg/mL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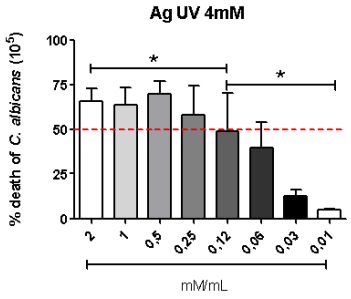
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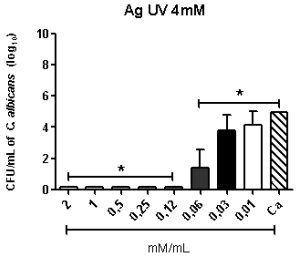
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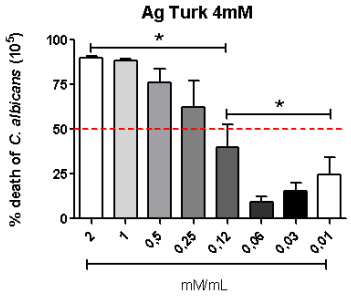
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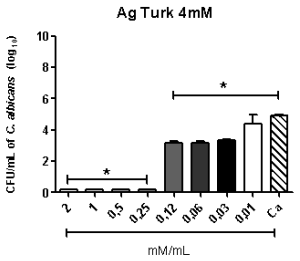
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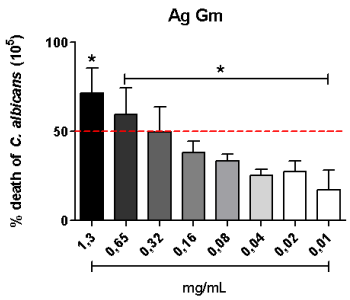
mM/mL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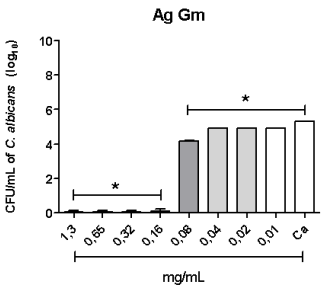
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CFU/mL of C. albicans (log10)
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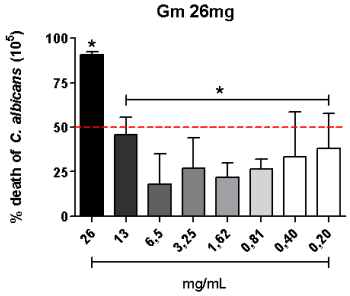
mg/mL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% death of C. albicans (10^5)
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mg/mL

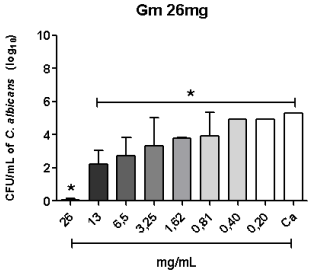
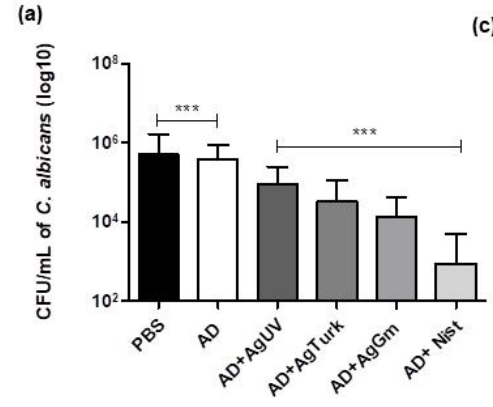
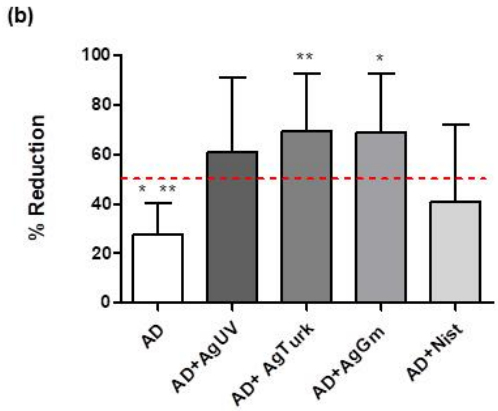

Gm 26mg
CFU/mL of C. albicans (log10)
26 13 6,5 3,25 1,62 0,81 0,40 0,20 Ca
mg/mL

Figure 1. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of nystatin and silver nanoparticles (Ag) synthesized by different routes (UV, Turk e Gm) against *Candida albicans* planktonic cells. According to the EUCAST protocol, both assays were performed with 10^6 cells/mL of *C. albicans* (SC5314) challenged with serial dilutions of nystatin, Ag UV, Ag Turk, Ag Gm and *G. max* extract only (Gm). On left the red dashed line represents 50% growth inhibition of *C. albicans* and the data were transported to the percentage of fungal death, according to the values obtained in the growth control (inoculum + RPMI-1640). In right Ca represents the mean values of the growth control group with only the inoculum of *C. albicans* and the results were logarithmically transformed (log10). Three independent experiments were performed in triplicate and both results were presented as mean ±standard deviation (SD) and submitted to one-way Analysis of Variance (ANOVA) followed by Tukey's HSD post-hoc test. Equal symbols (*) represent $p<0.05$.





(c)

Groups	Biofilm mass (µm3)	Viable (%)	Nonviable (%)
PBS	25892.8 ± 0.945	97.6	2.4
AD	7336.7 ± 0.143	98.8	1.2
AD+AgUV	859.3 ± 0.101	97.8	2.2
AD+AgTurk	216.3 ± 0.018	83.0	17.0
AD+AgGm	797.1 ± 0.020	41.7	58.3
AD+Nist	409.1 ± 0.016	98.3	1.7

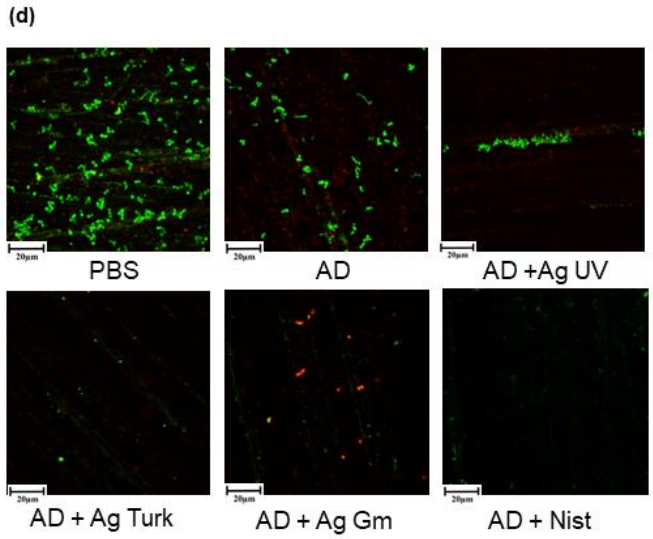
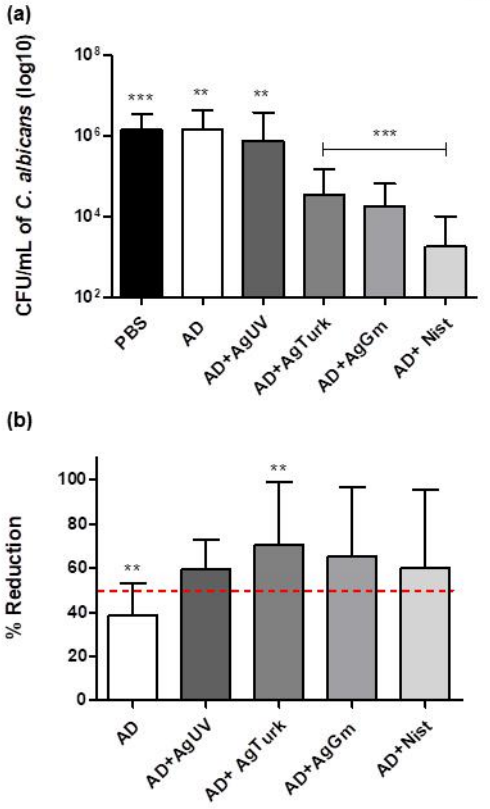


Figure 2. Evaluation of *C. albicans* biofilm after three hours of development on acrylic surfaces not treated (PBS) and treated with Corega® adhesive (AD) and association of AD and Ag UV, Ag Turk, Ag Gm, and Nist. (a) CFUs/mL on the surface of acrylic resin specimens (mean ± SD). **(b)** Percentage of reduction (mean ± SD) in the metabolic activity of *C. albicans* biofilms. **(c)** Biofilm mass in μm^3 (mean ± SD) and mean percentage of viable and non-viable cells. **(d)** CLSM images of viable (green) and non-viable (red) cells per group (20.0 µm image scale bar). In all tests, the controls were untreated (PBS) and treated (AD + Nist). Three independent tests were performed in duplicate (a) and triplicate (b, c, and d) forms. For all periods, the PBS group had a higher fungal charge (a) and lower death reduction percentage (b) than with nystatin. In **(a)** Equal symbols *** represents ($p < 0.0001$) in **(b)** ** $p<0.0027$ and * $p< 0.05$.



(c)

Groups	Biofilm mass (µm3)	Viable (%)	Nonviable (%)
PBS	66804.0 ± 0.001	97.0	3.0
AD	27225.0 ± 0.023	96.0	4.0
AD+AgUV	540.8 ± 0.045	97.6	2.4
AD+AgTurk	359.2 ± 0.042	91.6	8.4
AD+AgGm	242.2 ± 0.033	99.8	0.2
AD+Nist	115.4 ± 0.003	81.8	18.2

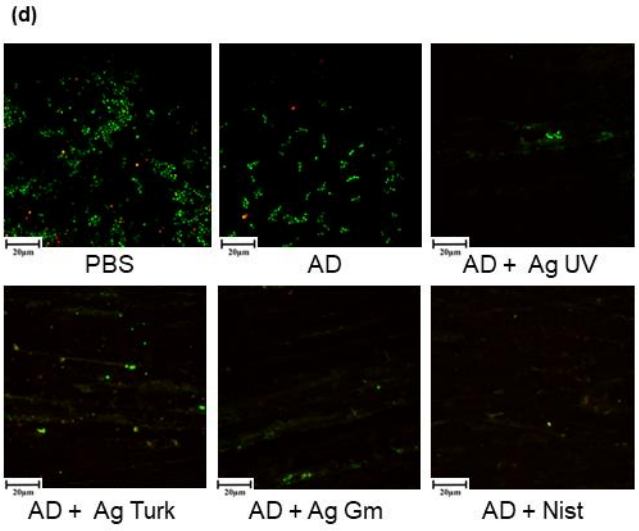
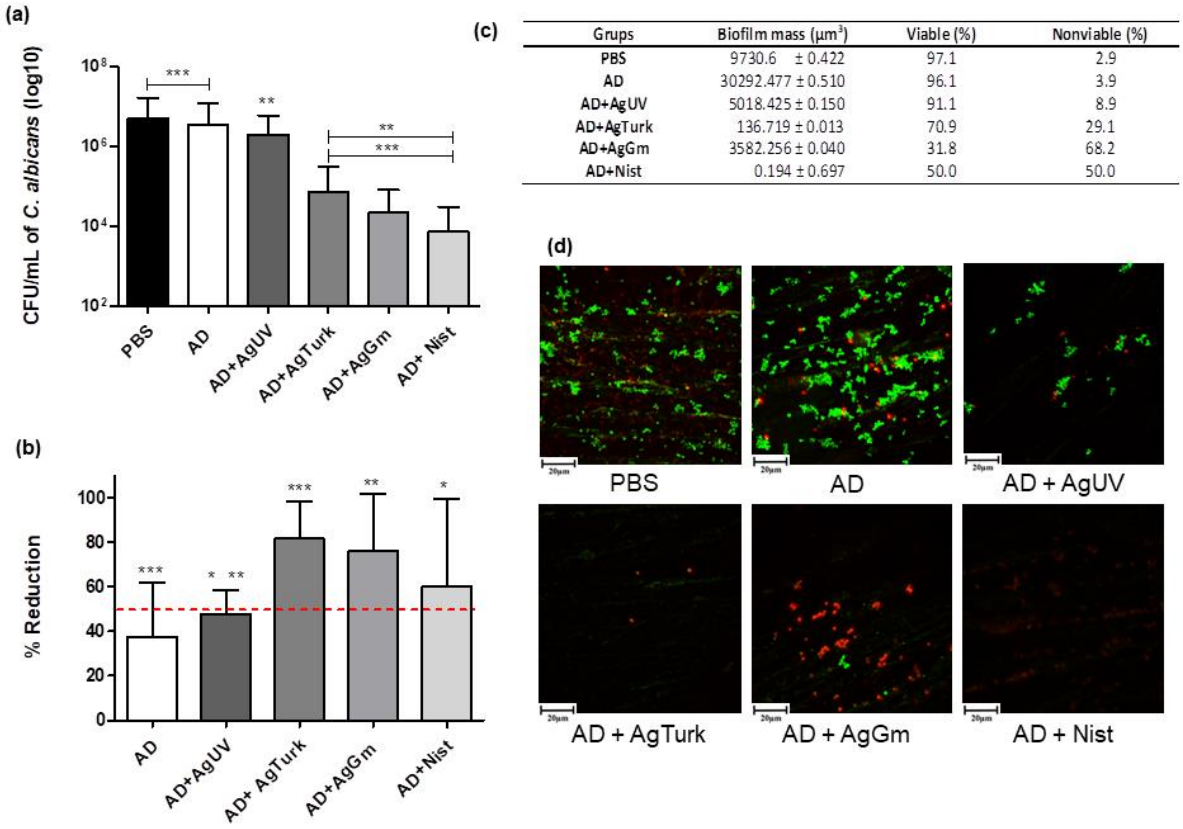


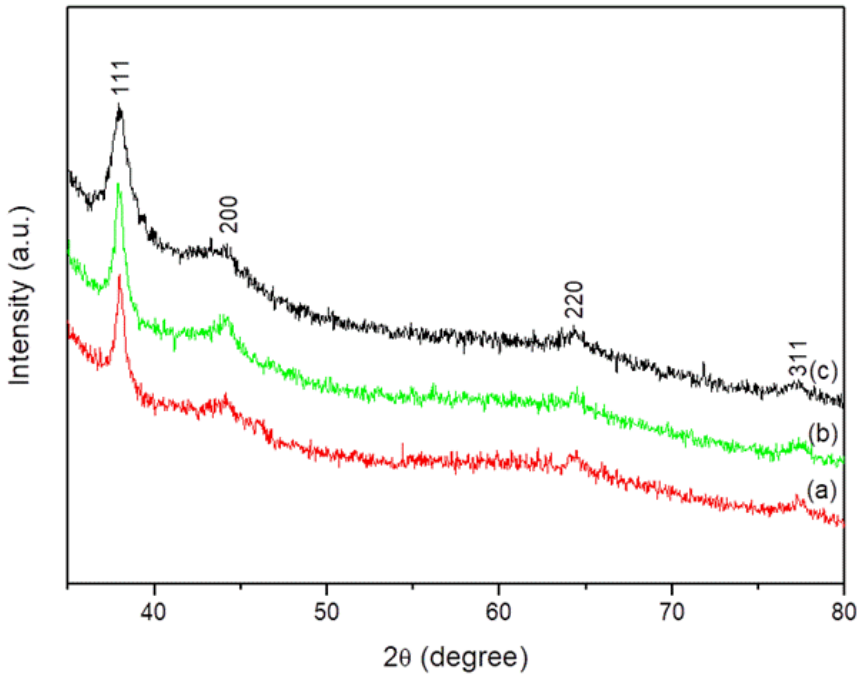
Figure 3. Evaluation of *C. albicans* biofilm after six hours of development on acrylic surfaces treated with Corega® adhesive (AD) and association of AD with Ag UV, Ag Turk, Ag Gm, and Nist. (a) CFUs/mL on the surface of acrylic resin specimens (mean ± SD). **(b)** Percentage of reduction (mean ± SD) in metabolic activity of *C. albicans* biofilms. **(c)** Biofilm mass in μm^3 (mean ± SD) and mean percentage of viable and non-viable cells. **(d)** CLSM images of viable (green) and non-viable (red) cells per group (20.0 µm scale bar). In all tests, the controls were untreated (PBS) and treated (AD + Nist). Three independent tests were performed in duplicate (a) and triplicate (b, c, and d) forms. For all periods, the PBS group had a higher fungal charge **(a)** and lower death reduction percentage **(b)** than with nystatin. In **(a)** *** represents ($p < 0.0001$) ** represents ($p < 0.005$) in **(b)** ** ($p<0.0146$).



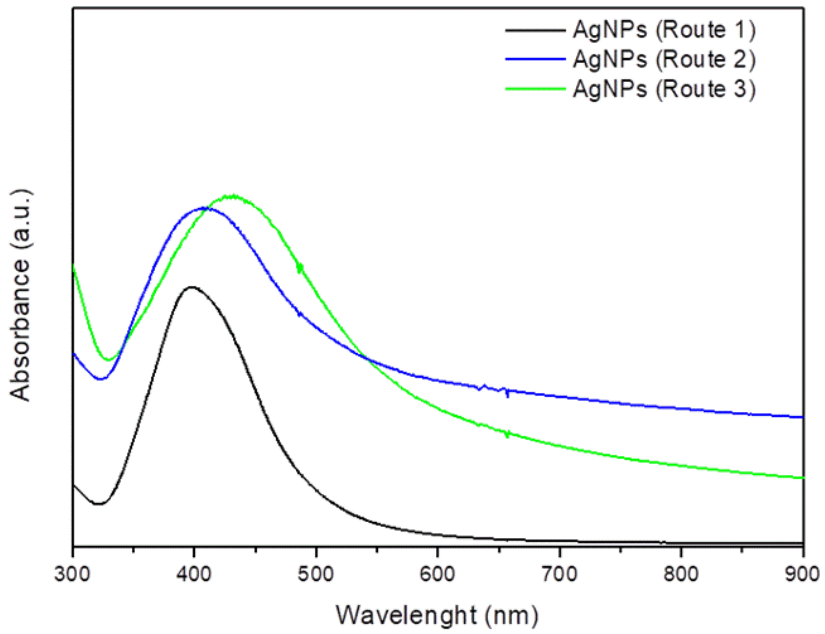
Grups	Biofilm mass (µm³)	Viable (%)	Nonviable (%)
PBS	9730.6 ± 0.422	97.1	2.9
AD	30292.477 ± 0.510	96.1	3.9
AD+AgUV	5018.425 ± 0.150	91.1	8.9
AD+AgTurk	136.719 ± 0.013	70.9	29.1
AD+AgGm	3582.256 ± 0.040	31.8	68.2
AD+Nist	0.194 ± 0.697	50.0	50.0

Figure 4. Evaluation of *C. albicans* biofilm after twelve hours of development on acrylic surfaces not treated (PBS) and treated with Corega® adhesive (AD) and association of AD with Ag UV, Ag Turk, Ag Gm, and Nist. (a) CFUs/mL (mean ± SD) on the surface of acrylic resin specimens. **(b)** Percentage of reduction (mean ± SD) in metabolic activity of *C. albicans* biofilms. **(c)** Biofilm mass in µm^3 (mean ± SD) and mean percentage of viable and non-viable cells. **(d)** CLSM images of viable (green) and non-viable (red) cells per group (20.0 µm scale bar). In all tests, the controls were untreated (PBS) and treated (AD + Nist). Three independent tests were performed in duplicate (a) and triplicate (b, c, and d) forms. For all periods, the PBS group had a higher fungal charge **(a)** and lower death reduction percentage **(b)** than nystatin. In **(a)** *** represents ($p < 0.0001$) ** represent ($p<0.05$) in **(b)** *** represents ($p<0.0002$) ** represents ($p<0.05$)

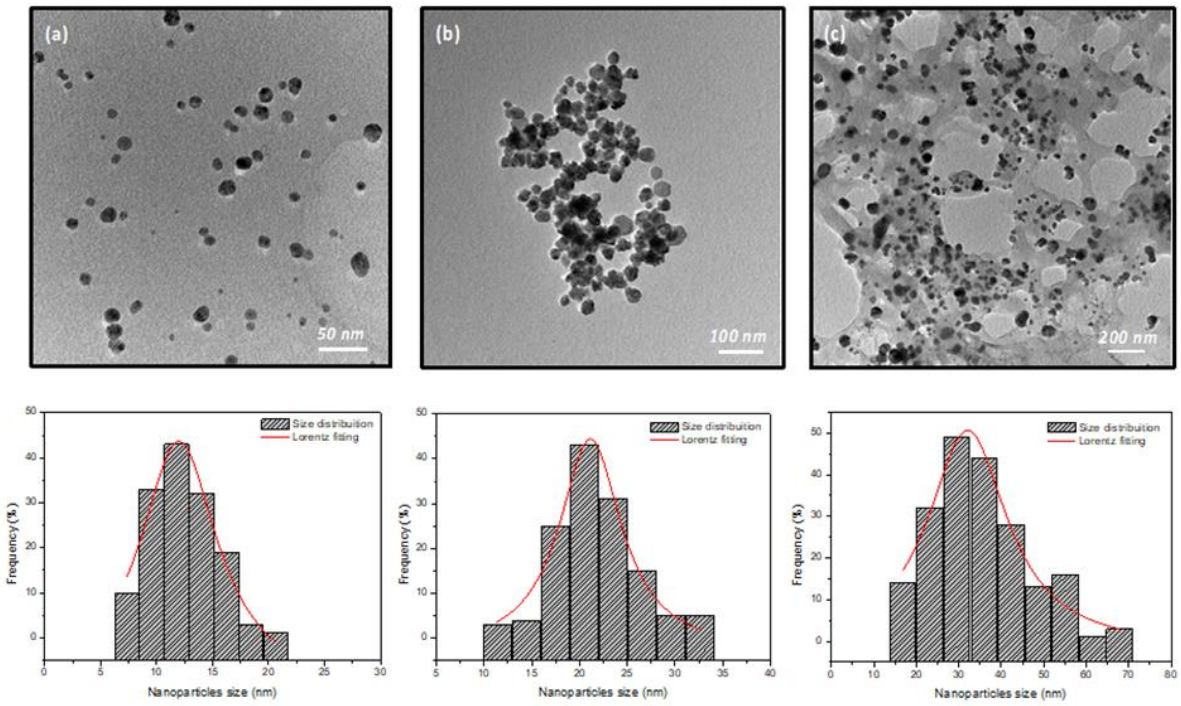
SUMPLEMENTARY FILES (SMF)



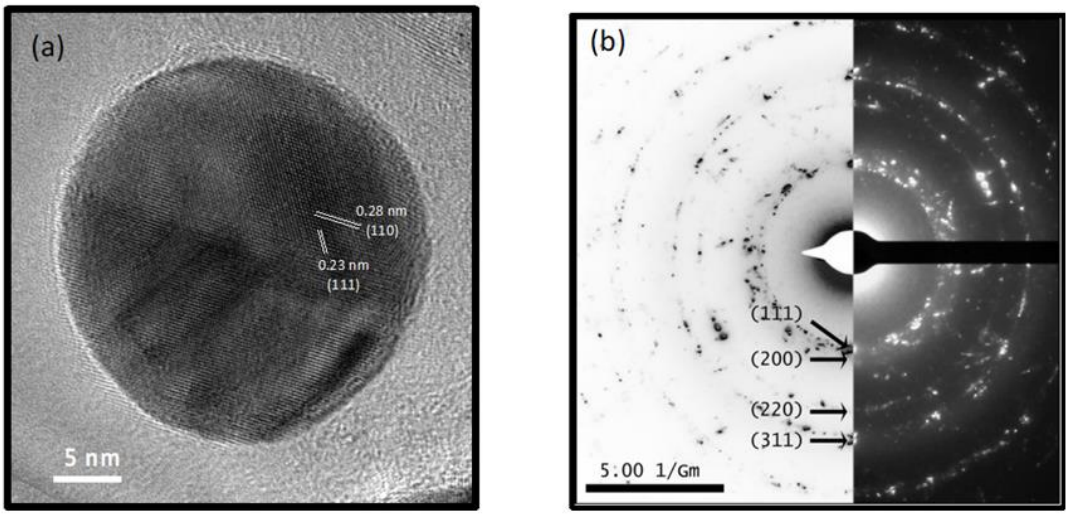
SMF 1: XRD patterns of Ag NPs obtained using **(a)** AgGm (Route 3), **(b)** AgTurk (Route 2) and **(c)** AgUV (Route 1).



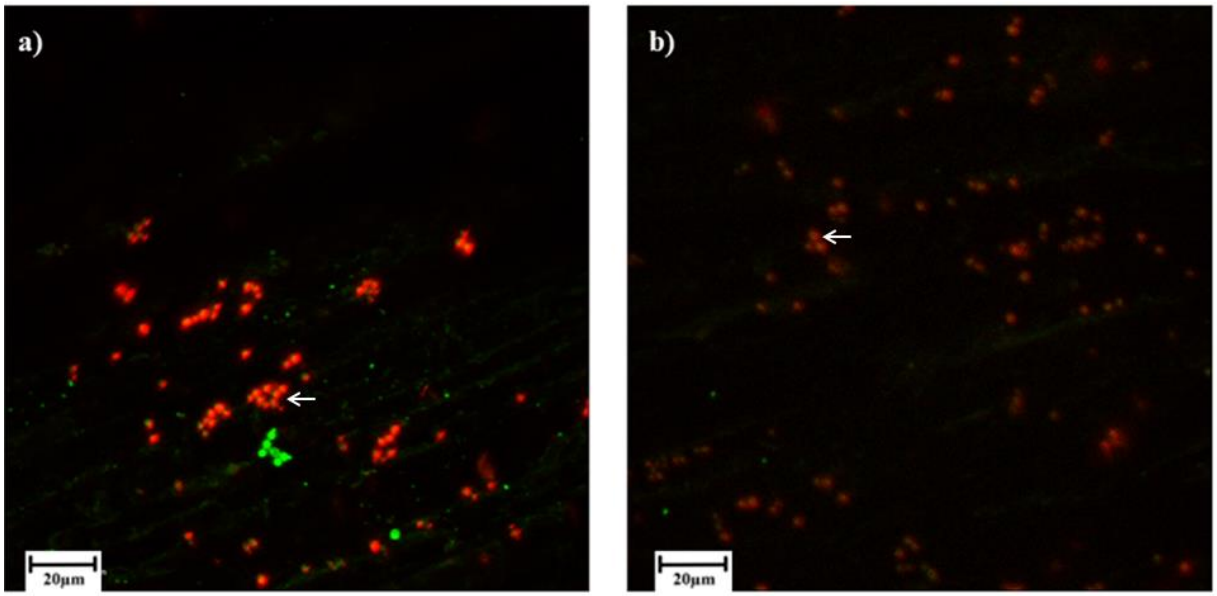
SMF 2: Absorption spectra of Ag NPs obtained using UV light (Route 1), Turkevich method (Route 2), and Green chemical method using *Glycine max* (Route 3).



SMF 3: TEM images and nanoparticles size distribution histogram of Ag NPs synthesized using **(a)** UV light (Route 1), **(b)** Turkevich method (Route 2), and **(c)** Green chemical method using *Glycine max* (Route 3).



SMF 4: (a) HRTEM image nanoparticle of Ag NPs synthesized using Turkevich method and **(b)** SAED.



SMF 5. Microscopic images obtained by the CLSM LIVE/DEAD assay. **a)** non-viable cells (in red) well circumscribed and delimited in group treated with AD + Ag Gm, **b)** diffuse areas around the red cells suggesting the disruption and/or disarray of the cell wall/ cell membrane in the AD + Nist group (20.0 µm scale bar) (view white arrows).

Referências

REFERÊNCIAS

ABASSI, E. *et al*. Silver nanoparticles: Synthesis methods, bio-applications and properties. **Crit Rev Microbiol** , v.42, n.2, p.173-180, 2016. DOI 10.3109/1040841X.2014.912200. Disponível em: https://doi.org/10.3109/1040841X.2014.912200

ABBASZADEGAN, A, *et al*. The effect of charge at the surface of silver nanoparticles on antimicrobial activity against gram-positive and gram-negative bacteria: a preliminary study. **J Nanomater**, v.2015, n.16, p.53. DOI 10.1155/2015/720654. Disponível em: https://doi.org/10.1155/2015/720654

AGNIHOTRI, R.; GAUR, S.; ALBIN, S. *Nanometals in Dentistry*: Applications and Toxicological Implications-a Systematic Review. **Biol Trace Elem Res**, v.197, n.1, p.70-88, Sep. 2020. DOI 10.1007/s12011-019-01986-y. Disponível em: https://doi.org/10.1007/s12011-019-01986-y

AHMAD, N.; JAFRI, Z.; KHAN, Z.H. Evaluation of nanomaterials to prevent oral Candidiasis in PMMA based denture wearing patients. A systematic analysis. **J Oral Biol Craniofac Res**, v.10, n.2, p.189-193, Apr./Jun. 2020. DOI 10.1016/j.jobcr.2020.04.012. Disponível em: https://doi.org/10.1016/j.jobcr.2020.04.012

AL-FATTANI, M.A.; DOUGAL, L.J. *Biofilm matrix of Candida albicans and Candida tropicalis*: chemical composition and role in drug resistance. **J Med Microbiol**, v.55, n. 8, p.999-1008, Aug. 2006. DOI 10.1099/jmm.0.46569-0. Disponível em: https://doi.org/10.1099/jmm.0.46569-0.

ALMATROUDI, A. *Silver nanoparticles*: synthesis, characterization and biomedical applications. **Open Life Sci**, v. 15, n.1, p. 819-839. Nov. 2020. DOI 10.1515/biol-2020-0094. Disponível em: https://doi.org/10.1099/jmm.0.46569-0.

ALMEIDA, N. L. M. *et al*. Antimicrobial activity of denture adhesive associated with *Equisetum giganteum*- and *Punica granatum*-enriched fractions against *Candida albicans* biofilms on acrylic resin surfaces, **Biofouling**, v. 34, n. 1, p. 62-73, 2018.Disponível em: https://doi.org/10.1080/08927014.2017.1407408

ALVES, A. C. *et al*. Quality of life related to complete denture. **Acta Odontolog Latinoam: AOL**, v. 31, n.2, p. 91-96, 2018. Disponível em: http://www.scielo.org.ar/pdf/aol/v31n2/v31n2a03.pdf

BAPAT, R.A. *et al*. An overview of application of silver nanoparticles for biomaterials in dentistry. **Mater Sci Eng C Mater Biol**, v.91, p.881-898, Oct. 2018. DOI 10.1016/j.msec.2018.05.069. Disponível em: https://doi.org/10.1016/j.msec.2018.05.069.

BHATTACHARYA, S.; SAE-TIA, S.; FRIES, B.C. Candidiasis and Mechanisms of Antifungal Resistance. **Antibiotics (Basel)**, v.9, n.6, p.312, 2020. Jun. 2020. DOI 10.3390/antibiotics9060312. Disponível em: https://doi.org/10.1128/JB.183.18.5385-5394.2001

BRANTES, M.F. *et al. Analysis of risk factors for maxillary denture-related oral mucosal lesions:* A cross-sectional study. **Med Oral Patol Oral Cir Bucal,** v. 24, n.3, p. e305-e313. May. 2019, DOI 10.4317/medoral.22826. Disponível em: https://doi.org/10.4317/medoral.22826.

CARDOSO, M. *et al. Edentulism in Brazil*: trends, projections and expectations until 2040. **Ciên Saúde Colet**, v.21, p.1239-1246. DOI 10.1590/1413-81232015214.13672015. Disponível em: https://doi.org/10.1590/1413-81232015214.13672015

CARLSSON, G.E.; OMAR, R. *The future of complete dentures in oral rehabilitation*. A critical review. **J Oral Rehabil**, v.37, n.2, p.143-56, Feb. 2010. DOI 10.1111/j.1365-2842.2009.02039.x. Disponível em: https://doi.org/10.1111/j.1365-2842.2009.02039.x.

CHANDRA, J. *et al.* Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. **J Bacteriol**, v.183, n.18, p. 5385-94, 2001a. DOI 10.1128/JB.183.18.5385-5394.2001. Disponível em: https://doi.org/10.1128/JB.183.18.5385-5394.2001

CONTALDO, M. *et al.* Association Between Denture Stomatitis, Candida Species and Diabetic Status. **J Biol Regul Homeost Agents**, v. 33, n. 3 Suppl. 1, p.35-41, 2019. DENTAL SUPPLEMENT. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31538448/

DE FOGGI, C.C. *et al.* Effect of surface roughness on the hydrophobicity of a denture-base acrylic resin and *Candida albicans* colonization. **J Investig Clin Dent**, v. 7, n.2, p.141-8, May.2016. DOI 10.1111/jicd.12125. Disponível em: https://doi.org/10.1111/jicd.12125.

DE SOUZA. R.F.*et al. Improving practice guidelines for the treatment of denture-related erythematous stomatitis:* a study protocol for a randomized controlled trial. **Trials**. v.18, n.1, p.211, May. 2017. DOI 10.1186/s13063-017-1947-y. Disponível em: https://doi.org/10.1186/s13063-017-1947-y

DI STASIO, D. *et al. Management of denture stomatitis*: a narrative review. **J Biol Regul Homeost Agents**, v. 32, n. 2 Suppl. 1, p. 113-116, 2018. Disponível em: https://www.researchgate.net/publication/323318333_Management_of_denture_stomatitis_A_narrative_review

DILNAWAZ, F.; ACHARYA, S.; SAHOO, S.K. Recent trends of nanomedicinal approaches in clinics. **Int J Pharm**, v. 538, n.1-2, p.263-278, Mar. 2018. DOI 10.1016/j.ijpharm.2018.01.016. Disponível em: https://doi.org/10.1016/j.ijpharm.2018.01.016

DOS SANTOS, C.A. *et al.* Silver nanoparticles: therapeutical uses, toxicity, and safety issues. **J Pharm Sci,** v. 103, n.7, p. 1931-1944, 2014. DOI 10.1002/jps.24001. Disponível em: https://doi.org/10.1002/jps.24001.

GAD, M. M.; FOUDA, S. M. Current perspectives and the future of *candida albicans*-associated denture stomatitis treatment. **Dent Med Probl**, v. 57, n. 1, p. 95–102, 2020. DOI 10.17219/dmp/112861. Disponível em: https://doi.org/10.17219/dmp/112861

GAD, M.M. *et al. Inhibitory effect of zirconium oxide nanoparticles on Candida albicans adhesion to repaired polymethyl methacrylate denture bases and interim removable prostheses:* a new approach for denture stomatitis prevention. **Inter J Nanomed**, v. 12, p. 5409-5419, Jul. 2017. DOI 10.2147/IJN.S142857 Disponível em: https://doi.org/10.2147/IJN.S142857

GARAICOA, J.L.*et al*. Promise of Combining Antifungal Agents in Denture Adhesives to Fight *Candida* Species Infections. **J Prosthodont**, v.27, n.8, p.755-762, Oct. 2018. DOI 10.1111/jopr.12565. Disponível em: https://doi.org/10.1111/jopr.12565.

GAUCH, L.M.R.*et al*. Isolation of *Candida* spp. from denture-related stomatitis in Pará, Brazil. **Braz J Microbiol**, v.49, n.1, p.148-151, Jan./Mar. 2018. DOI 10.1016/j.bjm.2017.07.001. Disponível em: https://doi.org/10.1016/j.bjm.2017.07.001.

GAVANJI, S.; LARKI, B. Comparative effect of propolis of honey bee and some herbal extracts on *Candida albicans*. **Chin J Integr Med**, v. 23, n.3, p. 201-207, Mar. 2017. DOI 10.1007/s11655-015-2074-9. Disponível em: https://doi.org/10.1007/s11655-015-2074-9. Acesso: 8 nov. 2020.

GLEIZNEYS, A.; ZDANAVIčIENÉ, E.; ŽILINSKAS, J. *Candida albicans* importance to denture wearers. A literature review. **Stomatologia**, v.17, n.2, p.54-66, 2015. Disponível em: https://sbdmj.lsmuni.lt/152/152-04.pdf.

GRASSO, J. E. Denture adhesives. **Dent Clin North Am**, v. 48, n.3, p. 721-33, 2004, DOI 10.1016/j.cden.2004.04.002. Disponível em: https://doi.org/10.1016/j.cden.2004.04.002.

HANNAH, V.E. *et al*. *Denture Stomatitis*: Causes, Cures and Prevention. **Prim Dent J**, v.6, n.4, p.46-51, Dec. 2017. DOI 10.1308/205016817822230175. Disponível em: https://doi.org/10.1308/205016817822230175

HOSEINZADEH, E. *et al. A Review on Nano-Antimicrobials:* Metal Nanoparticles, Methods and Mechanisms. **Current Drug Metabolism**, v. 18, n.2, p. 120-128, 2017. DOI 10.2174/1389200217666161201111146. Disponível em: https://doi.org/10.2174/1389200217666161201111146.

ILAKIYYA, E. Denture adhesive- a review. **Int J Sci Res (IJSR)**, v. 5, (issue 8), p. 272-273, 2016. Disponível em: https://www.ijsr.net/get_count_search.php?paper_id=NOV164502

IQBAL H.M.N. *et al.* Recent Trends in Nanotechnology-Based Drugs and Formulations for Targeted Therapeutic Delivery. **Recent Pat Inflamm Allergy Drug Discov**, v.10, n.2, p.86-93, 2017. DOI 10.2174/1872213X10666161213162823. Disponível em: https://doi.org/10.2174/1872213X10666161213162823.

KINOSHITA, H. *et al.* Cryptic antifungal compounds active by synergism with polyene antibiotics. **J Biosci Bioeng**, v.121, n.4, p.394-8, Apr. 2016. DOI 10.1016/j.jbiosc.2015.08.003. Disponível em: https://doi.org/10.1016/j.jbiosc.2015.08.003.

KUMAR, P. R. *et al.* Denture Adhesives in Prosthodontics: An Overview. **J Int Oral health**, v. 7, Suppl 1, p.93–95, 2015. Disponível em:

LEE, D.J.; SAPONARO P.C. Management of Edentulous Patients. **Dent Clin North Am**, v.63, n.2, p.249-261, Apr. 2019. Doi 10.1016/j.cden.2018.11.006. Disponível em: https://doi.org/10.1016/j.cden.2018.11.006.

LIU, D. *et al*. The Smart Drug Delivery System and Its Clinical Potential. **Theranostics**, v. 6, n. 9, p.1306-23, Jun. 2016. DOI 10.7150/thno.14858. Disponível em: https://doi.org/10.1016/j.cden.2018.11.006.

LIU, Y. *et al*. Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. **Chem Soc Rev**, v.48, n.2, p.428-446, Jan. 2019. DOI 10.1039/c7cs00807d. Disponível em: https://doi.org/10.1039/c7cs00807d.

LOYAGA-CASTILLO, M. *et al*. Antifungal Activity of Peruvian Banana Peel (*Musa paradisiaca L*.) on *Candida albicans*: An *In Vitro* Study. **J Contemp Dent Pract**, v.21, n.5, p.509-514, May. 2020. DOI 10.5005/jp-journals-10024-2827. Disponível em: https://doi.org/10.5005/jp-journals-10024-2827

MATALON, S. *et al*. *The effect of enriching denture adhesives with chlorhexidine diacetate on the proliferation of Candida albicans:* an *in vitro* analysis. **J Biol Regul Homeost Agents**, v.31, p. 45-52. Apr./Jun. 2017. 2 Suppl 1. Disponível em: https://europepmc.org/article/MED/28691453

MBA, I. E.; NWEZE, E. I. *The use of nanoparticles as alternative therapeutic agents against Candida infections*: an up-to-date overview and future perspectives. **World J Microbiol Biotechnol**, v.36, n.11, p.163, Sep. 2020. DOI 10.1007/s11274-020-02940-0. Disponível em: https://doi.org/10.1039/c7cs00807d.

MILLSOP, J.W.; FAZEL, N. Oral candidiasis. **Clin Dermatol**, v.34, n.4, p. 487-94, Jul./Aug. 2016. DOI 10.1016/j.clindermatol.2016.02.022. Disponível em: https://doi.org/10.1016/j.clindermatol.2016.02.022.

MORONES , J. R. *et al*. The bactericidal effect of silver nanoparticles. **Nanotechnology,** v. 16, n.10, p. 2346-53, 2005. DOI 10.1088/0957-4484/16/10/059. Disponível em: https://doi.org/10.1088/0957-4484/16/10/059.

MOUSAVI, S.A. *et al*. Antibacterial and antifungal impacts of combined silver, zinc oxide, and chitosan nanoparticles within tissue conditioners of complete dentures *in vitro*. **Irish j of med science**, v. 189, n.4, p. 1343-1350, 2020. DOI 10.1007/s11845-020-02243-1. Disponível em: https://doi.org/10.1007/s11845-020-02243-1.

NEWTON, A.V. Denture sore mouth. A possible etiology. **British Dental J**, v.112, p.357-360, 1962.

NOSRATZEHI, T. *et al*. The comparison of the effect of curcumin with nystatin on inhibition level of *Candida albicans*. **J Exp Pharmacol**, v.6, n.11, p. 93-97, Aug. 2019. DOI

10.2147/JEP.S215843.. Disponível em: https://doi.org/10.2147/JEP.S215843. Acesso: 8 nov. 2020.

ONOUE, S.; YAMADA, S.; CHAN, H.K. *Nanodrugs:* pharmacokinetics and safety. **Int J Nanomedicine**, v.20, n.9, p.1025-37, Feb. 2014. DOI 10.2147/IJN.S38378. Disponível em: https://doi.org/10.2147/IJN.S38378.

PAL, S. *et al*. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. **Appl Environ Microbiol**, v. 73, n.6, p.1712-20, 2007. DOI 10.1128/AEM.02218-06. Disponível em: https://doi.org/10.1128/AEM.02218-06.

PANKHURST, C. L. Candidiasis (oropharyngeal). **BMJ Clin Evid**, v. 2013, 1304, 8 Nov. 2013. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3821534/. Acesso: 8 nov. 2020.

PARK, J.B. *et al*. Development of an antifungal denture adhesive film for oral candidiasis utilizing hot melt extrusion technology. **Expert Opin Drug Deliv**, v.12, n.1, p.1-13, Jan. 2015. DOI 10.1517/17425247.2014.949235. Disponível em: https://doi.org/10.1517/17425247.2014.949235.

PATIL, M.P., KIM, GD. Eco-friendly approach for nanoparticles synthesis and mechanism behind antibacterial activity of silver and anticancer activity of gold nanoparticles. **Appl Microbiol Biotechnol**, v. 101, p. 79–92, 2017. DOI 10.1007/s00253-016-8012-8. Disponível em: https://doi.org/10.1007/s00253-016-8012-8.

PERO, A.C. *et al*. Measurement of interfacial porosity at the acrylic resin/denture tooth interface. **J Prosthodont**, v.19, n.1, p.42-6, Jan. 2010. DOI 10.1111/j.1532-849X.2009.00511.x. Disponível em: https://doi.org/10.1111/j.1532-849X.2009.00511.x.

POMA-CASTILLO, L. *et al.* Antifungal Activity of Ethanol-extracted Bixa orellana (L) (*Achiote*) on *Candida albicans*, at Six Different Concentrations. **J Contemp Dent Pract**, v.20, n.10, p. 1159-1163, Oct. 2019. DOI 10.5005/jp-journals-10024-2672. Disponível em: https://doi.org/10.5005/jp-journals-10024-2672 . Acesso: 8 nov. 2020.

PRADÍES, G. *et al.* Clinical study comparing the efficacy of two denture adhesives in complete denture patients. **Int J Prosthodontics**, v. 22, n.4, p.361-7, 2009.

PRASAD, R.; NAIR, R.; BANERJEE, A. Emerging Mechanisms of Drug Resistance in *Candida albicans*. **Prog Mol Subcell Biol**, v.58, p.135-153, 2019. DOI 10.1007/978-3-030-13035-0_6. Disponível em: https://doi.org/10.1007/978-3-030-13035-0_6.

PURYER, J. Denture Stomatitis – A Clinical Update. **Dent Update**, v.43, n.6, p.529-30, 533-5, Jul./Aug. 2016. DOI 10.12968/denu.2016.43.6.529. Disponível em: https://doi.org/10.12968/denu.2016.43.6.529.

RAJARAM, A.; MANOJ, S.S. Influence of 3 different forms of a commercially available denture adhesive material on the growth of *Candida* species: An *in vitro* study. **J Prosthet**

Dent, v.118, n.3, p.379-385, Sep. 2017. DOI 10.1016/j.prosdent.2016.11.015. Disponível em: https://doi.org/10.1016/j.prosdent.2016.11.015.

RAUTEMAA, R.; RAMAGE, G. Oral candidosis clinical challenges of a biofilm disease. **Crit Rev Microbiol**, v. 37, n. 4, p.328–336, 2011. DOI 10.3109/10408 41X.2011.585606. Disponível em: https://doi.org/10.3109/10408 41X.2011.585606.

RAZA, A. *et al*. *Solid nanoparticles for oral antimicrobial drug delivery*: a review. **Drug Discov Today**, v.24, n.3, p.858-866, Mar. 2019. DOI 10.1016/j.drudis.2019.01.004. Disponível em: https://doi.org/10.1016/j.drudis.2019.01.004.

RIVERA, L.; RAMOS, A. Actividad antimicótica de nanopartículas. **Mundo Nano. Rev Interd Nanocien Nanotecnol**, v. 7, n. 12, Ene./Jun. 2014. 7. DOI 10.22201/ceiich.24485691e.2014.12.49099. Disponível em: https://doi.org/10.22201/ceiich.24485691e.2014.12.49099.

RÓNAVÁRI, A. *et al. Green Silver and Gold Nanoparticles:* Biological Synthesis Approaches and Potentials for Biomedical Applications. **Molecules (Basel, Switzerland)**, v.26, n.4, p. 844. Feb. 2021. DOI 10.3390/molecules26040844. Disponível em: https://doi.org/10.3390/molecules26040844.

SAKAGUCHI H. Treatment and Prevention of Oral Candidiasis in Elderly Patients. **Med Mycol J**, v.58, n.2, p.J43-J49, 2017. Japanese. DOI 10.3314/mmj.17.004. PMID: 28566666.

SALERNO, C. *et al*. Candida-associated denture stomatitis. **Med Oral Patol Oral Cir Bucal**, v.16, n.2, p.e139-43, Mar. 2011. DOI 10.4317/medoral.16.e139.

SAMPAIO-MAIA B. *et al*. The effect of denture adhesives on *Candida albicans* growth *in vitro*. **Gerodontology**, v.29, n.2, p.e348-56, Jun. 2012. DOI 10.1111/j.1741-2358.2011.00478.x. Disponível em: https://doi.org/10.1111/j.1741-2358.2011.00478.x.

SÁNCHEZ-LÓPEZ, E. *et al. Metal-Based Nanoparticles as Antimicrobial Agents*: An Overview. **Nanomaterials (Basel, Switzerland)**, v. 10, n.2, p. 292. Feb. 2020, DOI 10.3390/nano10020292. Disponível em: https://doi.org/10.3390/nano10020292.

SCHEIBLER, E. *et al*. Use of nystatin and chlorhexidine in oral medicine: Properties, indications and pitfalls with focus on geriatric patients. **Gerodontology**, v.34, n.3, p.291-298, Sep. 2017. DOI 10.1111/ger.12278. Disponível em: https://doi.org/10.1111/ger.12278.

SHAMSOLKETABI, S.; NILI, M. The effect of denture adhesive on the efficiency of complete denture in patients with different alveolar ridges. **Dent Res J** (Isfahan), v.15, n.4, p.271-275, Jul./Aug. 2018.

SHANMUGANATHAN, R. *et al*. Synthesis of Silver Nanoparticles and their Biomedical Applications - A Comprehensive Review. **Curr Pharm Des**, v. 25, n.24, p. 2650-2660, 2019. DOI 10.2174/1381612825666190708185506.
Disponível em: https://doi.org/10.2174/1381612825666190708185506.

SHARON, V.; FAZEL, N. Oral candidiasis and angular cheilitis. **Dermatol Ther**, v.23, n,3, p.230-42, May./Jun. 2010. DOI 10.1111/j.529-8019.2010.01320.x. Disponível em: https://doi.org/10.1111/j.529-8019.2010.01320.x.

SIDDIQUI, K.S. *et al.* A review on biosynthesis of silver nanoparticles and their biocidal properties. **J Nanobiotechnol**. v. 16,1 14. 16 Feb. 2018, DOI 10.1186/s12951-018-0334-5. Disponível em: https://doi.org/10.1186/s12951-018-0334-5.

SINGH, R. *et al. Bacteriagenic silver nanoparticles:* synthesis, mechanism, and applications. **Appl Microbiol Biotechnol**, [s. l.], v. 99, n. 11, p. 4579–4593, 2015. DOI 10.1007/s00253-015-6622-1. Disponível em: https://doi.org/10.1007/s00253-015-6622-1. Acesso em: 28 jun. 2021.

SLADE, G.D.; AKINKUGBE, A.A.; SANDERS, A.E. Projections of U.S. edentulism prevalence following 5 decades of decline. **J Dent Res**, v.93, n.10, p.959–65, 2014. DOI 10.1177/0022034514546165. Disponível em: https://doi.org/ 10.1177/0022034514546165.

SLAVIN, Y.N. *et al. Metal nanoparticles*: understanding the mechanisms behind antibacterial activity. **J Nanobiotechnol**, v. 15, n. 65 ,2017. DOI 10.1186/s12951-017-0308-z. Disponível em: https://doi.org/10.1186/s12951-017-0308-z.

SOUSA, M.G.C. *et al*. Nanofibers as drug-delivery systems for infection control in dentistry. **Expert Opin Drug Deliv**, v.17, n.7, p. 919-930, Jul. 2020. DOI 10.1080/17425247.2020.1762564. Disponível em: https://doi.org/10.1080/17425247.2020.1762564.

SUN, Y C *et al*. Research and development of digital design and fabrication of complete denture. **Chin J Stomatology**, v. 53, n.1, p.60-65, 2018. DOI 10.3760/cma.j.issn.1002-0098.2018.01.013. Disponível em: https://doi.org/10.3760/cma.j.issn.1002-0098.2018.01.013.

TAKAMIYA, A.S. *et al*. Biocompatible silver nanoparticles incorporated in acrylic resin for dental application inhibit *Candida albicans* biofilm. **Mater Sci Eng C Mater Biol**, v.118, p.111341, Jan. 2021. DOI 10.1016/j.msec.2020.111341. Disponível em: https://doi.org/10.1016/j.msec.2020.111341.

TANG, S.; ZHENG, J. Antibacterial Activity of Silver Nanoparticles: Structural Effects." **Adv Healthc Mat,**v. 7, n.13, p.e1701503, 2018. DOI 10.1002/adhm.201701503. Disponível em: https://doi.org/10.1002/adhm.201701503.

WANG, L.; HU, C.; SHAO, L. *The antimicrobial activity of nanoparticles*: present situation and prospects for the future. **Int J Nanomedicine**, v.12, p.1227-1249, Feb. 2017. DOI 10.2147/IJN.S121956. Disponível em: https://doi.org/10.2147/IJN.S121956.

WEBB *et al. Candida-associated denture stomatitis*. Aetiology and management:a revie. Part . Treatment of oral candidosis. **Aust Dent J**, v.43, n.4. 1998. DOI 10.1111/j.1834-7819.1998.tb00172.x. Disponível em: https://doi.org/ j.1834-7819.1998.tb00172.x.

WEI, L. *et al. Silver nanoparticles*: synthesis, properties, and therapeutic applications. **Drug discovery today**, v.20, n.5, p. 595-601, 2015. DOI 10.1016/j.drudis.2014.11.014. Disponível em: https://doi.org/10.1016/j.drudis.2014.11.014.

XU, L. *et al. Silver nanoparticles*: Synthesis, medical applications and biosafety. **Theranostics,** v. 10, n. 20, p. 8996-9031. Jul. 2020, DOI 10.7150/thno.45413. Disponível em: https://doi.org/10.7150/thno.45413.

YARBOROUGH, A, *et al.* Evidence Regarding the Treatment of Denture Stomatitis. **J Prosthodont**, v.25, n.4, p.288-301, Jun. 2016. DOI 10.1111/jopr.12454. Disponível em: https://doi.org/10.1111/jopr.12454.

YIN, I.X. *et al.* The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. **Int J Nanomedicine**, v.15, p.2555-2562, Apr. 2020. DOI 10.2147/IJN.S246764. Disponível em: https://doi.org/10.2147/IJN.S246764.

ZHANG, L. *et al.* Development of Nanoparticles for Antimicrobial Drug Delivery. **Curr Med Chem**, v.17, n.6, p.585–594, 2010. DOI 10.2174/092986710790416290 Disponível em: https://doi.org//10.2174/092986710790416290

ZHANG, X.F. *et al. Silver Nanoparticles*: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. **Int J Mol Sci**, v. 17, n.9, p.1534, Sep. 2016. DOI 10.3390/ijms17091534. Disponível em: https://doi.org/10.3390/ijms1709