**UNIVERSIDADE ESTADUAL DE CAMPINAS**
**FACULDADE DE ODONTOLOGIA DE PIRACICABA**

**LARISSA PAVANELLO**

# *Desenvolvimento, caracterização e atividade antimicrobiana de nanopartículas de sílica multicamada com clorexidina incorporadas a compósitos dentais*

# *Development, characterization and antimicrobial activity of multilayer silica nanoparticles with chlorhexidine incorporated into dental composites*

**Piracicaba –SP**

**2022**

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Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Odontologia, na Área de Farmacologia, Anestesiologia e Terapêutica.

Dissertation presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Pharmacology, Anesthesiology and Therapeutics.

**Orientadora:** Profª Drª Karina Cogo-Müller

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**Professora Dra. Karina Cogo-Müller**
Presidente da Comissão Examinadora

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**Professora Dra. Michelle Franz Montan Braga Leite**

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## RESUMO

A aplicação de nanopartículas de sílica (SNPs) em materiais restauradores, como as resinas compostas, é bastante ampla e, de modo geral, objetiva aprimorar as propriedades gerais e nanocarrear fármacos, como antimicrobianos e substâncias osteogênicas. Apesar dos constantes aprimoramentos, o desempenho clínico e a longevidade dos compósitos resinosos ainda é influenciado negativamente pelos constituintes da matriz orgânica e pelo dinâmico ambiente oral. Para superar as principais limitações na interface dente-compósito e, consequentemente, cáries recorrentes, a incorporação de nanopartículas de carga com antimicrobiano surgem como uma abordagem promissora. Assim, o objetivo do presente estudo foi desenvolver e caracterizar um compósito experimental com diferentes concentrações de SNPs carregadas com clorexidina (CHX), produzidas pelo sistema *layer by layer* (CHX-SNPs), afim de promover ação antimicrobiana contra a adesão do biofilme de *Streptococcus mutans*, bem como propriedades físico-químicas satisfatórias. Para tal, sete grupos experimentais foram preparados com BisGMA/TEGDMA e SNPs com ou sem CHX (0, 10, 20 ou 30% em peso). A atividade antimicrobiana foi avaliada através do método de difusão em ágar (n = 5) contra *Streptococcus mutans*, *Streptococcus mitis* e *Streptococcus gordonii*. Além disso, a inibição da formação de biofilme (n = 6) dos compósitos foi avaliada contra *Streptococcus mutans* em 24 h e 72 h. As propriedades físico-químicas dos materiais experimentais foram avaliadas pelo grau de conversão (n = 3), módulo de elasticidade e resistência à flexão (n = 10), rugosidade superficial (n = 10) e contração volumétrica pós-gel (n = 5). Os resultados do grau de conversão, rugosidade superficial, contração volumétrica pós-gel e halo de inibição foram analisados por One-Way ANOVA/Tukey (α = 5%) e as demais variáveis por Two-Way ANOVA/Tukey (α = 5%). A adição de CHX-SNPs resultou em uma resina com propriedades antimicrobianas aumentadas em relação ao grupo controle e SNPs. Os compósitos com CHX formaram zonas de inibição para todas as bactérias testadas de modo concentração-dependente ($p < 0,05$) e reduziram a adesão do biofilme de *Streptococcus mutans*, de forma mais significativa após 24 h da formação. Nos dois tempos analisados, CHX-SNPs à 30% promoveu a maior redução de UFC/mL ($p < 0,05$). Em relação as propriedades físico-químicas, não foi observado diferença significativa ($p > 0,05$) entre os grupos para o grau de conversão. Os grupos com CHX apresentaram os maiores valores de contração volumétrica pós-gel ($p < 0,05$) e, à 30% apresentaram maiores valores de resistência à flexão e módulo de elasticidade. O armazenamento dos espécimes em água por 60 dias influenciou significativamente ($p < 0,05$) os resultados, aumentando ou reduzindo os valores a depender do grupo para estas últimas

variáveis. Por fim, a rugosidade superficial aumentou de forma concentração-dependente, sem diferença estatística entre os grupos com e sem CHX. Portanto, as CHX-SNPs forneceram ao material restaurador atividade antimicrobiana e antibiofilme contra bactérias do gênero *Streptococcus* e propriedades físico-químicas satisfatórias.

**Palavras-chave:** Resina composta. Nanopartículas. Sílica. Clorexidina. Odontologia.

## ABSTRACT

The application of silica nanoparticles (SNPs) in restorative materials, such as composite resins, is very vast and, in general, aims to improve the general properties and the nanocarry drugs, such as antimicrobials and substances osteogenic. Despite constant improvements, the clinical performance and longevity of resin composites is still negatively influenced by the organic matrix constituents and the dynamic oral environment. To overcome the main limitations at the tooth-composite interface and, consequently, recurrent caries, the incorporation of filler nanoparticles with antimicrobial appears as a promising approach. Thus, the objective of the present study was to develop and characterize an experimental composite with different concentrations of chlorhexidine-loaded SNPs, produced by the *layer by layer* system (CHX-SNPs), in order to promote antimicrobial action against the adhesion of the *Streptococcus mutans* biofilm, as well as satisfactory physicochemical properties. For this, seven experimental groups were prepared with BisGMA/TEGDMA and SNPs with or without chlorhexidine (CHX - 0, 10, 20 or 30% by weight). Antimicrobial activity was evaluated by agar diffusion method (n = 5) against *Streptococcus mutans*, *Streptococcus mitis* and *Streptococcus gordonii*. Furthermore, the inhibition of biofilm formation (n = 6) of the composites was evaluated against *Streptococcus mutans* at 24 h and 72 h. The physicochemical properties of the experimental materials were evaluated by the degree of conversion (n = 3), flexural modulus and flexural strength (n = 10), surface roughness (n = 10) and post-gel volumetric shrinkage (n = 5). The results of the degree of conversion, surface roughness, post-gel volumetric shrinkage and inhibition zone were analyzed by One-Way ANOVA/Tukey ($\alpha = 5\%$) and the other variables by Two-Way ANOVA/Tukey ($\alpha = 5\%$). The addition of CHX-SNPs resulted in a resin with increased antimicrobial properties compared to the control group and SNPs. The CHX composites formed zones of inhibition for all bacteria tested in a concentration-dependent manner ($p < 0.05$) and reduced the adhesion of the *Streptococcus mutans* biofilm, more significantly after 24 h of formation. In the two analyzed times, CHX-SNPs 30% promoted the highest reduction of CFU/mL ($p < 0.05$). Regarding the physicochemical properties, no significant difference ($p > 0.05$) was observed between the groups for the degree of conversion. The groups with CHX had the highest post-gel volumetric shrinkage values ($p < 0.05$) and, at 30% they had the highest values of flexural strength and flexural modulus. The storage of specimens in water for 60 days significantly influenced ($p < 0.05$) the results, increasing or reducing the values depending on the group for these last variables. Finally, the surface roughness increased in a concentration-dependent manner, with no statistical difference

between the groups with and without CHX. Therefore, CHX-SNPs showed the restorative material with antimicrobial and antibiofilm activity against bacteria of the *Streptococcus* genus and satisfactory physicochemical properties.

**Key words:** Composite resin. Silica. Nanoparticle. Chlorhexidine. Dentistry.

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**Artigo 1: Silica nanoparticles: Applications in dentistry**



**Artigo 2: Development, characterization and antimicrobial activity of multilayer silica nanoparticles with chlorhexidine incorporated into dental composites**

## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| μL | Microlitro |
| μm | Micrômetro |
| μSBS | Resistência ao microcisalhamento |
| μTBS | Força de ligação |
| ACP | Fosfato de cálcio amorfo |
| Ag-BGN-MSN | Nanopartículas de sílica mesoporosa revestidas de vidro bioativo e carregadas com prata |
| AgNPs/$SiO_2$ | Nanopartículas porosa de sílica shell dopadas com nanopartículas de prata |
| b | Largura da amostra em milímetro |
| BA | Baicalina |
| BE | Baicaleína |
| BisGMA | *Bisphenol A Bis (2-hydroxy-3-methacryloxypropyl) Ether* |
| BG | Vidro bioativo |
| C | Carga registrada |
| *C. albicans* | *Candida albicans* |
| Ca | Cálcio |
| $Ca_3(PO_4)_2$ | Fosfato de cálcio |
| $Ca_3(PO_4)_2$-MSNs | Nanopartículas de sílica dopadas com fosfato de cálcio |
| CAD/CAM | Computer-aided design/Computer-aided manufacturing |
| CCK – 8 | Cell Counting Kit-8 |
| CHX | Clorexidina |
| CHX/MSN-PLGA | Nanopartículas de sílica mesoporosa dopadas com clorexidina modificada com poli- (ácido lático-co-glicólico) |
| CHX-MSN | Nanopartícula de sílica mesoporosa dopadas com clorexidina |
| CHX-SNPs | Nanopartículas de sílica dopadas com clorexidina |
| Cm | Centímetro |
| Cu-MBGN | Nanoesferas de vidro bioativo mesoporoso dopado com cobre |
| $CO_2$ | Dióxido de carbono |
| d | Deflexão correspondente a carga registrada em milímetros |
| DH | Hipersensibilidade dentinária |

| | |
|---|---|
| DMAEMA | *2-(Dimethylamino) ethyl methacrylate* |
| E | Módulo de elasticidade |
| *E. faecalis* | *Enterococcus faecalis* |
| ECA | Adesivo composto experimental |
| EDTA | Ácido Etilenodiamino Tetra-Acético |
| EPD | Deposição Eletroforética |
| F | Carga máxima registrada antes da fratura do corpo de prova |
| FTIR | Fourier transform infrared |
| g | Gramas |
| GEN | Gentamicina |
| GEN-SNPs | Nanopartículas de sílica dopadas com gentamicina |
| GF | Nanofilme à base de sílica |
| GPa | Gigapascal |
| GS | Partículas de alumina |
| h | Altura da amostra em milímetros |
| HA | Hidroxiapatita |
| HCA | Apatita de hidroxicarbonato |
| hGECs | Células epiteliais gengivais humanas |
| IL-1β | Interleucina beta 1 |
| L | Distância entre os apoios em milímetros |
| *L. casei* | *Lactobacillus casei* |
| LbL | *layer by layer* |
| LDH | *Pierce lactate dehydrogenase.* |
| M41S | Família de nanopartículas de sílica mesoporosa |
| Ma | Magnésio |
| MCM | *Mobil composition of matter* |
| mg/L | Miligrama por litro |
| $Mg_3(PO_4)_2$ | Fosfato de magnésio |
| mL/min | Mililitro por minuto |
| MOX | Moxifloxacino |
| MPa | Megapascal |
| MPS | γ-metacriloxipropiltrimetoxisilano |
| MSNs | Nanopartículas de sílica mesoporosa |

| | |
|---|---|
| MTT | *Brometo de [3-(4,5-dimetiltiazol-2yl)-2,5-difenil tetrazolium* |
| N | Newton (unidade de medida de força) |
| Nano - BA | Nanopartículas de sílica mesoporosa dopadas com baicalina |
| Nano – BE | Nanopartículas de sílica mesoporosa dopadas com baicaleína |
| Nano-D | Nanopartículas de diamante |
| Nano-$ZrO_2$ | Nanopartículas de dióxido de zircônio |
| NaOCl | Hipoclorito de sódio |
| NCMS | Nanopartículas de sílica dopadas com óxido de cálcio |
| nHA | Nano-hidroxiapatita |
| nHA-MSNs | Nanopartículas de sílica associada a nano-hidroxiapatita |
| nm | Nanômetro |
| NPs | Nanopartículas |
| NPs $Ta_2O_5/SiO_2$ | Nanopartículas de sílica com butóxido de tântalo |
| mm | Milímetro |
| O/A | Óleo/Água |
| OCT | Dicloridrato De Octenidina |
| OCT-SNPs | Nanopartícula de sílica dopadas com dicloridrato de octenidina |
| OH | Hidroxila |
| ORMOSIL | Organically modified silica |
| PDI | Infecções articulares protéticas |
| PHF | Fluoropolímero funcionalizado com hidroxila |
| PMMA | Poli(metil)metacrilate |
| ppm | Partes por milhão |
| Pre-PU | Oligômero de Poliuretano |
| QAC | Compostos de amônio quaternário |
| QASi | Dióxido de sílica de amônio quaternário |
| RMGI | Material restaurador de ionômero de vidro modificado por resina |
| ROS | Espécies reativas de oxigênio |
| rpm | Rotações por minuto |
| *S. gordonii* | *Streptococcus gordonii* |
| *S. mutans* | *Streptococcus mutans* |
| *S. mitis* | *Streptococcus mitis* |

| | |
|---|---|
| *S. oralis* | *Streptococcus oralis* |
| *S. sanguinis* | *Streptococcus sanguinis* |
| SBA | Santa Barbara Amorphous |
| SBS | Resistência ao cisalhamento |
| $SiO_2$ | Sílica |
| SNPs | Nanopartículas de sílica |
| SNPs-ACP | Nanopartícula de sílica com fosfato de cálcio amorfo |
| Sr | Estrôncio |
| S-rGO/$SiO_2$ | Óxido de grafeno silanizado associado a nanopartículas de sílica |
| TEGDMA | *Triethyleneglycol Dimethacrylate* |
| TPU | Elastômero de Poliuretano Termoplástico |
| T-Sil | Nanopartículas de sílica modificadas com trietoxivinilsilano |
| UHA | *Urchin-Like Hydroxyapatite* |
| UV | Radiação ultravioleta |
| WST-1 | *Water-soluble tetrazolium* |
| wt% | Peso em porcentagem |
| Y-TZP | Zircônia tetragonal estabilizada com ítrio policristalino |
| w/w | Peso por peso |
| Zn-MSNs | Nanopartículas de sílica mesoporosa dopadas com zinco |

**SUMÁRIO**

## 1 INTRODUÇÃO

O campo da odontologia restauradora passou por constantes aprimoramentos ao longo dos anos, onde inúmeros materiais foram projetados e aplicados clinicamente com o propósito de reconstruir e/ou preservar a função e a estética dental (Marghalani, 2016; Tammaro et al., 2020).

Dentre estes materiais, os compósitos destacam-se devido as suas boas propriedades físico-químicas, biocompatibilidade, facilidade de manipulação, custo acessível e ampla aplicação clínica (Zagho et al., 2018). Por aderir-se a estrutura dental de forma satisfatória e esteticamente aceitável, estes materiais são utilizados para restaurar tecidos dentários anteriores e posteriores acometidos por doenças, traumas e/ou cariados e próteses fraturadas, como restaurações provisórias, dispositivos ortodônticos, selante radicular, selante de fossas e fissuras e também para produção de outros materiais resinosos como adesivos odontológicos, cimentos resinosos e ionômeros de vidros modificados por resina (Ferracane, 2011; Marghalani, 2016; Alzraikat et al., 2018).

Basicamente, os compósitos resinosos são constituídos por uma *matriz polimérica orgânica* composta por uma mistura de monômeros de dimetacrilato, uma *matriz inorgânica* composta por partículas de carga, *agentes de união* que integram as duas matrizes e um *sistema iniciador/ativador/modulador* da reação de polimerização (Marghalani, 2016; Nikolaidis et al., 2019). Alterações nesta composição permitem direcionar os materiais resinosos para aplicação em cimentação, selamento, restaurações, dentre outros (Ferracane 2011).

Apesar dos constantes aprimoramentos, o desempenho clínico das resinas compostas ainda é influenciado pelos constituintes orgânicos da formulação (Alzraikat e al., 2018). Para superar as principais limitações na interface dente-compósito, como baixa resistência ao desgaste e contração de polimerização que pode gerar microinfiltrações e episódios de cáries recorrentes, a incorporação de partículas de carga inorgânica surgem como uma alternativa eficaz para fornecer o reforço necessário às aplicações odontológicas (Wang et al., 2016; Aydınoğlu & Yoruç, 2017; Topa & Ortyl; 2020; Tammaro et al., 2020).

Nesta perspectiva, a nanotecnologia constitui uma abordagem promissora para o aprimoramento dos compósitos odontológicos, por melhorar as propriedades físico-químicas, como reologia, coeficiente de expansão térmica, cor e efeitos ópticos, resistência ao desgaste resistência a flexão, resistência a compressão, resistência ao cisalhamento e tração, tenacidade a fratura e módulo de elasticidade, além de apresentar alta capacidade de carregamento e liberação controlada de fármacos (Anusavice et al., 2013; Alkahtani, 2018; Yang et al., 2021).

É proposto que as melhorias observadas com a adição de partículas de carga inorgânica como vidro, quartzo ou sílica em escala nanométrica às resinas compostas é devido sua grande área superficial e pela presença de grupos funcionais de superfície que podem modificar a estrutura e as propriedades macroscópicas deste material (Wang et al., 2016; Nikolaidis et al., 2019).

Dentre as nanopartículas (NPs) empregadas em odontologia, as NPs de sílica (SNPs) tem chamado atenção devido as suas características, como biocompatibilidade, baixo custo, alta estabilidade bioquímica, grande área superficial, capacidade de funcionalização e de adsorção de substâncias (Priyadarsini et al., 2018; Hoang Thi et al., 2019), sendo utilizadas principalmente para melhorar o desempenho clínico dos materiais dentários e para o nanocarreamento de fármacos (Priyadarsini et al., 2018).

Considerando o dinamismo do microbioma oral e a possibilidade de falhas das resinas compostas que podem resultar na adesão e colonização de sua superfície por biofilmes cariogênicos, o desenvolvimento de compósitos que apresentem atividade antimicrobianas para reduzir o impacto patogênico dos biofilmes, sem alterar drasticamente o microbioma e a viabilidade das células orais, é desejável (Zhang et al., 2014; Boaro et al., 2019; Tammaro et al., 2020; Yang et al., 2021).

Dentre os antimicrobianos utilizados em odontologia, a clorexidina (CHX), uma bisguanida de amplo espectro, destaca-se (Wood et al., 2015; Carvalho et al., 2021). A CHX apresenta efeito bactericida e bacteriostático, a depender de sua concentração e atividade antibiofilme na cavidade oral (Reda et al., 2021). Há relatos que adição de CHX à resina composta apresenta liberação inicial em altas concentrações, seguida de um efeito terapêutico de curto prazo (Wood et al., 2015; Yang et al., 2021), além de propriedades físico-químicas reduzidas pela formação de agregados de CHX na matriz resinosa, o que é explicado por esta substância ser imiscível na matriz orgânica do material odontológico. Sendo assim, compósitos odontológicos sintetizados com CHX tendem a apresentar baixo desempenho clínico (Zhang et al., 2014).

Neste contexto, o desenvolvimento da nanotecnologia, com por exemplo, a síntese de NPs carregadas com CHX surge como uma solução viável para obtenção de um material restaurador com efeito antibacteriano estável e duradouro, devido ao potencial de carrear e liberar fármacos de modo controlado e sustentado, além de melhorar as propriedades mecânicas da resina composta e, consequentemente, aumentar seu desempenho clínico e longevidade (Yang et al., 2021).

Existem diversos métodos para preparar um sistema de liberação de fármacos antimicrobianas, dentre eles, a deposição do fármaco em uma superfície ou a uma partícula pela

técnica de montagem *layer by layer* (LbL) (Perni et al., 2017). A LbL consiste em uma técnica de revestimento que permite a montagem de uma estrutura multicamada pela deposição de compostos multivalentes, como os polieletrólitos, de modo alternado a um substrato. A formação de sistemas LbL é atribuída às interações eletrostáticas entre compostos de cargas opostas, tais como ligações de hidrogênio ou covalente, interações hidrofóbicas e Van der Waals (Alkekhia et al., 2020).

Frente a estas características, a LbL é amplamente aplicada para entrega de fármacos (Al Thaher et al., 2018; Alkekhia et al., 2020). Os fármacos podem ser adicionados ao sistema LbL como uma camada, isto é, substituindo o polieletrólito ou conjugado a eles. Na primeira ocasião, caracteriza-se um sistema reservatório para liberação prolongada. A composição e espessura das camadas é controlável e juntamente com a quantidade e interações eletrostáticas entre si, temperatura, oscilações de pH, luminosidade e ondas sonoras, influenciam o perfil de liberação do fármaco (Perni et al., 2017).

Recentemente, pesquisadores da Universidade de Cardiff (Reino Unido) desenvolveram um estudo propondo o uso de SNPs carregadas com gentamicina (GEN) LbL a fim de promover atividade antimicrobiana e prolongar a liberação de GEN de cementos ósseos. As SNPs foram compatíveis com os cementos ósseos testados, que continham em sua composição polimetilmetacrilato (PMMA) e dimetilacrilato, preservando as propriedades do material como resistência à compressão, força e módulo de elasticidade e tenacidade à fratura. Além disso, a GEN apresentou boa difusividade através do PMMA, atividade antimicrobiana prolongada e citocompatibilidade (Al Thaher et al., 2018). Visto que o perfil de liberação de antibióticos é caracterizado por uma alta liberação nas primeiras horas, seguida por uma queda acentuada na taxa de liberação pelos próximos dias geralmente abaixo da concentração inibitória mínima (CIM), as SNPs carregadas com GEN conseguiram manter uma liberação sustentada superior ao fármaco livre incorporado ao cemento, o que é desejável para um cemento ósseo (Al Thaher et al., 2018).

Com base nisto, esta dissertação foi dividida em dois capítulos, onde o capítulo 1 traz um artigo de revisão da literatura acerca da aplicação das SNPs em odontologia e o capítulo 2 traz os resultados do desenvolvimento e caracterização (*in vitro*) de um compósito experimental com diferentes concentrações de SNPs carregadas com CHX, produzidas pelo sistema LbL (CHX-SNPs). A adição das CHX-SNPs objetivou fornecer ao material resinoso ação antimicrobiana e antibiofilme contra *Streptococcus mutans*, bem como propriedades físico-químicas satisfatórias.

# 2 ARTIGOS

## 2.1 Artigo: Silica nanoparticles: Applications in dentistry

Artigo submetido ao periódico *European Journal of Oral Sciences* (Anexo I)

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## ABSTRACT

**Objective:** Silica nanoparticles (SNPs) have been extensively studied and used in different dental specialties to promote improved physicochemical properties, high loading efficiency, and controlled drug-delivery with therapeutic and/or preventive effects. Therefore, this study aimed to review the SNPs applications in nanomaterials and nanoformulations, subcategorized in dental materials and other applications, discussing general characteristics and biocompatibility. **Methods**: A literature searches were carried out in the PubMed database with the keywords "silica nanoparticle" or "SNPs" and "dentistry." After the search, the articles were separated by dental specialty and allocated to the respective topics of this review. **Results:** In dentistry, SNPs were developed and studied for application mainly in restorative dentistry, endodontics, dental implants, orthodontics, periodontics, and prosthesis areas. Generally, SNPs improved physicochemical properties of dental biomaterials, acted as efficient nanocarriers of antimicrobial and osteogenic substances, and showed excellent biocompatibility *in vitro*. **Significance:** Overall, SNPs are a promising drug delivery system that can improve dental materials biological and mechanical properties. However, more studies are needed to elucidate SNPs short- and long-term effects in the oral cavity, mainly on *in vivo* and clinical studies, to prove their effectiveness and safety.

**Keywords:** Nanotechnology. Nanoparticles. Silica. Dentistry.

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## 1. Introduction

Nanotechnology has revolutionized modern dentistry by introducing nanomaterials and nanoformulations with improved physicochemical properties, high loading efficiency, and controlled release of substances, such as antimicrobial and osteogenic substances [1,2], which can be successfully applied in diagnostic, preventive, restorative, and conservative dentistry [3].

Currently, many studies investigate the use of nanoparticles (NPs) in different dental specialties. Among these, silica NPs (SNPs), especially mesoporous nanoparticles (MSNs) (Table 1), stand out due to their unique properties, such as excellent physicochemical stability, biocompatibility, adjustable morphology, possibility of modifying and functionalizing the surface for loading and releasing drugs in a targeted and controlled manner [4, 1].

Considering the multi-ecosystem environment and the adversities of the oral cavity, SNPs in dentistry are mainly used as a filler in dental materials, to increase their clinical performance, and as a drug-delivery system with therapeutic and/or preventive effects [3].

In this context, this review overviews the perspectives and applications of SNPs in nanomaterials and nanoformulations, subcategorized by their use in (1) dental materials, including dental specialties such as prothesis, restorative dentistry and orthodontics; and (2) other applications, which include SNPs use in formulations or biomaterials related to specialties such as endodontics, implantology and periodontics, for example, but that do not involve incorporating NPs into dental materials. Furthermore, the general characteristics and *in vitro* cellular biocompatibility of SNPs are discussed.

## 2. Silica nanoparticles (SNPs): Types and general biomedical applications

In the last few years, the growing scientific evolution in the field of nanotechnology has brought promising perspectives to modern medicine, allowing greater understanding and agility in the diagnosis and specific treatment of certain pathologies at the nanoscale (particle size of 10-100 nanometers) [5, 6].

There is a vast arsenal of nanomaterials that can be used in biomedical applications, offering, among other advantages, diagnostic capacities and the ability to signal, transport, and release drugs, genes, and proteins in a controlled and targeted way, overcoming the drawbacks of conventional systemic treatments [7, 5]. Examples of these nanomaterials are organic nanoparticles (NPs), such as dendrimers, liposomes, polymers, and micelles, among others, and inorganic NPs, such as those composed of iron oxide, graphene, gold, silver, and titanium, as well as SNPs [4, 8, 6].

Among the various types of NPs, SNPs have received particular attention for applications in dentistry and medical sciences in general [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. Different types of SNPs (Figure 1) can be synthesized using various raw materials and methods [20]. The Stöber synthesis method is the pioneer and is widely used [20, 21]. Other synthesis methods reported in the literature include the sol-gel process [22, 20, 7], liquid crystal templating [7], reverse microemulsion [7], evaporation-induced self-assembly [20], self-assembly and soft-template [23, 24], solution casting [25], aerosol-based synthesis, and dissolving reconstruction [26].

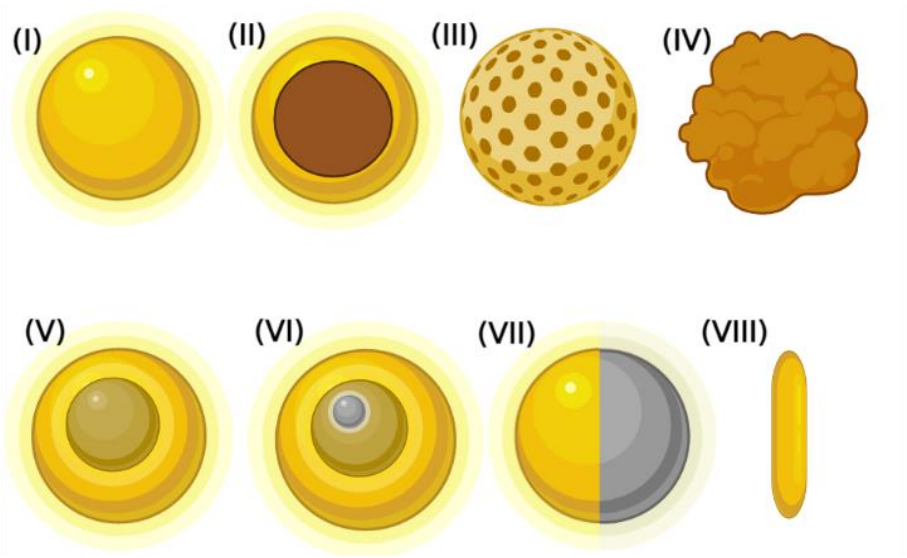


**Figure 1.** Illustration of the different types of SNPs: (I) non-porous (compact NPs, no pores); (II) hollow/rattle (large central cavity); (III) mesoporous (pores with variable and adjustable sizes); (IV) amorphous (shapeless); (V) core-shell (silica core or outer shell); (VI) yolk/shell (hybrid structures with a core inside a cavity of the same or different material); (VII) Janus (heterogeneous surface); and (VIII) rod-shaped SNPs (flattened, non-spherical NPs). Adaptaded of Mebert et al. [4]

Studies reported in the literature have investigated the use of SNPs for the treatment of many types of diseases, such as cancer [27, 28, 29, 30, 31,10], eye diseases [32, 33, 34], cardiovascular disease [35, 36, 37, 16], orthopedic problems [38, 39, 40, 18], degenerative conditions [41, 11], and endocrine disorders [42, 43]. There are also reports of the application of SNPs for inflammatory conditions [44, 45], infectious processes [46, 47, 48, 49, 50, 51, 52, 53], and for wound healing [54].

Use in conjunction with radioisotopes and metal ions constitutes the second major biomedical application of SNPs, in bioimaging (optical, magnetic resonance, and tomography), where they act as contrast agents with high selectivity and sensitivity [8, 6]. Furthermore, they can be used for photodynamic and photothermal therapy [6].

Among the SNPs, the most used are the mesoporous SNPs, due to their excellent physicochemical properties, uniform pore distribution (pore diameters between 2 and 50 nm), and *in vitro* and *in vivo* biocompatibility. These nanoparticles have the advantages of simple and economical synthesis, easy scale-up, and the possibility of surface modification with functional molecules to improve stability. They are suitable for use as nanocarriers for adsorbed/loaded hydrophilic and hydrophobic bioactive substances, having adjustable shape, size, and volume [55, 56, 57, 6, 41]. These characteristics favor their use in diagnostic and therapeutic nanosystems, providing controlled and efficient release of drugs responsive to physiological and/or photochemical stimuli at the target site [5, 58, 26]. Table 1 presents some of the main characteristics of these NPs, subdivided into families (M41S, Santa Barbara amorphous (SBA), organically modified silica (ORMOSIL), and hollow/rattle types), together with the main methods of synthesis.

Nanoparticles such as SNPs have been extensively studied and used in dentistry, notably in specialty dental biomaterials employed in restorative dentistry, endodontics, dental implants, orthodontics, periodontics, and prostheses. The main purposes of incorporating SNPs in dental materials are to improve their physicochemical properties and interaction with the oral cavity, as well as to act as nanocarriers of substances with therapeutic effects [2]. Their use has also been tested in other formulations as nanocarriers of substances such as antimicrobials and osteogenic substances, for example. This topic is discussed in the following sections.

**Table 1.** Different types of MSNs and their characteristics

| MSN family | MSN categories | Synthesis Method | Pore structure | Pore size (nm) | Main characteristics | References |
|---|---|---|---|---|---|---|
| **M41S** | MCM-41 | Stöber method | 2D hexagonal | 2.5 – 6 | M41S plus study for biomedical applications. They have good thermal stability, large surface area (700 $m^2 g^{-1}$), limited pore size distribution, and ability to conjugate with metal ions (improve MCM-41 properties for imaging features such as magnetic resonance imaging). | [26, 23, 59] |
| | MCM-48 | Modified Stöber method | 3D cubic | 2 – 5 | They have surface area greater than MCM-41 (1600 $m^2 g^{-1}$), improved chemical stability by pore branching, and greater efficiency in drug delivery due to the bicontinuous feature of mesoporous channels. | [26, 23, 20, 59] |
| | MCM-50 | - | Lamellar | 2 – 5 | They are composed of layers of silicates or aluminosilicates and a layer of surfactant molecules; they can be used as catalysts and sorbents in the manufacturing processes of other mesoporous solids. | [26, 20, 59] |
| **SBA** | SBA-11<br>SBA-12<br>SBA-15<br>SBA-16 | Requires a source of silica, acidic condition, and a directing agent of the organic structure | 3D cubic<br>3D hexagonal<br>2D hexagonal<br>Cubic cage-structured | 2.1 – 3.6<br>3.1<br>5 – 30<br>5 – 15 | They have large surface area (400 – 900 $m^2 g^{-1}$), large and adjustable pores, excellent thermal and mechanical hydro stability. Among SBAs, SBA-15 is widely employed as a drug delivery system. | [26, 23, 20, 59, 60] |
| **ORMOSIL** | - | O/A microemulsion and subsequent purification | - | - | Hybrid SNPs (particle size between 10 – 100 nm). The surface can be functionalized with different targeting groups compounds (thiol/hydroxyl, carboxyl group, etc.), they are biodegradable by cleavage of the silica-carbon bond, inert and transparent, and are used with fluorophores to generate optical images, stable over long periods of storage. | [26, 59] |

**Table 1.** Different types of MSNs and their characteristics (continuation)

| MSN family | MSN categories | Synthesis Method | Pore structure | Pore size (nm) | Main characteristics | References |
|---|---|---|---|---|---|---|
| **Hollow/ Rattle** | - | Dual template method with a hard template or soft template | - | - | They have interstitial hollow space and mesoporous shell with uniform morphology, low density, high specific area and pore volume, high efficiency of drug loading (1 g of drug per 1 g of silica) and controlled release, easy surface functionalization. | [26, 23, 59] |

MCM – Mobil composition of matter; SBA – Santa Barbara amorphous; ORMOSIL – Organically modified silica; O/A – Oil/water

## 3. Use of SNPs in dental materials by dental specialty

### 3.1. Prosthodontics

Poly(methyl)methacrylate (PMMA) is widely used for denture base prosthesis and provisional crowns. The material displays interesting features, such as easy handling, low cost, stability in the oral cavity and, acceptable esthetic [13]. However, PMMA has some drawbacks attributed to low mechanical properties that can cause denture base fractures [61]. Over the years, many studies have been conducted to improve PMMA properties by adding different types of NPs, such as SNPs [62, 13, 14, 15, 17, 63]. The SNPs exhibit high surface activity and solid interfacial interaction with the organic matrix [64] and at appropriate concentrations can enhance the general properties of the organic polymer [63].

*In vitro* studies have investigated the most appropriate SNP concentration in PMMA for denture base and provisional crowns, and most reports suggest the use of low [63; 17] over the high concentrations [65]. Balos et al. [62] observed that 0.23% of SNPs by volume in the liquid component of PMMA increased the maximum hardness and fracture toughness of the material. The results showed that higher concentrations (0.91%) caused extensive agglomeration, whereas no agglomeration was noted at 0.23% in the PMMA matrix, indicating that low concentrations are prone to a more homogenous distribution of the NPs in the polymer [62]. Others have found that incorporating low concentrations of SNPs (0.05 wt%) increased the flexural strength and elastic modulus of modified denture base resins, and the flexural strength decreased as NPs concentration increased [66]. Similarly, Sodagar et al. [64] reported that higher concentrations of SNPs and nano-$TiO_2$ NPs (0.5 and 1 wt%), combined or not, and incorporated into PMMA resin promoted the lowest flexural strength values. Comparing the NPs, SNPs showed higher flexural strength.

The propagation cracks resistance under load of a material, also known as fracture toughness, depends on the total energy absorbed to deform plastically [67]. Adding fillers to PMMA aims at raising resistance to crack propagation, rigidity, hardness, and durability of the inorganic NPs and the polymer flexibility [68]. Moreover, the uniform particle dispersion in the matrix [62] is crucial to avoid the development of stress concentration areas, impairing the physicochemical properties of the material [69].

Since the bonding between the reinforcement particles and the PMMA polymer affects the fracture toughness of the dental polymer materials [70], modifying the SNPs with triethoxyvinylsilane (T-Sil) has been suggested to improve the interface between the modified SNPs and the polymer chains. Authors have demonstrated that incorporating pure silica and T-

Sil (0.25; 0.5; 0.75; and 1%) NPs increased the fracture toughness of a provisional PMMA material [14] and that the lowest concentration (0.25%) improved the overall PMMA mechanical properties. Others synthesized and analyzed the mechanical properties of γ-methacryloxypropyltrimethoxysilane (MPS, 0.25; 0.5; 1; 5; 10; and 15%) on experimental nanosilica-reinforced PMMA and concluded that 1% of silanized NPs provided the highest flexural strength, flexural modulus, fracture toughness, and clinically acceptable color change [15].

The impact of other NPs, such as zirconium dioxide (nano-$ZrO_2$) and diamond (nano-D) incorporated in PMMA-based denture material, has also been evaluated and compared with the SNPs [63]. The addition of nano-$ZrO_2$, SNPs, and nano-D at any tested concentration (0, 0.5, 1.0, 2.5 5.0 wt%) increased hardness. Nonetheless, additions above 0.5 wt% of NPs, regardless of the type, increased surface roughness above the clinically acceptable value (0.2 μm) [71, 72] and could be unfavorable to the material properties. The authors agree that concentrations above 0.5% affect NPs uniform distribution within the resin matrix and NPs agglomeration rate [63]. As apprised, the homogeneous distribution, the degree of agglomeration, and the bonding of NPs with the polymer chains influence PMMA mechanical properties [62, 63, 17]. Moreover, the shape, size, and distribution of NPs also impact PMMA esthetic properties by influencing the material's translucence [63].

Since those materials could be placed in esthetic locations, investigating the effects of SNPs incorporation on their optical properties is also important. In a recent study, Gad et al. [17] incorporated nano-$ZrO_2$, SNPs, and nano-D particles (ranging from 15 – 150 nm) in acrylic PMMA powder to evaluate the material's translucency. As observed, the NPs nano-range size allowed them to fill the gaps between the polymer chains, producing a homogeneous PMMA/NPs matrix. Although the modified PMMA exhibited a homogeneous matrix, adding more than 0.5 wt% of nano-$ZrO_2$ or SNPs decreased the material's translucence. Furthermore, regardless of the concentration, nano-D adversely affected the denture esthetics due to the crystalline nature structure, grey color, and morphology of this nanoparticle [17]. The authors concluded that the proportion of the NPs should be minimum to guarantee uniform distribution without agglomerations. In this context, silanization could enhance the dispersion based on the chemical interactions between the OH– of the SNPs particles and the hydrolysable groups of the silane coupling agent. Thus, modifying the interface could increase the distance between NPs, allowing light to pass through. Although 0.25 wt% SNPs has been reported to affect the color of autopolymerizing PMMA, authors observed that color changes were not clinically perceptible [73].

Another important aspect of SNPs incorporation into PMMA is the antibacterial effects provided by its addition [53], since the potential for biofilm accumulation due to the surface porosities and food-retentive configuration is a common issue faced by patients wearing PMMA-based prostheses or provisional crowns and bridges. The biofilm formation, in turn, increases the possibility of *Candida albicans* (*C. albicans*) adhesion, eventually leading to denture stomatitis [63]. Therefore, adding NPs with antimicrobial potential is highly desirable. However, the required NPs concentration, type, and overall features to decrease the biofilm formation cannot be detrimental to the mechanical properties. In this context, the nano-filled-reinforced PMMA must exhibit an antimicrobial potential without interfering or, preferably, while increasing the mechanical aspects.

Alzayyat et al. [66] noted that incorporating low-concentration (0.05, 0.25, 0.5, and 1.0 wt%) silanized SNPs to PMMA denture-based material decreased *C. albicans* adhesion. The direct contact of SNPs with the cell may inhibit the budding process since the NPs can interrupt the fungus's metabolic pathway after penetrating its cell membrane. The lowest *C. albicans* adhesion were reported with 1.0% SNPs. On the other hand, the surface roughness of the PMMA material increased alongside the concentration, possibly due to a higher rate of NPs agglomeration. Nevertheless, surface roughness did not exceed the 0.2 μm threshold at which biofilm formation tends to occur [71, 72].

Also, the contact angle of the PMMA nano-filled material decreased, increasing the acrylic resin wettability, thus reducing the ability of *C. albicans* to adhere to a hydrophobic surface. Authors also reported that the SNPs increased the PMMA surface hardness, which may be related to the presence of hard particles uniformly distributed within the denture base material. The addition of 0.05 wt% SNPs to PMMA promoted the highest translucence, and the 1.0 wt% SNPs, the lowest. At 1.0 wt%, the absorbed light did not pass through due to the SNPs crystallinity (high opacity) [66].

Due to their high surface area and total pore volume, MSNs have also been used as fillers in PMMA resins. The pores facilitate the delivery of antimicrobial biomolecules, and the nanoscale morphology is an advantage for anchorage in the PMMA matrix [74, 75]. Lee et al. [75] developed an antimicrobial PMMA with different MSNs concentrations (0.5, 1, 2.5, and 5 wt%) with or without amphotericin B loading. According to the results, 5% of MSNs decreased the flexural strength and increased roughness of the PMMA material. The 2.5 and 5 wt% concentrations increased the surface free energy, leading to more hydrophilicity. Therefore, microbial species that have a hydrophobic profile, such as *C. albicans* and *Streptococcus oralis (S. oralis)* cannot easily adhere to the hydrophilic surface of the MSN-

incorporated PMMA. The long-term antimicrobial effect was only observed with 2.5 wt% of MSN-incorporated PMMA when amphotericin B was loaded.

Another study [76] incorporated MSNs loaded with silver-sulfadiazine (0.5, 1, 2.5, and 5 wt%) to PMMA material to promote long-term antimicrobial and rechargeable features. The authors observed that incorporating silver-sulfadiazine-MSN did not compromise the flexural strength of PMMA but increased the surface hardness. The immediate and long-term anti-adhesive effects against *C. albicans* and *S. oralis* were due to silver ion release and changes in the surface hydrophilicity. The enhanced mechanical properties and the microbial anti-adhesive effects immediately, in the long-term and after reloading the drug, suggest silver-sulfadiazine-MSNs into PMMA could be an effective approach for a new antimicrobial biomaterial.

#### *3.1.1. Coating agents*

Coating agents have been developed to reduce the surface roughness of digitally fabricated PMMA CAD/CAM prostheses [77]. The SNPs were added to these materials to decrease the surface energy, hindering biofilm adhesion [78]. Yoshizaki et al. [82] evaluated the appropriate SNPs content concentration in a methanol-based coating agent and found that 0.5 or 0.75 wt% provided higher hydrophobicity for the PMMA surface, which could clinically affect the biofilm adhesion.

Cheng et al. [80] developed a superhydrophobic coating for denture base materials composed of hydroxyl functionalized fluoropolymer (PHF), polyurethane oligomer (Pre-PU), and functionalized SNPs. Results show that this coating displayed appropriate translucency, not compromising the optical properties or clinical esthetics of the PMMA material. Besides, this coating exhibited excellent hydrophobicity inhibiting bacterial adhesion [80].

The advantage of the coating materials is changing the surface properties of denture-bases and provisional crowns and bridges [79] but without compromising PMMA bulk properties. Furthermore, investigations have demonstrated that SNPs added to the coating agents can hamper biofilm formation by changing PMMA surface hydrophobicity [80] and enhance the mechanical properties of these agents [81]. The results of a previous investigation demonstrate that reinforced-glaze-containing SNPs produced higher surface hardness and elastic modulus than conventional glaze. Besides, surface roughness did not significantly increase after aging for 6 and 12 months. The specimens were glaze coated with the denture glaze material prior to being polymerized for 90 s in a light polymerizing unit [81]. These results signal that the coating agent containing SNPs could resist over time. Similarly,

Yoshizaki et al. [82] noted that adding platinum and diamond NPs to the silica coating agents increased the coating layer durability after brushing-wear simulation.

In the long-term, the coating agent applied to the PMMA surface is expected to wear out due to the intraoral chemical and mechanical degradation. However, another advantage of the coating material is the possibility of reapplying it to the PMMA surface. In this context, incorporating SNPs into PMMA denture base materials seems to be a feasible alternative to extend the SNPs beneficial effects [80].

### 3.2 Restorative dentistry

For years, dentistry has benefited from materials to restore the aesthetics and function of the dental elements. The clinical performance and longevity of dental restorations are related to the procedure, the material used, and patient factors [83]. In this section, material-related factors will be discussed, such as physicochemical and optical properties, antimicrobial and remineralizing activity of restorative materials in dentistry, and the effect of SNPs on these parameters. In restorative dentistry, composite resin, adhesives, cements, and occlusion of dentinal tubules materials will be discussed.

#### *3.2.1. Composite resins*

Composite resins are materials widely used for restorative dentistry due to presenting, among other advantages, similar dental aesthetics, easy application, good mechanical properties, and biocompatibility [85, 101]. The longevity and clinical success of this restorative material is influenced by oral cavity conditions, such as pH fluctuations, masticatory force, and oral microbiome action. Therefore, adding reinforcing agents, such as SNPs, is a viable strategy to address these adversities [86].

The influence of different SNPs concentrations in experimental composite resins, alone [87] or combined with other substances, such as graphene [88], urchin-like hydroxyapatite (UHA) [89], amorphous calcium phosphate (ACP) [90], copper-doped mesoporous bioactive glass nanospheres (Cu-MBGN) [91] has been evaluated.

By increasing the SNPs concentration from 20 to 30 and 40%, Hosseinalipour et al. [87] observed a significant increase ($p < 0.05$) in fracture toughness and flexural strength. On the other hand, fracture toughness stabilizes as concentration increases from 40 to 50%, whereas flexural strength decreases dramatically ($p < 0.05$). This difference is due to the presence of SNPs clusters at 50%, which constitutes one of the types of intrinsic failures of experimental composites that directly affect their strength. The Vickers microhardness results were

significantly different for all groups ($p < 0.05$) and is concentration dependent. Therefore, the different SNPs concentrations directly influenced the mechanical properties evaluated [87].

Excellent physical, mechanical and rheological properties were obtained by adding different concentrations (0.0 – 4.0 wt.%) of silanized reduced graphene oxide/silica (S-rGO/$SiO_2$) in experimental nanocomposites. Increasing the S-rGO/$SiO_2$ concentration, the viscosity of the material decreased, consequently, improving nano-hardness, elastic modulus, degree of conversion, and crosslinking density of the nanocomposites, resulting in a compact and resistant composite structure, with high clinical practice performance [88]. A significant increase ($p < 0.05$) in compressive strength, flexural strength, and elastic modulus also resulted from incorporating 10% silanized UHA in composite resins with SNPs when compared with composites filled with silica alone (60 wt.%). The higher packing density provided by SPNs explains this result [89].

Positive results were also obtained by combining SNPs and Cu-MBGN. The values of degree of conversion, flexural strength, elastic modulus, and Vickers microhardness increased with SNPs addition to experimental Cu-MBGN composites. The specimens did not deteriorate with aging, showing statistically similar results at 1 and 28 days for flexural strength, flexural modulus, and Vickers microhardness [91].

On the other hand, the concentration of SNPs did not show a positive correlation with the flexural strength in the study by Marovic et al. [90]. Incorporating 5 and 10% of silanized SNPs into the experimental ACP composites, a direct precursor of hydroxyapatite (HA), did not significantly increase flexural strength ($p > 0.05$). Whereas 5% SNPs significantly increased the elastic modulus compared with the control, 10% SNPs did not interfere with this parameter. The authors argue that this may be due to the lower degree of conversion and crosslinking density of the polymeric network of the 5% SNPs group. Note that calcium and phosphate ions were released in the static and dynamic system after 7, 14, and 28 days in the 5 and 10% SNPs groups ($p < 0.05$) compared with the control, indicating the remineralizing potential of the experimental material [91].

Wang et al. [92] evaluated the influences on the mechanical properties of SNPs, nanofibers, and $SiO_2$ microparticles. Interestingly, experimental composites with only $SiO_2$ microparticles showed the lowest flexural strength, elastic modulus, and wear resistance, but greater polymerization shrinkage. By adding 5% SNPs or $SiO_2$ nanofibers to the material, the results improved significantly ($p < 0.05$), and the last was the most relevant. This is explained by the extra reinforcement provided by SNPs and $SiO_2$ nanofibers to the composites, providing improved mechanical properties and wear resistance [92].

In addition to the mechanical properties, the optical properties must also be considered, mainly due to their influence on the aesthetics of composite resins. Kim et al. [93] evaluated the effect of different $SiO_2$ nanofillers concentrations (1 – 6% by weight) on experimental composites translucency. The authors observed that, by increasing the concentrations, the contrast ratio tended to decrease, and was more significant with 3% or more of nanofillers ($p < 0.05$) when compared with the control (no nanofiller). This results from the translucency of composites depending on the size and distribution of particles in the resin matrix, therefore, composites with NPs show superior translucency and acceptable aesthetics [93].

The development of nanocomposites with antimicrobial activity to reduce the oral biofilm adhesion and pathogenicity, without altering the composite-tooth microbiome has been extensively studied [94]. In this context, research has been carried out to develop dental composite resins with high clinical performance and antimicrobial activity, by associating antimicrobial agents, metal ions, and quaternary ammonium salts [95].

Composite resins with MSNs (SBA-15) doped with chlorhexidine (CHX) (0, 3, 5, and 6.3 wt.%) (MNS-CHX) showed sustained and controlled release of CHX for 16 days, acting as a CHX reservoir to efficiently control cariogenic biofilm formation. This study showed antimicrobial activity against plankton and biofilm growth of *Lactobacillus casei* (*L. casei*) and *Streptococcus mutans* (*S. mutans*) with 5% MSN-CHX and individual CHX. Furthermore, the flexural strength and the elastic modulus increased ($p < 0.05$) and, even after one month of immersion in water, surface roughness was constant ($3.4 \pm 1.42$ μm) when compared with the direct CHX incorporation to the composites ($14.47 \pm 3.40$ μm) ($p < 0.05$), especially for the 5% group [96].

On the other hand, adding SNPs functionalized with quaternary ammonium compounds (QAC) has shown an effect against oral biofilm [86, 97], increasing the mechanical properties of experimental composites [86]. Rechmann et al. [97] observed *in situ* that quaternary ammonium silica dioxide (QASi) composites significantly ($p < 0.05$) reduced enamel demineralization compared with a conventional composite for 4 weeks, meaning the material has potential for reducing the occurrence of secondary caries. As for mechanical properties, Nikolaidis et al. [86] observed an increase in the gel-effect and in the degree of conversion of composites with QASi. When QASi was silanized, the elastic modulus, flexural and compressive strength increased by 74, 19, and 19%, respectively. These findings may be interesting for clinical dental practice.

MSNs doped with zinc (Zn-MSNs) were synthesized and the antimicrobial and mechanical effect on composite resins was investigated [95]. With increasing concentrations (0

– 15 wt.%), flexural strength, elastic modulus, compressive strength, and Vickers microhardness gradually increased when compared with the control (without Zn-MSN). Furthermore, antibacterial activity against *S. mutans* was observed in a concentration-dependent manner ($p < 0.05$), with an antibacterial rate of 100% at 15%. Therefore, composites with Zn-MSN may have improved mechanical properties and exhibit contact antimicrobial activity against oral biofilm adhesion [95].

### *3.2.2. Dental adhesives*

In addition to composite resins, incorporating inorganic filler particles can be a strategy to increase the physicochemical properties of the adhesive layer and the bond strength of dental adhesives, used mainly to ensure adequate adhesion of restorative composites to tooth structure and to avoid deficiencies that can lead to failure of restorations [98].

The influence of different concentrations of SNPs (0, 0.2, 0.5, 1, 2, and 5% wt.%) [97] on the mechanical properties of dental adhesives were investigated. At a concentration of 0.2%, the adhesives showed improved flexural strength and microsilage ($p < 0.05$). However, with SNPs increase, these properties decreased, probably due to the formation of SNPs agglomerates in the resin matrix and formation of cracks [97]. Mazloom-Jalali et al. [99] found similar results. The flexural strength, fracture toughness and micro-tensile bond strength (μTBS) showed the highest values ($p < 0.05$) with the addition of 0.2% (wt. %) silanized SNPs, whereas only the elastic modulus increased ($p > 0.05$) with the incorporation of higher SNPs concentrations. Also, at 0.2% the surface of the experimental adhesives showed less roughness [99].

The analysis by Alhenaki et al. [100] found a positive linear correlation between increasing SNPs concentrations and μTBS. Incorporating 15% (wt.%) of SNPs to experimental adhesives resulted in higher μTBS, that is, better bond strength to dentin, in addition to the acceptable degree of conversion.

Promising results were obtained when evaluating the antimicrobial effect of MSNs with octenidine dihydrochloride (OCT) in a commercial adhesive. An accelerated release was observed during the first five days of immersion of the material in phosphate-buffered saline (PBS) followed by a steady release up to 90 days, with a cumulative release of 0.43% w/w of the total OCT payload, indicating potential for long-term release. The material showed an antimicrobial effect by contact against the *S. mutans* biofilm on the adhesive surface with a reduction of 4.4 log ($p < 0.05$) in viable bacterial cells after 30 days. These findings reveal the potential of the material studied to increase the longevity of dental restorations and maybe also have other biomedical applications [84].

A pH-sensitive chlorhexidine-loaded MSN modified with poly-(lactic-*co*-glycolic acid) (CHX/MSN-PLGA) was incorporated into dentin adhesives to investigate the pH responsive behavior and CHX controlled release from the material [102]. After 30 days, the nanocarriers exhibited a constant and pH-dependent CHX release behavior. The 5% MSN/CHX-PLGA group showed significant ($p < 0.05$) reduction in *S. mutans* biofilm viability and higher μ-TBS (even after 6 months in artificial saliva with pH fluctuations), without significant change in the degree of conversion when compared with the control (no filler). These results are promising for the dentin-resin bond preservation considering the pH variations at this interface caused by the adhesion of the oral biofilm [102].

To increase confidence in the detection of recurrent caries lesions, restorative materials must have a radiopacity greater than that of the surrounding dental tissue [103]. Schulz et al. [103] developed radiopaque adhesives with $Ta_2O_5/SiO_2$ NPs (tantalum butoxide). The material showed similar radiopacity to dentin and enamel and excellent adhesive strength, being considered, therefore, as a dental adhesive with great potential for clinical use.

### *3.2.3. Dental cements*

As adhesives, dental cements play an important role in the clinical performance and longevity of restorations. In recent work, Rezvani et al. [104] concluded that the addition of 0.5 wt.% of SNPs in commercial RMGI powder (Fuji II LC) significantly increased ($p > 0.05$) the micro-shear bond strength (μSBS) over different storage periods (one day, one week, and one month) in an incubator. There was no statistical difference ($p > 0.05$) between the RMGI material and the control (no SNPs). It was suggested that the increase of μSBS over the time studied may have been related to incomplete maturation of the RMGI after 24 h of storage, while cement maturation would be complete after one month of aging [104].

The adhesive strength, durability, and failure types of resin-based materials bonded to yttrium-stabilized tetragonal zirconia polycrystalline (Y-TZP) ceramic were evaluated by Queiroz et al. [105]. The strength of the bond between the materials was influenced by the type of cement, as well as by surface treatment and aging. For the non-aged condition, treatment using air-abrasion with 45 μm alumina particles (GS), associated with Relyx U100 cement, presented a significantly higher shear strength (SBS) value ($p < 0.05$), while aging resulted in significant reductions of the values for all types of cement ($p < 0.05$). When silica-based nanofilm (GF) treatment was used, the resin cements showed micrometric defects such as microcracks and detachment at the edges of the specimens, presenting mixed failures, with and without aging. Although there was no significant difference between GS and GF in terms of

bond strength, the authors suggested the need to improve the deposition method for GF, in order to avoid surface detachment [105].

### *3.2.4. Dentin Hypersensitivity*

The MSNs are also applied to occlusion of dentinal tubules in severe dentinal hypersensitivity (DH) treatment, a common problem in clinical dentistry with an incidence of 4 to 74% [106, 107]. Previous studies have investigated the efficacy of MSNs associated with calcium [106, 108], hydroxyapatite (HA) [109], bioactive glass (BG) [110], and with electrophoretic deposition (EPD) [111] for this purpose.

*In vitro*, MSNs containing calcium oxide (NCMS) and 30% phosphoric acid reduced dentin permeability ($p < 0.05$), due to precipitation and release of calcium ions, and occluded dentinal tubules significantly ($p < 0.05$) when compared with a commercial desensitizer (Seal & Protect®) and BG [108]. Tian et al. [106] found similar results when comparing MSNs loaded with calcium phosphate ($Ca_3(PO_4)_2$-MSNs) with Green-Or™. The $Ca_3(PO_4)_2$-MSNs effectively occluded the dentinal tubules ($p < 0.05$), with a mean infiltration of 105 μm in depth, whereas, with Green-Or™, the depth was only 5 μm [106]. Although associating MSNs with calcium particles constitutes a potential application for the DH treatment given their rapid diffusion and superior obstructive capacity in the dentinal tubules when compared with commercial products [108], note that studies did not investigate the long-term effects of the oral cavity and daily practices on biomaterials on efficacy and longevity.

Similar results were obtained with HA NPs associated with MSNs (nHA-MSNs). When compared with the control (no treatment) and with the desensitizer NovaMin, the MSNs and nHA-MSNs groups completely occluded the dentinal tubules *in vitro* ($p < 0.05$) by forming precipitates. After exposure to 6% citric acid (1 min), these groups still occluded most tubules, which was more noticeable for nHA-MSNs, showing the membrane covering the dentin surface. This finding denotes the stability, acid resistance, presented by the MSN-based biomaterial and the potential of nHA-MSNs remaining in the root canal, since nHA can act as a $Ca_3(PO_4)_2$ reservoir. Furthermore, nHA-MSNs did not influence μTBS between dentin and adhesive ($p > 0,05$) [109].

The MSNs were also tested *in vitro* for their ability to occlude dentinal tubules for DH treatment and their effect on shear bond strength (SBS) after using the electrophoretic deposition (EPD) technique. Findings show effective occlusion of dentinal tubules after EPD treatment with MSNs and significant reduction in permeability ($p < 0.05$) after addition of 37% phosphoric acid. The MSNs infiltrated at approximately 7 – 8 μm into the tubules and were

tightly bound to their walls. This result suggests that the synthesized MSNs were effectively conducted and deposited in the tubules. The SBS showed no significant difference ($p > 0.05$) [111].

The use of bioactive glass-coated and silver-loaded mesoporous silica nanoparticles (Ag-BGN-MSN) at 1, 3, and 5% for occlusion of the dentinal tubule proved to be effective. The Ag-BGN-MSN 3% significantly ($p < 0.05$) occluded the tubules when compared with the control group (no treatment), BG, and MSN and a surface membrane in the dentin was formed even after acid treatment (6% citric acid) meaning that the nanomaterial has acid-resistant stability. The Ag-BGN-MSN, BG, and MSN groups showed significant difference in terms of μTBS ($p < 0.05$). Silver showed antimicrobial activity against *L. casei* [110].

### 3.3 Orthodontics

In the oral cavity, despite the numerous advantages, fixed orthodontic appliances modify the symbiotic microbiome by accumulating cariogenic biofilm that reduces the oral pH to acidic levels and, consequently, causes dental demineralization, appearance of white spot lesions around the orthodontic brackets, inflammation of the periodontium, and increase in the risk of caries, particularly in patients with poor oral hygiene during treatment [112, 113, 114].

Orthodontic adhesives are a reported risk factor for adhesion and bacterial multiplication since they have surface roughness and may have areas with gaps around the brackets at the adhesive-enamel interface. An alternative to this situation is incorporating antimicrobial drugs to orthodontic adhesives to prevent the accumulation of biofilm without negatively interfering with the mechanical properties of the material [115].

In this context, incorporating SNPs and silver NPs (0 ppm, 250 ppm, and 500 ppm) to experimental composite adhesives (ECA) was investigated [115]. As a result, incorporating silver NPs did not significantly ($p > 0.05$) affect the mechanical properties, such as SBS and the patterns of displacement when compared with two conventional adhesives (non-fluoride-releasing composite and a RMGI). The ECA with 250 ppm and 500 ppm of silver NPs showed less bacterial adhesion and antimicrobial activity against cariogenic streptococci in a concentration-dependent manner. Also, bacterial suspension with ECA (250 ppm and 500 ppm) showed slower bacterial growth than those containing conventional adhesives ($p > 0.05$). The ECA without silver NPs also has antimicrobial activity, but smaller than the adhesive containing silver nanoparticles. Despite that, no ECAs shows bacterial growth after 48h of bacterial incubation by inhibition halo tests in agar [115].

The SNPs were also investigated when added to elastomeric ligatures of fixed appliances that hold the orthodontic wire to the bracket, aiming to overcome the main limitations such as water absorption and tension relaxation and improve the mechanical properties of this material [25]. For successful orthodontic treatment, elastomeric ligatures must provide sufficient strength for corrective tooth movement with a minimal tension relaxation to avoid causing pain to the patient, and have acceptable elasticity and stretching to not fail during treatment period [25, 116]. Due to conditions of the oral cavity and changes in the molecular structure, elastomeric ligatures may show a reduced viscoelastic strength, which compromise tooth movements and the success of orthodontic treatment. Therefore, materials that have elastic memory effect are clinically desirable for stabilizing the degree of strength for a longer period [116].

To this end, thermoplastic polyurethane (TPU) elastomer with 1% SNPs was developed and its applicability in orthodontic ligatures was evaluated by mechanical and hydrophobicity tests. The results illustrate that adding SNPs increased the hydrophobicity (water absorption content of $0.70 \pm 0.38\%$) of TPU, improved elasticity and tension relaxation, and provided shape memory effect (100%), also, that the material showed the ability to fully recover its original forms. Furthermore, the sample showed an acceptable initial force for tooth movement of 4.87 N in a simulated oral environment (reference value between 2.67 and 6.60 N) and, therefore, this material can be successfully introduced into clinical practice [25].

Table 2 summarizes the main results presented in the articles discussed in this topic.

**Table 2.** Summary of the results of the articles covered in topic 3

| Prosthesis | | | | | | |
|---|---|---|---|---|---|---|
| **Material** | **NPs** | **Better %** | **Antibacterial activity** | **Mechanical and optical properties** | **Dentin hypersensitivity and remineralizing activity** | **References** |
| **PMMA** | T-Sil | 0.25 | - | ↑ fracture toughness and dynamic mechanical properties | - | [14] |
| | MPS | 1 | - | ↑ flexural strength, flexural modulus, and clinically acceptable color change | - | [15] |
| | SNPs | 0.5 | - | ↑ hardness and more than 0.5% ↑ surface roughness and ↓ translucence | - | [17; 63] |
| | SNPs | 0.23 | - | ↑ hardness and fracture toughness | - | [62] |
| | SNPs | 0.5 | - | ↑ flexural strength | - | [64] |
| | SNPs | 0.05 – 1 | ↓ *C. albicans* adhesion for slide count and direct culture methods | ↑ surface roughness and surface hardness, ↓ contact angle and translucency (concentration-dependent) | - | [66] |
| | MSNs with amphotericin B | 2.5 – 5 | ↓ *C. albicans* and *S. oralis* adhesion | ↓ flexural strength, ↑ surface roughness (5%) and hydrophobicity | - | [75] |
| | MSNs with silver-sulfadiazine | - | Immediate and long-term anti-adhesive effects against *C. albicans* and *S. oralis* | ↑ surface hardness | - | [76] |
| | SNPs | 0.8 | ↓ *S. aureus* adhesion | ↑ hydrophobicity | - | [80] |
| | SNPs | 2.5 – 10% | - | ↑ surface hardness and elastic modulus | - | [81] |
| | SNPs | 0.5 – 0.75 | - | ↑ hydrophobicity | - | [82] |

**Table 2.** Summary of the results of the articles covered in topic 3 (continuation)

| Restorative dentistry | | | | | | |
|---|---|---|---|---|---|---|
| **Material** | **NPs** | **Better %** | **Antibacterial activity** | **Mechanical properties** | **Dentin hypersensitivity and remineralizing activity** | **References** |
| **Composite resin** | SNPs | 30 – 40% | - | ↑ fracture toughness, flexural strength, and Vickers microhardness | - | [87] |
| | S-rGO-SNPs | 4 | - | ↑ elastic modulus, nano-hardness, degree of conversion, and crosslinking density | - | [88] |
| | UHA-SNPs | 10 | - | ↑ compressive strength, flexural strength, and elastic modulus | - | [89] |
| | ACP-SNPs | 5 | - | ↑ elastic modulus | ↑ release of calcium and phosphate ions after 7, 14, and 28 days (5 and 10%) | [90] |
| | Cu-MBGN | 1 and 5 | - | ↑ degree of conversion, flexural strength, flexural modulus, and Vickers microhardness | - | [91] |
| | SNPs | 5 | - | ↑ flexural strength, flexural modulus, and wear resistance | - | [92] |
| | $SiO_2$ nanofiller | 1 – 6 | - | ↓ contrast ratio being more significant with 3% or more of the material | - | [93] |
| | Zn-MSN | 15 | Effective against *S. mutans* (surface plate test and gram staining method) | ↑ flexural strength, flexural modulus, compressive strength, and Vickers microhardness | - | [95] |
| | CHX-MSN | 5 | Effective against plankton and biofilm growth of *L. casei* and *S. mutans* | ↑ flexural strength, flexural modulus, and constant surface roughness (after 1 month in water) | - | [96] |
| | QASi | | - | - | ↓ enamel demineralization | [97] |

**Table 2.** Summary of the results of the articles covered in topic 3 (continuation)

| Restorative dentistry | | | | | | |
|---|---|---|---|---|---|---|
| **Material** | **NPs** | **Better %** | **Antibacterial activity** | **Mechanical properties** | **Dentin hypersensitivity and remineralizing activity** | **References** |
| **Dental adhesives** | OCT-SNPs | 10% | Effective against biofilm of *S. mutans* | - | - | [84] |
| | SNPs | 0.2 | - | ↑ flexural strength and microsilage | - | [98] |
| | Silanized SNPs | 0.2 | - | ↑ flexural strength, fracture toughness and μTBS. Also, showed less roughness and few adhered fragments | - | [99] |
| | SNPs | 15 | - | ↑ μTBS and acceptable degree of conversion | - | [100] |
| | MSN/CHX-PLGA | 5% | Effective against biofilm of *S. mutans* | ↑ μTBS | - | [102] |
| | $Ta_2O_5/SiO_2$ NPs | 20% | - | Radiopacity comparable to that of enamel | - | [103] |
| **Dental cements** | SNPs in commercial RMGI | 0.5% | - | ↑ μSBS | - | [104] |
| | Relyx U100 cement | - | - | GS ↑ SBS and GF treatment showed micrometric defects | - | [105] |
| **Dentin hypersensitivity** | $Ca_3(PO_4)_2$-MSNs | - | - | - | Occluded the dentinal tubules and formed a deeper seal which penetrated about 105 μm deep | [106] |
| | NCMS paste | - | - | - | Formed a $CaHPO_4 \cdot 2H_2O$ precipitation with a 100 μm depth and ↓ dentin permeability | [108] |
| | nHA-MSN | - | - | Did not influence μTBS between dentin/adhesive | Completely occluded the dentinal tubules by forming precipitates | [109] |

**Table 2.** Summary of the results of the articles covered in topic 3 (continuation)

| Restorative dentistry | | | | | | |
|---|---|---|---|---|---|---|
| **Material** | **NPs** | **Better %** | **Antibacterial activity** | **Mechanical properties** | **Dentin hypersensitivity and remineralizing activity** | **References** |
| **Dentin hypersensitivity** | Ag-BGN-MSN | 3% | ↓ optical density of *L. casei* growth | μTBS similar to the control, but significantly different to the BG and MSN group | Occluded the dentinal tubule and formed a membrane-like layer | [110] |
| | MSNs with EPD treatment | - | - | No significant difference for SBS | Occluded the dentinal tubule with infiltration of 7 – 8 μm and tightly associated with the tubular inwalls | [111] |
| **Orthodontics** | | | | | | |
| **Material** | **NPs** | **Better %** | **Antibacterial activity** | **Mechanical properties** | **Dentin hypersensitivity and remineralizing activity** | **References** |
| **Composite adhesives** | Silver SNPs | 250 and 500 ppm | ↓ optical density of *S. mutans* and *S. sobrinus*. No inhibition zones observed after 48 h | Addition of silver SNPs ↑ surface roughness. No significant difference in SBS. | - | [25] |
| **TPU** | SNPs | 1% | - | ↑ hydrophobicity, improved elasticity, tension relaxation, and provided shape memory effect | - | [115] |

PMMA - poly(methyl)methacrylate; SNPs – silica nanoparticle; MSNs - Mesoporous silica nanoparticle; T-Sil - silica nanoparticle modified with triethoxyvinylsilane; MPS - γ-methacryloxypropyltrimethoxysilane; UHA-SNPs – urchin-like hudroxyapatite; S-rGO-SNPs – silanized reduced graphene oxide/silica; Cu-MBGN – copper-doped MSN bioactive glass nanospheres; - SNPs-ACP – amorphous calcium phosphate with silica nanoparticle; CHX-MSN –chlorhexidine-loaded mesoporous silica nanoparticle; QASi – quartenary ammonium compounds; Zn-MSN – zinc-loaded mesoporous silica nanoparticle; OCT-SNPs – octenidine dihydrochloride-loaded silica nanoparticle; MSN/CHX-PLGA - chlorhexidine-loaded mesoporous silica nanoparticle modified with poly-(latic-*co*-glycolic acid); NCMS – calcium oxide-loaded mesoporous silica nanoparticle; EPD – electrophoretic deposition; Ag-BGN-MSN - silver-doped bioactive glass/mesoporous silica nanoparticle; nHA-MSN - nanohydroxyapatite/mesoporous silica nanoparticle; TPU - Thermoplastic Polyurethane Elastomer.

**4. Other applications of SNPs**

In addition to the application of SNPs in dental materials, they can also be used for other purposes in dentistry, performing anti-inflammatory, antimicrobial and osteogenic. In addition, improves the mechanical properties of aesthetic and functional materials used extraorally, such as maxillofacial prosthesis [9, 12, 19, 117, 118, 119].

The anti-inflammatory effect of baicalin (BA) and baicalin (BE) encapsulated in amine-modified MSNs (nano-BA and nano-BE, respectively) was investigated in human gingival epithelial cells (hGECs) treated with interleukin 1β (IL-1β) through the gene expression of pro-inflammatory cytokines. The experimental nanoparticles were internalized by hGECs and retained for approximately 24 h without cellular morphological change. The substances showed a prolonged release profile, for up to 9 days (accumulated % of 89.9% and 41.7% for nano-BA and Nano-BE, respectively). While nano-BE effectively regulated the expression of IL-6 and IL-8 stimulated by IL-1β at 50 μg/mL ($p < 0.05$) and 25 μg/mL ($p < 0.01$), respectively, while nano-BA did not significantly affect its expression [9].

Pouroutzidou et al. [19] investigated the efficiency of moxifloxacin (MOX) loading, delivery and regenerative activity of human periodontal ligament fibroblasts (hPDLFs) from MSNs doped with calcium (Ca), magnesium (Mg) and strontium (Sr). In general, the MOX loading rate and controlled release (after 7 days) was more expressive for the Ca groups. Except for the Sr group (more than MOX 90% was released after ~2 days), release lasted approximately 7 days and the rates ranged from 26 to 94%. A possible explanation for this finding is the adsorption of MOX on the surface of the Ca group mesopores, an alkaline earth metal (II), by electrostatic bonds to carboxylic groups, which can influence both charge and release kinetics. In addition, the hydroxycarbonate apatite (HCA) formation was observed on the experimental specimen's surface for the Ca and Mg groups, indicating the potential of this study for future application in regeneration strategies of periodontal tissues [19].

In **endodontics**, the application of antimicrobial irrigating substances is desirable. An irrigating solution of silver NPs-encapsulated porous silica shell ($AgNPs/SiO_2$) showed effective antimicrobial activity ($p < 0.05$) against monospecies (*Enterococcus faecalis)* and multispecies (*Fusobacterium nucleatum*, *Actinomyces naeslundii*, *E. faecalis*, *Streptococcus sanguinis* and *Streptococcus sobrinus*) biofilms, even after 168 h of incubation, while the conventional treatment with ethylenediamine tetraacetic acid (EDTA) and sodium hypochlorite (NaOCl) showed reduced activity in ~ 48 h [120].

NPs was also tested in **implantology**, for example, as components of implant cleaning creams in order to remove organic residues and reduce the abrasion often caused by the use of conventional toothpastes [118]. Furthermore, they have been tested in the production of biomaterials for use in bone grafts as bone substitutes [117].

Al-Hashedi et al. [118] developed an organic-free implant-paste based on two-dimensional nanocrystalline magnesium phosphate ($Mg_3(PO_4)_{2)}$ gel and hydrated SNPs (20–30% w/w), and investigated its effects on reduced bacterial adhesion to implant surface, organic contaminants removal and reduced abrasion caused in titanium. The result was compared with two conventional toothpastes - Colgate Total (Colgate-Palmolive, New York) and Blue M (Bluem® Zwolle, Netherlands).

Conventional toothpastes contain abrasives, surfactants, organic thickeners and antimicrobials substances [118]. The presence of organic compounds, as well as other compounds such as fluorine ions and abrasives, can affect surface stability, chemical properties, cause corrosion, increase roughness and cause significant damage to the implant surface [121, 122]. As a result, the paste containing $Mg_3(PO_4)_2$ and SNPs was shown to be superior to conventional toothpastes in terms of abrasion protection and reduction of organic contaminants, as carbon ($p< 0,05$). For the number of bacteria, both conventional toothpastes and the cream developed in the study showed significant bacterial removal ($p< 0,05$) [118].

In the **osteogenesis area** (for bone grafts), nanomaterials have also been studied, such as NanoBones ® (made up of nanocrystalline HA in a porous matrix of nanostructured silica gel). This technology has demonstrated osteoconductive and biomimetic properties, promoting early osteogenesis around the material, and could be an option for bone substitute biomaterial [117]. In addition, SNPs loaded with carboxymethyl chitosan and clindamycin were developed and tested for their ability to control the release of the actives, to promote antimicrobial activity against *S. sanguinis* and to influence of oxygen plasma treatment of NPs, and to enhance the surface hydrophilicity. Clindamycin was incorporated into NPs (MCM-41), treated with plasma, and then incorporated into carboxymethyl chitosan. As a result, the hydrogel formed containing the nanoparticles showed, *in vitro*, a slow degradation rate, antimicrobial activity for 14 days, in addition to induction of mineralization of human mesenchymal stem cells. Such results point to a possible use of these NPs in the treatment of intraoral bone infections [123].

In the **maxillofacial prosthesis area**, used to assist in the aesthetic reconstruction of congenital or acquired facial defects due to trauma, cancer or other conditions that affect the head and neck region [124], the use of SNPs has been tested, for example, aiming at improvements in material properties [12, 65]. Previously, a silicone elastomer, used in

maxillofacial prostheses in order to mimic soft tissues, using different concentrations of SNPs, showed significant improvements in parameters such as tear and tensile strength [12]. The addition of SNPs and titanium dioxide improved the mechanical properties, such as tear strength, when compared to a control group (elastomer only) [119].

**5. Biocompatibility and toxicity of SNPs**

The expansion of SNPs production, commercialization, and use of for the most diverse applications have increased the intentional exposure of human beings to this substance. In this context, SNPs must be biocompatible *in vitro* and *in vivo* and not cause deleterious effects to the organism [23, 125].

Studies to date are inconclusive and, in some cases, controversial. Cohesive data on pharmacokinetics is lacking, considering the different variables that interfere with biological effects [4, 6, 8]. On the other hand, the adverse influence of SNPs production method and physicochemical properties, especially the nanometric particle size on human safety and health is cited [23, 125].

The particle size is very important to measure the biocompatibility of nanomaterials and nanoformulations. The basis of this hypothesis is that NPs with smaller sizes have greater cytotoxicity since they diffuse more rapidly to tissues. However, there are no consensus on the effect of size on toxicity [23]. Table 3 shows results from SNPs biocompatibility in dentistry, associating the NPs size, time, and dose of exposure with relative cytotoxicity. Despite the different SNPs sizes studied, no case showed cytotoxicity.

Although not well established, the main mechanism involved in the cytotoxicity of SNPs is believed to be the induction of oxidative stress (generation of reactive oxygen species – ROS), that can lead to cell necrosis or apoptosis; the shape, particle, and pore size in turn influence the level of intracellular ROS and, consequently, toxicity [8, 125].

The systematic review by Murugadoss et al. [125] found that *in vitro*, SNPs showed cytotoxicity and genotoxicity in different cell lines and induced ROS, apoptosis, and autophagy (intrinsic or mitochondrial pathway) in a size and dose-dependent manner. Furthermore, it induced ROS and adversely affected the cardiovascular system, resulting in platelet aggregation, endothelial dysfunction with pro-inflammatory signs, and red blood cell hemolysis. *In vivo*, rats and mice were exposed to SNPs by the administration routes – oral, inhalation, topical, and parenteral, single dose or long-term. The administration route and the SNPs physicochemical properties influenced the toxicokinetics with adverse effects mainly on the lungs, kidneys, liver, and brain. Interestingly, toxic effects occurred in animals exposed to

a single dose whereas those exposed chronically showed no local or systemic toxicity. The authors conclude that the correlation between the findings remains unestablished due to the different methodologies used [125].

Besides, adverse immune reactions and production of inflammatory mediators were also observed, depending on the size, dose, and surface charge of the nanoparticles, and the type of immune cell. A lower ROS induction in macrophages is observed with NPs with larger pores size (> 30 nm), in addition, these NPs do not show pro-inflammatory effects *in vitro* and *in vivo* [8].

## 6. Concluding remarks

In conclusion, studies show that SNPs are promising for dental applications. In general, SNPs are effective in nano-carrying drugs, such as antimicrobials [66, 75, 76, 84, 95, 96, 102, 110, 115, 120, 123], anti-inflammatories [9], osteogenic substances [117], tubular occlusion agents [106, 108; 109, 110] and coating for dentures [80, 81, 82], in nanomaterials and nanoformulations, intra- or extraorally.

They also improve the physicochemical properties of dental biomaterials. Based on the restorative materials characteristics and their clinical application, different concentrations of filler particles, associated or not with other substances, were added to PMMA, adhesives, dental cements, and composite resins. In general, at low concentrations, the general properties of PMMA, dental cements, and adhesives, such as flexural strength, flexural modulus, microtensile bond strength (μTBS), and microshear bond strength (μSBS) [15, 64, 66, 98, 99, 102, 103]. Increasing the SNPs content, associated or not with other substances, reduces the general properties of these materials, probably due to the heterogeneous distribution of nanoparticles and nanoclusters formation. On the other hand, for composite resins, intermediate and high concentrations of SNPs increased general properties, such as fracture toughness, flexural strength, degree of conversion, and flexural modulus [87, 88, 89, 95, 96].

Although SNPs have been shown to be promising nanocarriers in dentistry, further studies are needed to elucidate their long-term physicochemical and biological effects in the oral cavity, considering variations in the oral environment, such as pH fluctuations, masticatory force, and the multispecies biofilms presence. Further investigation is also needed on the long-term release profile of substances associated with SNPs and their biocompatibility. Generally, studies focus on evaluating physicochemical properties and short-term nanocarriers of substances. Note that few *in vitro* studies address biocompatibility in oral cells and, consequently, *in situ* evidence to support SNPs clinical safety is lacking.

Silica is considered a non-cytotoxic substance for the body and has applications in several areas. The literature indicates that SNPs biocompatibility may be associated with especially the particle size. The basis of this hypothesis is that NPs with smaller sizes have greater cytotoxicity since they diffuse more rapidly to tissues [23, 125]. Each nanoparticle (for example, mesoporous, core-shell SNPs) has unique characteristics and can cause a unique effect. Therefore, knowing which type of SNPs is being studied, with or without association with other substances, and which synthesis method is used, can lead to important information about the substance release profile and biological activity, such as cytotoxicity. In this context, the literature had a gap regarding this information and the possible mechanisms related to SNPs biological effects. Associated with this, NPs shape, size, and distribution also impact the aesthetic properties of restorative materials [63], which is rarely addressed in studies.

In conclusion, SNPs can function as fillers for composite resins and other materials, sustaining release of substances with different biological activities, especially antimicrobial activity. However, for this application to happen, further studies to verify their long-term biological and physicochemical properties of dental materials need to be carried out. Furthermore, in situ studies are essential to verify the properties of materials added with SNPs under the conditions of the oral cavity.

**Table 3.** Summary of studies on SNP toxicity

| Material type | SNPs type | Cell or animal species studied | Particle*/poro size• (nm) | Exposure dose and exposure time | Experimental methods | Results | Reference |
|---|---|---|---|---|---|---|---|
| - | MSNs – MCM-41 (nano-BE and nano-BA) | Human gingival epithelial cells | 367 ± 94* | 12.5, 25, 50, 100, and 200 μg $mL^{-1}$ 24 hours rated | CCK-8 | Biocompatible * | [9] |
| **Superhydrophobic coating material for denture surface** | Not specified | Dental pulp cells | Not informed | 0.5, 0.8, or 1.0% 24, 48, and 72 hours rated | MTT | Biocompatible | [16] |
| - | MSN (MSN Mg- and Sr-doped NPs for Moxifloxacin) | Periodontal ligament fibroblasts | 151.9 to 534.7 | 60, 125, and 250 μg $mL^{-1}$ 24 hours rated | MTT | Biocompatible | [19] |
| **Commercial bone cements (CMW Smart GHV and Simplex P) dopped with gentamicin** | MSN | 3T3 mouse fibroblasts cells | 100 – 400 nm in length and 100 nm in diameter* | 8.15, 5.44 or 2.72% of MSNs and 2.72% of drug 24 hours rated | MMT | Biocompatible | [74] |
| **PMMA** | MSN | Immortalized human oral keratinocytes | 85.2 ± 7.7* 3.54 ± 0.41• | 0, 0.5, 1, 2.5 or 5% 24 hours rated | WST | Biocompatible | [75] |
| **PMMA** | MSN (AgMSNs) | Immortalized human oral keratinocytes | 85.2 ± 7.7* 3.54 ± 0.41 to 3.50 ± 0.31• | 0.5, 1, 2.5, or 5% 24 hours rated | WST | Biocompatible | [76] |
| **Dental Adhesive** | Octenidine dihydrochloride - loaded MSN | Human gingival fibroblasts | 424 ± 75* | 0, 5, 15 or 50ng/$mL^{-1}$ 24 h rated | WST | Biocompatible | [84] |
| **Composite resins** | MSN (Zn-MSN) | Osteoblasts MC3T3-E1 | 138* | 0, 2, 5, 10 and 15% 1, 3, and 5 days rated | Live/Dead staining and MTT | Biocompatible | [95] |

**Table 3.** Summary of studies on SNP toxicity (continuation)

| Material type | SNPs type | Cell or animal species studied | Particle*/poro size• (nm) | Exposure dose and exposure time | Experimental methods | Results | Reference |
|---|---|---|---|---|---|---|---|
| **Dental Adhesive** | CHX/MSN-PLGA | Dental pulp stem cells | ~ 78* | 5 or 10%<br>Exposure time uninformed | MTT | Biocompatible | [102] |
| **Composite resins** | MSN (nHA@MSN) | Human dental pulp cells | 150 – 350 | 0, 10, 20, 40, 80, 160, 320, 640 μg $mL^{-1}$<br>24 hours rated | CCK-8 | Biocompatible | [109] |
| **Composite resins** | MSN (Ag-BGN@MSN) | | 100 – 350* | 1, 3 and 5%<br>24, 48 and 72 h rated | MTT | Biocompatible | [110] |
| **Orthodontic brackets** | Zeolite-zinc oxide nanoparticles | HuGu cells | 52.85* | 0,5, 1, and $2 \times 10^{-4}$ g $L^{-1}$<br>24 hours rated | MTT and SRB | Biocompatible | [113] |
| **Clindamycin-releasing mesoporous silica/carboxymethyl chitosan composite hydrogels** | Clindamycin-loaded MCM-41 | Human mesenchymal stem cells | 98 ± 21 μm• | Exposure dose not informed<br>1, 3, and 7 days rated | MTT | Biocompatible | [123] |

Experimental methods: MTT – [3-(4,5-dimethylthyazol-2-yl)-2,5-diphenyltetrazolium] bromide, WST-1 – water-soluble tetrazolium, CCK-8 – Cell Counting Kit-8; LDH – Pierce lactate dehydrogenase.
*At high concentrations (200 μg $mL^{-1}$), nano-BE reduced cell viability, but biocompatibility was confirmed by the stability of the LDH test

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**Conflict of interest**

The authors declare no conflict of interest.

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**Figure 1 caption:** Illustration of the different types of SNPs: (I) non-porous (compact NPs, no pores); (II) hollow/rattle (large central cavity); (III) mesoporous (pores with variable and adjustable sizes); (IV) amorphous (shapeless); (V) core-shell (silica core or outer shell); (VI) yolk/shell (hybrid structures with a movable core inside a hollow shell of the same or different material); (VII) Janus (heterogeneous surface); and (VIII) rod-shaped SNPs (flattened, non-spherical NPs). Adaptaded of Mebert et al. [4]

## 2.2 Artigo: Development, characterization and antimicrobial activity of multilayer silica nanoparticles with chlorhexidine incorporated into dental composites

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## ABSTRACT

**Objectives:** This study aimed to develop and characterize an experimental composite containing silica nanoparticles (SNPs) loaded with chlorhexidine (CHX) at different concentrations. **Methods:** Seven experimental composite groups were produced using an organic matrix of BisGMA/TEGDMA and SNPs with or without CHX (0, 10, 20, or 30 wt%). The physicochemical properties of the experimental materials were evaluated considering the degree of conversion ($n$ = 3), elastic modulus and flexural strength ($n$ = 10), surface roughness ($n$ = 10), and post-gel volumetric shrinkage ($n$ = 5). The agar diffusion method ($n$ = 5) was used to test antibacterial activity against *Streptococcus mutans*, *Streptococcus mitis*, and *Streptococcus gordonii*. In addition, the action of the composites in inhibition of biofilm formation ($n$ = 6) was evaluated against *Streptococcus mutans*. The data for degree of conversion, surface roughness, and post-gel volumetric shrinkage were analyzed by one-way ANOVA/Tukey's test (α = 5%), while two-way ANOVA/Tukey's test (α = 5%) was used for the other variables. **Results:** The degree of conversion ranged from 69.3 to 76.3, with no statistical difference between the groups ($p$ > 0.05). The surface roughness increased in a concentration-dependent manner, with no statistical difference between the groups with and without CHX. In general, groups with CHX showed the highest post-gel volumetric shrinkage values ($p$ < 0.05). Higher flexural strength and elastic modulus values were observed for the groups with CHX, especially at 30 wt%. Storage in distilled water significantly affected the results for this variable. The composites containing CHX formed zones of inhibition for all the bacteria tested, in a concentration-dependent manner ($p$ < 0.05), and reduced the adhesion of the *Streptococcus mutans* biofilm at 24 h and 72 h, especially at the earlier time. The 30% CHX-SNPs group showed the greatest CFU/mL reduction ($p$ < 0.05) at both times. **Conclusions:** The nanoparticles affected the physicochemical properties of the composite, except for the degree of conversion, and the nanomaterial with CHX showed antimicrobial activity against *Streptococcus*. Therefore, this initial study is a step forward in the synthesis of experimental composites with improved performance using CHX-SNPs.


**Keywords:** Composite resin. Nanoparticle. Silica. Chlorhexidine. Dentistry.

### 1. Introduction

Among the many applications of nanotechnology in dentistry, its use for the production of dental materials such as nanocomposites can be highlighted. Different types of nanoparticles (NPs) can be added to dental composites, such as silica NPs (SNPs). SNPs have a range of

properties that favor their applications, such as excellent physicochemical characteristics, biocompatibility, simple and economical synthesis, adjustable morphology, and the possibility of modifications and surface functionalization, among others (Mebert et al., 2017; Alkahtani, 2018; Li et al., 2019; Li, 2020). These features favor their use to optimize optical, physicochemical, and mechanical properties, as well as morphophysiology and dental aesthetics. They can be used to obtain therapeutic effects when employed as nanocarriers for the sustained release of active substances during the diagnosis or treatment of certain clinical conditions (Gonçalves, 2018; Manzano & Vallet-Regí, 2018; Chen et al., 2018; Aquib et al., 2019; Jandt & Watts, 2020).

Considering the necessity for replacement of direct restorations, the complex and dynamic nature of the oral microbiome, and the negative impact of dysbiosis in restorative dental procedures, the association of nanocomposites with antimicrobial agents is a viable prophylactic strategy for recurrent oral infectious complications (Belibasakis et al., 2019; Boaro et al., 2019; Jandt & Watts, 2020).

Several antimicrobials have been tested in dental nanocomposites, including chlorhexidine (CHX), a broad-spectrum bisbiguanide antiseptic that is widely used in clinical dental practice for the prevention and treatment of oral infections (Raszewski et al., 2019; Zhang et al., 2014). Ideally, nanocomposites containing CHX should present a sustained release profile of the antiseptic agent at the tooth/nanocomposite interface, reducing bacterial adhesion on the resin material, without affecting the homeostasis and viability of oral cells (Boaro et al., 2019).

The *layer by layer* technique (LbL) has great potential in the sustained delivery of drugs, since it can create multiple nanostructures that act as a drug reservoir (Al Thaher et al., 2018). A recent study investigated the incorporation of gentamicin-loaded SNPs (GEN-SNPs), synthesized by the LbL technique, into polymethylmethacrylate (PMMA) bone cements, for prophylaxis of prosthetic joint infection (PJI) (Al Thaher et al., 2018). The GEN-SNPs showed prolonged and superior antimicrobial activity, compared to the free drug, as well as cytocompatibility, good diffusivity, and preservation of the properties of the bone cements tested, and could potentially be used in the prevention or treatment of PJI.

Aiming at obtaining a restorative nanomaterial with antimicrobial activity and improved mechanical properties, it was proposed to develop and characterize an experimental composite with different concentrations of CHX-loaded SNPs (CHX-SNPs) synthesized using the LbL method. To this end, the null hypotheses tested were (I) that the incorporation of CHX-SNPs would not influence the physicochemical properties of the experimental composite, and (II) that

the incorporation of CHX-SNPs in the experimental composite would not show antimicrobial activity.

## 2. Materials and methods

The experimental design of this study was divided into three stages: (I) preparation of the experimental composites, (II) evaluation of their physicochemical properties, and (III) evaluation of the antimicrobial activity of the experimental composites (Figure 1).

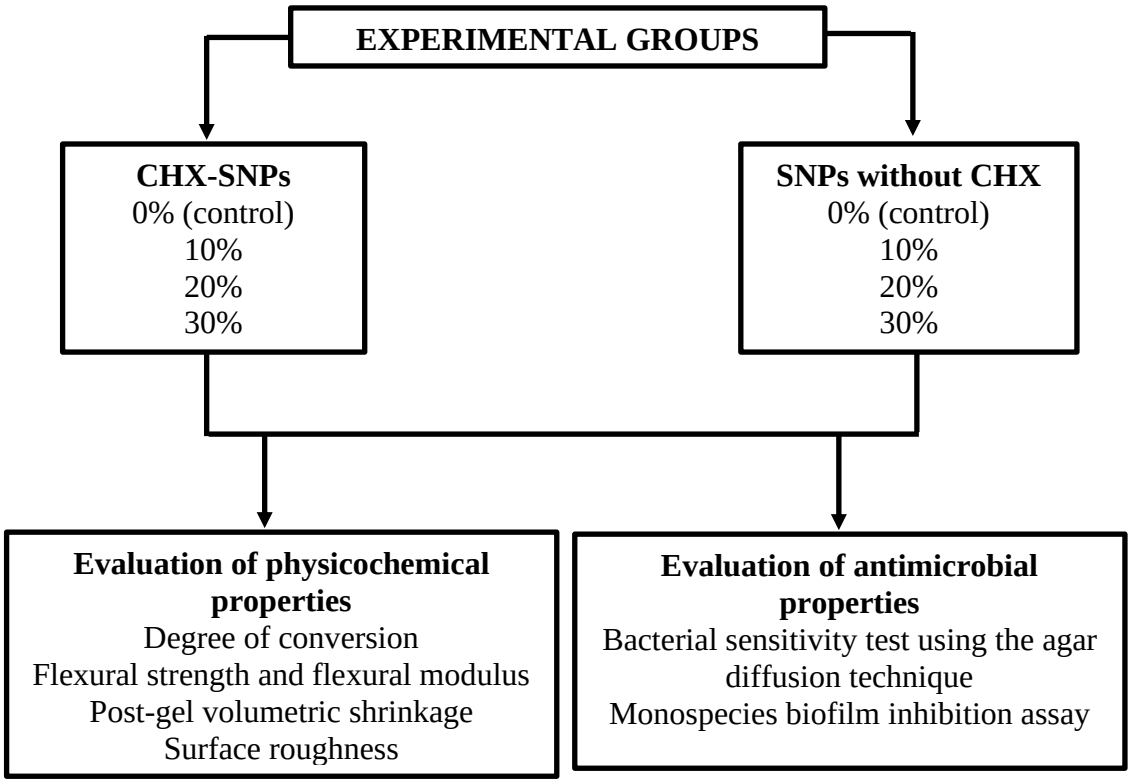


**Figure 1.** Experimental design of the study.

### 2.1. Experimental dental composites preparation

Seven groups of experimental composites were prepared, using a resin matrix based on Bis-GMA (Bisphenol A bis(2-hydroxy-3-methacryloxypropyl) ether) (Esstech, Essington, Pennsylvania, USA) and TEGDMA (triethyleneglycol dimethacrylate) (Esstech, Essington, Pennsylvania, USA), in equal weight proportions. The photoinitiator system used was composed of 1% (w/w) DMAEMA (2-(dimethylamino) ethyl methacrylate) (98%, Sigma-Aldrich, Steinheim, Germany) and 0.8% (w/w) camphorquinone (97%, Sigma-Aldrich, Steinheim, Germany) (Boaro et al., 2019). SNPs with the presence or absence of CHX, synthesized and previously characterized by Al Thaher et al. (2021), were added to the composites in proportions of 0 (control group), 10, 20, or 30% (w/w), in a dark room with

yellow lighting to avoid polymerization during the manufacturing process, with mixing for 3 min using an automatic vacuum mixer (SpeedMixer DAC 150 FVZ, Hauschild) at 3500 rpm.

The samples were light-cured on top for 40 s, using an LED device (VALO Cordless, Ultradent Products Inc., South Jordan, UT, USA) operating in a standard mode (1000 mW/cm$^2$), between two glass slides in a silicone mold. The specimens were made 24 h before use and were stored dry at 37 °C in hermetically closed dark flasks until the time of use.

### 2.2. Evaluation of physicochemical properties

#### 2.2.1. Degree of conversion

Fourier transform infrared (FTIR) spectroscopy (Vertex 70, Bruker, Germany) was used for analysis of the degree of conversion. The unpolymerized specimens ($n$ = 3, 7 mm diameter x 1 mm thick) were placed into the FTIR spectrophotometer so that the laser beam passed through the center of the specimen. Spectra were recorded for the uncured composites and for the samples immediately after photopolymerization. Determination was made of the area under the peak at 6165 cm$^{-1}$, corresponding to the vinyl bond (Tantbirojn et al., 2011, Boaro et al., 2019). The degree of conversion was calculated as a function of the area of the vinyl peak (DC = (1 – polymerized / not polymerized) × 100%).

#### 2.2.2. Flexural strength and flexural modulus

The flexural strength and flexural modulus of the samples were evaluated after 24 h of photoactivation ($n$ = 10) and after 60 days ($n$ = 10) of immersion in distilled water in an incubator (TE-392/2, Tecnal, São Paulo, Brazil) at 37 °C. For each composite, 10 bar-shaped specimens were made using a split steel mold (10 mm × 2 mm × 1 mm) (Muench et al., 2005; Boaro et al., 2019). The specimens were submitted to three-point flexure, using a universal testing machine (model 5565, Instron, Canton, MA, USA), with 8 mm distance between the supports and crosshead speed of 0.5 mm/min, until failure. The maximal FS was calculated according to the following equation:

$$\sigma = \frac{3FL}{2bh^2}$$

where $\sigma$ is the flexural strength (MPa), $F$ is the maximum load recorded before the specimen fractured (N), $L$ is the distance between the supports (mm), $b$ is the width of the specimen (mm), and $h$ is the height of the specimen (mm), respectively and FM was calculated as:

$$E = \frac{CL^3}{4bh^3D}10^{-3}$$

where *E* is the flexural modulus (GPa), *C* is the load recorded (N), *L* is the span between the supports (mm), *b* is the width of the specimen (mm), *h* is the height of the specimen (mm) and *D* is the deflection corresponding to C (mm).

### 2.2.3. Post-gel volumetric shrinkage

Post-gel volumetric shrinkage was measured using the biaxial strain gauge method (Sakaguchi et al., 1997; Boaro et al., 2010). The specimens ($n$ = 5) were weighed (~0.1 g) and placed on a unidirectional strain gauge (PA-06-060BG-350LEN, Excel, São Paulo, Brazil) connected to a strain gauge adapter (Instron). A microcomputer operating with BlueHill software was used to receive the signals from the data acquisition board. The specimens were light-activated under the same conditions described above, with the contraction due to photoactivation being monitored for 5 min. The deformation resulting from contraction of the material was measured in two perpendicular directions.

### 2.2.4. Surface roughness

The surface roughness ($n$ = 10, 7 mm x 1 mm) of each specimen was evaluated using a surface tester (Surftest SJ-410, Mitutoyo, São Paulo, Brazil). Three measurements were performed for each specimen, by rotating the specimen through 45°, with a cut-off at 0.8 mm and speed of 0.5 mm/s.

## 2.3. Evaluation of antimicrobial properties

### 2.3.1. Bacterial sensitivity test using the agar diffusion technique

The agar dilution method was performed according to the Clinical and Laboratory Standards Institute protocol M2-A8 (CLSI, 2003), with some modifications, using three bacterial strains: *Streptococcus mutans* (UA 159), *Streptococcus mitis* (ATCC 49456), and *Streptococcus gordonii* (ATCC 35105).

Overnight cultures were grown using 30 µL of the frozen stocks in glass tubes containing 9 mL of BHI (*Brain Heart Infusion) broth (*Condalab, Madrid, Spain). The cultures were placed in an incubator (MCO-19AIC, Sanyo Electric Co., Japan) for 24 h, at 37 °C, in a 10% $CO_2$ atmosphere. To prepare the bacterial inoculum, 300 µL of the overnight mixture were suspended in a glass tube containing 5 mL of 0.9% saline solution, to reach the density equivalent to the turbidity of the 0.5 McFarland standard solution (at a wavelength of 625 nm) (CLSI, 2003).

The bacterial suspension was spread over the surface of Müeller-Hinton agar (HiMedia Laboratories Pvt. Ltd, Mumbai, India) supplemented with 5% sheep blood (Anilab Animais para Pesquisa Criação e Comérico Ltda., Paulínia, São Paulo, Brazil), using a sterile swab. Specimens ($n$ = 5, 7 mm x 1 mm) were disinfected using a gauze soaked in 70° alcohol (5 strokes forward and backward on the surface) and UV light irradiation for 1 min (mercury vapor lamp with >90% radiation at 253.7 nm) on both sides (André et al., 2021), and were positioned over the agar and the plates were incubated for 24 h, at 37 °C, in a 10% $CO_2$ atmosphere. After this period, the total inhibition zone diameter was measured using a digital caliper, disregarding the diameter of the specimens (CLSI, 2003).

### 2.3.2. Monospecies biofilm inhibition assay

The biofilm assay was adapted from methods described previously (Duarte et al., 2006; Sugii et al., 2017). An inoculum of *S. mutans* was prepared by transferring 30 µL of the frozen stock to a glass tube containing 9 mL of BHI broth, followed by incubation for 18 h, at 37 °C, in a 10% $CO_2$ atmosphere. The optical density was adjusted to approximately 0.7, at a wavelength of 660 nm.

Previously sterilized specimens ($n$ = 6, 7 mm x 1 mm, under the same conditions described above) were embedded in a metallic apparatus and placed in 24-well polystyrene plates, in a vertical position. Each well received 2.85 mL of BHI broth, 0.15 mL of 20% sucrose solution, and 12 µL of bacterial inoculum. To control for experimental contamination, groups containing only the specimens and culture medium were also tested ($n$ = 2). The plates were incubated for 24 h or 72 h, at 37 °C, in a 10% $CO_2$ atmosphere, and the culture medium was renewed daily (in the case of the 72 h biofilm).

After the incubation periods (24 h or 72 h), discs were removed, washed, and prepared, in order to determine viable cells by the plating method. To remove non-adherent bacterial cells, the biofilms were gently washed (1x) in a 24-well plate with 3 mL of 0.9% sterile saline. Subsequently, the discs were transferred to polystyrene tubes containing 5 mL of sterile saline, vortexed for 1 min, and sonicated for 1 min (5% amplitude, 6 pulses, 9.9 s for each pulse, and 5 s intervals), at 4 °C (Vibra-Cell VCX400 sonicator, Sonics, USA). Serial dilutions from this suspension were plated on BHI agar. The plates were incubated for 48 h, followed by counting of the colonies to obtain the number of colony forming units per mL (CFU/mL). The experiments were performed in duplicate.

### 2.4. Statistical analysis

The data were analyzed using R software (R Foundation for Statistical Computing, Vienna, Austria). The degree of conversion, post-gel volumetric shrinkage, surface roughness results and inhibition zone were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The monospecies biofilm inhibition, flexural strength, and flexural modulus results were analyzed by two-way ANOVA followed by Tukey's multiple comparison test, according to normality and homoscedasticity conditions. For all tests, a significance level of 5% was considered.

## 3. Results

### 3.1. Evaluation of physicochemical properties

#### 3.1.1. Degree of conversion

No significant differences were found among the groups ($p > 0.05$, Table 1). The degree of conversion ranged from 69.3% (20% SNPs) to 76.3% (30% SNPs and 20% CHX-SNPs).

#### 3.1.2. Post-gel volumetric shrinkage

The post-gel volumetric shrinkage values ranged from 0.3% (10% SNPs) to 0.71% (10% CHX-SNPs) (Table 1). In general, the CHX-SNPs group showed higher values, compared to the control and SNPs groups ($p < 0.05$). Differences were found between the SNPs and CHX-SNPs groups, when comparing the same concentrations ($p < 0.05$), except for the 30% group. The control group differed statistically ($p < 0.05$) from the 10% and 20% CHX-SNPs groups, which exhibited higher post-gel volumetric shrinkage.

#### 3.1.3. Surface roughness

The surface roughness of the experimental composites gradually increased with increasing percentages of SNPs and CHX-SNPs ($p < 0.05$, Table 1), with values ranging from 0.018 μm (10% SNPs and 10% CHX-SNPs) to 0.038 μm (30% CHX-SNPs). There was no statistical difference between the SNPs and CHX-SNPs groups, when comparing the same concentrations ($p > 0.05$). The control group only differed statistically ($p < 0.05$) from the 30% CHX-SNPs group, which exhibited higher surface roughness.

**Table 1.** Means and standard deviations for the degree of conversion (n = 3), post-gel volumetric shrinkage (n = 5), and surface roughness (n = 10)

| Groups | Degree of conversion (%) | Post-gel volumetric shrinkage (%) | Surface roughness (µm) |
|---|---|---|---|
| Control | 73.4 ± 0.51 [a] | 0.390 ± 0.0 [bc] | 0.023 ± 0.008 [bcd] |
| CHX-SNPs 10% | 73.1 ± 4.91 [a] | 0.714 ± 0.24 [a] | 0.018 ± 0.002 [cd] |
| CHX-SNPs 20% | 76.3 ± 4.26 [a] | 0.696 ± 0.13 [a] | 0.030 ±0.007 [ab] |
| CHX-SNPs 30% | 72.3 ± 0.45 [a] | 0.570 ± 0.05 [ab] | 0.038 ± 0.01 [a] |
| SNPs 10% | 72.5 ± 1.21 [a] | 0.3 ± 0.04 [c] | 0.018 ± 0.005 [d] |
| SNPs 20% | 69.3 ± 1.98 [a] | 0.306 ± 0.11 [bc] | 0.028 ± 0.006 [bc] |
| SNPs 30% | 76.3 ± 3.27 [a] | 0.402 ± 0.13 [bc] | 0.032 ± 0.008 [ab] |

Mean values followed by the same superscript letters are statistically similar ($p > 0.05$; one-way ANOVA followed by Tukey's test).

### 3.1.4. Flexural strength and flexural modulus

The means and standard deviations (SD) for flexural strength (MPa) and flexural modulus (GPa) are shown in Table 2 for the two times of analysis.

For flexural strength, the CHX-SNPs group showed higher values, compared to the control and SNPs groups, 24 h after polymerization. The highest flexural strength was obtained for the 30% CHX-SNPs group, which was the only group statistically different from the control ($p < 0.05$). Comparing the same concentrations for the groups with and without CHX, a significant difference was only observed for the groups with 30% of NPs ($p < 0.05$). The storage time in water was significant for the control and 10% CHX-SNPs groups ($p < 0.05$). After storage, these groups showed higher flexural strength, compared to the other groups. When comparing the same concentrations, a significant difference was observed for the groups with 10% and 30% NPs ($p < 0.05$).

In general, for the flexural modulus, the 30% CHX-SNPs group showed the highest values ($p < 0.05$), for both times of analysis. At 24 h after polymerization, no difference was found between the SNPs and CHX-SNPs groups, when comparing the same concentrations ($p > 0.05$), and at 30% both groups differed statistically from the control. On the other hand, after storage in distilled water, there was a statistical difference ($p < 0.05$) when comparing the same concentrations, except for the 20% group. The 10% and 30% CHX-SNPs groups differed significantly from the control group. The storage time was significant for the control, 10% CHX-SNPs, and 10% SNPs groups ($p < 0.05$).

**Table 2.** Means and standard deviations for flexural strength (GPa) and flexural modulus (MPa) after 24 h of polymerization (n = 10) and after 60 days immersed in distilled water (n = 10)

| | After 24 h of polymerization | | After 60 days immersed in water | |
|---|---|---|---|---|
| **Groups** | **Flexural strength (mean ± SD)** | **Flexural modulus (mean ± SD)** | **Flexural strength (mean ± SD)** | **Flexural modulus (mean ± SD)** |
| Control | 29.96 ± 4.92 $^{bcB}$ | 0.339 ± 0.09 $^{cB}$ | 35.89 ± 2.21 $^{aA}$ | 0.527 ± 0.07 $^{cdA}$ |
| CHX-SNPs 10% | 31.29 ± 5.08 $^{abcA}$ | 0.395 ± 0.12 $^{bcB}$ | 33.35 ± 3.66 $^{abA}$ | 0.647 ± 0.08 $^{abA}$ |
| CHX-SNPs 20% | 34.4 ± 6.15 $^{abA}$ | 0.474 ± 0.37 $^{bcA}$ | 30.38 ± 3.04 $^{bcdA}$ | 0.614 ± 0.07 $^{abcA}$ |
| CHX-SNPs 30% | 37.39 ± 5.56 $^{aB}$ | 0.702 ± 0.13 $^{aA}$ | 30.67 ± 2.69 $^{bcA}$ | 0.688± 0.11 $^{aA}$ |
| SNPs 10% | 26.72 ± 6.02 $^{cA}$ | 0.463 ± 0.13 $^{bcA}$ | 28.37 ± 4.76 $^{cdA}$ | 0.271 ± 0.14 $^{eB}$ |
| SNPs 20% | 31.64 ± 4.49 $^{abcA}$ | 0.481 ± 0.17 $^{bcA}$ | 30.62 ± 3.08 $^{bcA}$ | 0.559 ± 0.07 $^{bcA}$ |
| SNPs 30% | 30.18 ± 4.43 $^{bcA}$ | 0.555 ± 0.07 $^{abA}$ | 26.64 ± 2.68 $^{dA}$ | 0.439 ± 0.09 $^{dA}$ |

Mean values followed by the same superscript letters are statistically similar within the same period analyzed ($p > 0.05$; two-way ANOVA followed by Tukey's test) Lowercase letters represent statistical difference between rows, while uppercase letters represent statistical difference between columns (influence of storage time).

### 3.2. Evaluation of antimicrobial properties

#### 3.2.1. Bacterial sensitivity test using the agar diffusion technique

The agar diffusion method was used to evaluate the antimicrobial activities of the CHX-SNPs/dental resin composites against strains of *S. mutans*, *S. mitis*, and *S. gordonii*. The results (Fig. 3) showed that the CHX-SNPs groups formed inhibition zones for all the bacteria, with the 30% CHX-SNPs group presenting higher antimicrobial activity, compared to the other concentrations ($p < 0.05$). No inhibition zones were observed for the SNPs and control groups.

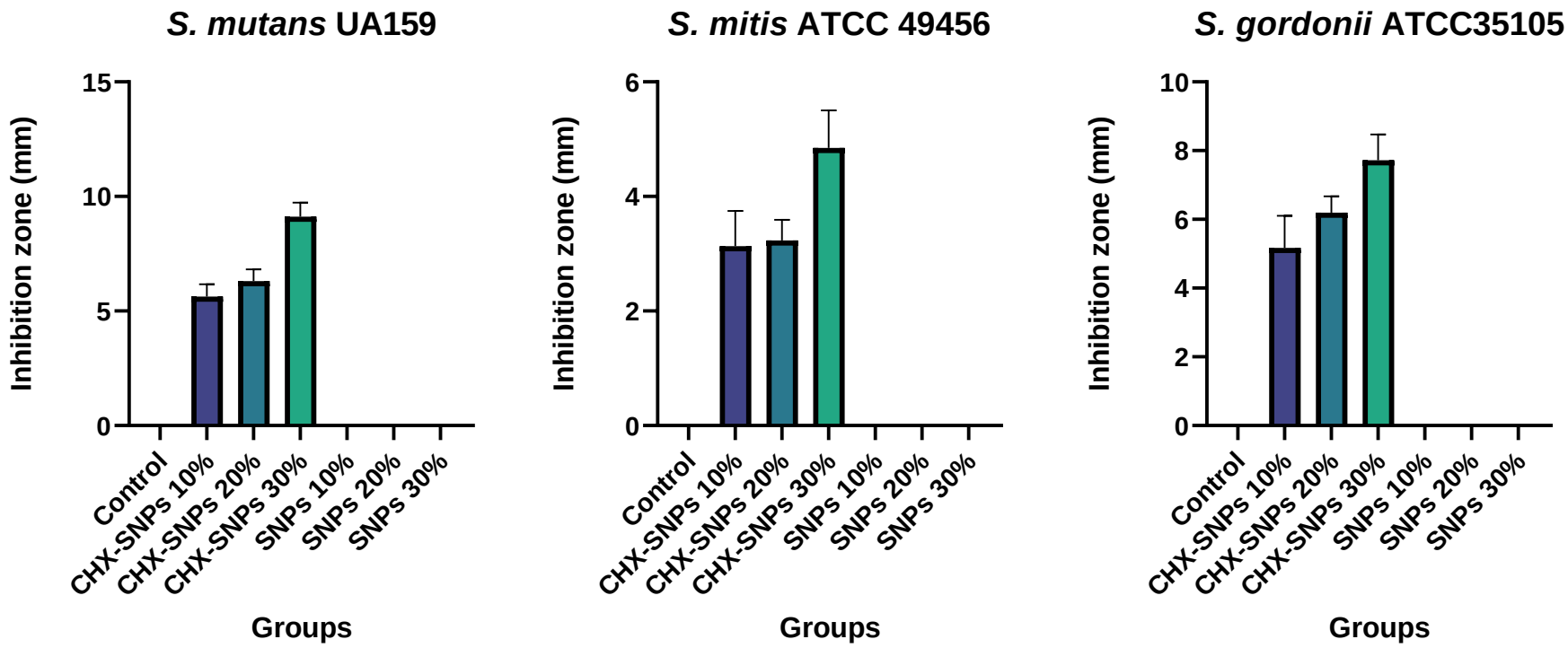


**Figure 2.** Means and standard deviations obtained for the agar dilution assays. Mean values with the same letter are statistically similar ($p > 0.05$, One-Way ANOVA/Tukey's test)

### 3.2.2. Monospecies biofilm inhibition assay

The *S. mutans* biofilm inhibition assay was evaluated at 24 h and 72 h. For both times, the number of colony forming units per mL (CFU/mL) was reduced when the CHX-SNPs were incorporated in the resin composites (Fig. 2), with the 30% CHX-SNPs group showing the highest inhibition ($p < 0.05$). Significant differences ($p < 0.05$) were found between the SNPs and CHX-SNPs groups, when comparing the same concentrations. At 24 h, the 20% and 30% concentrations were significantly different between the groups, while at 72 h, only the 30% concentration showed a significant difference. The time of biofilm formation significantly influenced the results for the 20% and 30% CHX-SNPs groups.

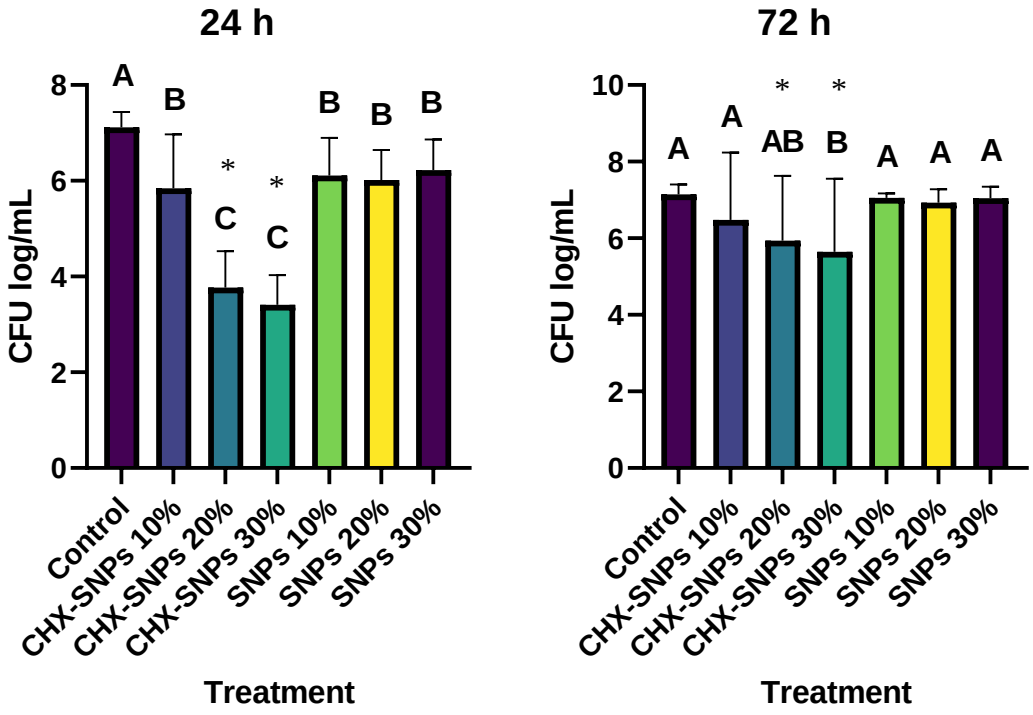


**Figure 3.** Means and standard deviations obtained in the biofilm inhibition assays. Mean values followed by the same letter are statistically similar ($p > 0.05$, Two-Way ANOVA/Tukey's test). Asterisks indicate statistical differences between the groups for the two

## 4. Discussion

The appearance of marginal cracks at the tooth/restoration interface and, consequently, recurrent episodes of caries, are the main factors that negatively affect the long-term clinical performance of composite resin restorations. These factors are primarily related to the characteristics of the composite resin, especially its physicochemical properties (Bai et al., 2020; Veloso et al., 2019). In order to overcome these limitations and considering the reinforcing effect of SNPs, as well as the broad-spectrum antimicrobial activity of CHX, the purpose of this *in vitro* study was to develop a composite resin containing multilayers of CHX-

SNPs, at different concentrations, and to investigate the performance of this new composite. The results indicated that addition of the CHX-SNPs provided antimicrobial activity against the cariogenic bacteria and influenced the mechanical properties of the experimental composites, with the exception of the degree of conversion.

Considering their physicochemical properties, the composites containing SNPs or CHX-SNPs could be considered satisfactory in terms of the degree of conversion, in relation to the clinical standard of 39-79% (Sideridou et al., 2002; Yang et al., 2021), with no difference in polymerization, independent of the addition of these nanoparticles. It is suggested that the higher the degree of conversion of the polymer matrix, the greater the mechanical strength presented by the composite and, consequently, the higher the clinical performance (Cunha et al., 2018). Furthermore, this reduces the occurrence of residual monomers that can solubilize in the oral environment, resulting in degradation of the restorative material, recurrent episodes of caries, and cytotoxicity (Marovic et al., 2013).

Proportionally to increase of the degree of conversion, there are also increases of other mechanical properties (Braga et al., 2005; Cunha et al., 2018). Although the groups did not differ statistically from each other in terms of the degree of conversion, higher values of post-gel volumetric shrinkage and flexural strength (after 24 h of light curing) were observed for the CHX-SNPs groups. The modulus of elasticity was significantly higher for the 30% CHX-SNPs group, even after 60 days of immersion in water.

The post-gel volumetric shrinkage results for the SNPs groups, compared to the control group, supported the notion that an increase of inorganic filler particles in resin composites reduces the polymerization shrinkage (Al Sunbul et al., 2016). Therefore, for the CHX-SNPs groups, it was assumed that CHX could increase the intermolecular interactions between the monomers during the photopolymerization of the nanocomposite, accelerating this reaction and, consequently, resulting in greater volumetric deformation. This characteristic is not desirable for a restorative material, since the amount of stress at the tooth/resin interface, generated by polymerization shrinkage, can cause the adhesive system to rupture, leading to undesired clinical consequences such as fissures and secondary caries (Cunha et al., 2018).

For flexural strength, the particle content was not significant in the groups with CHX (after 24 h of light curing), but these groups showed a statistical difference when compared with the SNPs and control groups. This suggested that experimental composites with CHX-SNPs may be more resistant to cohesive fracture. It should be noted that the results obtained here were below the requirement of the ISO 4049/2000 standard ($\geq$ 80 MPa), probably due to the low concentration of filler particles incorporated into the resin material. On the other hand, the

filler particles were statistically significant for the modulus of elasticity. The composites with 30% SNPs and CHX-SNPs showed higher values for this variable 24 h after light curing, which was consistent with the suggestion that higher modulus proportionally reflects a higher content of inorganic filler particles in the restorative material (Labella et al., 1999; Cunha, et al., 2018).

For some groups, the flexural strength and elastic modulus were influenced by the period during which the specimens were immersed in distilled water, showing an increase or decrease for these properties, depending on the group. This could be explained by the ability of resin materials to absorb water, which leads to plasticization, weakening of the three-dimensional polymeric network, and, in the long term, hydrolytic degradation of the organic matrix. This degradation increases the solubility and absorption of more fluids, consequently further weakening the mechanical properties of the resin (Ferracane, 2006; Nagano et al., 2018; Rummani et al., 2021). According to Van Landuyt et al. (2011), water solubility and sorption in resin composites are influenced by the level of residual monomers and, consequently, by degradation. Despite the good degree of conversion results for all groups, this hypothesis could not be excluded. For the modulus of elasticity, only the groups with 10% NPs were influenced by the storage time, so it could be speculated that these results may have been related to the low content of nanoparticles incorporated into the resin material. On the other hand, for the flexural strength, it was not possible to make this association, because the 30% CHX-SNPs group was influenced by the storage time.

For all the groups, increasing the concentration of filler particles led to a gradual increase in surface roughness. However, despite this increase, no group presented values higher than the critical limit of 0.2 μm (Rummani et al., 2021), suggesting that there was no influence of surface roughness on adhesion and accumulation of bacteria on the surfaces of the experimental composites. This could be observed for the SNPs groups, where, despite the increase in surface roughness, there was no difference between the SNPs concentrations in terms of biofilm formation, at both 24 h and 72 h.

Considering the complexity and dynamism of the oral symbiotic microbiome, it is desirable that in addition to excellent mechanical properties, composite resins should have the potential to combat the accumulation and proliferation of cariogenic bacteria at the edges of dental restorations. The antimicrobial activities of the experimental composites were investigated using bacteria of the genus *Streptococcus*, which are primary colonizers of the oral biofilm, accounting for 60-80% of all bacteria in the first hours of biofilm formation (Huang et al., 2011; Koo et al., 2013; Samaranayake & Matsubara, 2017). The addition of CHX-SNPs in the experimental resin resulted in a restorative material with increased antimicrobial properties,

compared to the control and SNPs groups. The antimicrobial activities presented by the nanocomposites were dependent on the concentration of CHX-SNPs, the bacterial species, and, for the biofilms, the time of analysis.

In the agar diffusion tests, only the composites containing CHX-SNPs showed antimicrobial activity, with the antimicrobial performance of the nanocomposites improving as the concentration of CHX-SNPs was increased. These results suggested that CHX was released from the restorative material and diffused into the agar medium, significantly inhibiting the growth of all the evaluated bacteria around the specimen. Considering the degree of conversion of the groups with CHX, as well as the absence of formation of zones of inhibition for the control and SNPs groups, the hypothesis that bacterial inhibition was due to the accumulation of residual cytotoxic monomers could be excluded. Furthermore, the diffusion of CHX from the nanocomposites confirmed the effective deposition of polyelectrolytes and CHX on the surface of functionalized SNPs, as described by Al Thaher et al. (2021), enabling use of the LbL system as a drug nanocarrier.

In addition to antimicrobial action, CHX also has broad-spectrum antibiofilm activity (Reda et al., 2020). The antibiofilm efficacy of resin composites is highly dependent on the time that the antimicrobial remains in the material and, consequently, on its release rate. Therefore, a significant reduction in drug release exposes the surfaces of restorations to colonization by cariogenic bacteria (Zhang et al., 2014).

According to the literature, SNPs and the LbL system act as a reservoir of substances and enable their controlled and sustained release (Priyadarsini et al., 2018; Perni et al., 2017; Hoang Thi et al., 2019). In this study, there was a notable reduction in formation of the *S. mutans* biofilm when the CHX-SNPs was added to the nanocomposites, especially at 30%, at times of 24 h and 72 h, with the most significant effect observed at 24 h, as shown by a greater reduction of CFU/mL. Based on the characteristics of the nanoparticles studied, it was expected that the nanocomposites with CHX-SNPs would present similar inhibition profiles of *S. mutans* biofilm formation at 24 h and 72 h. It is possible that the release of CHX from the nanocomposites decreased over time, due to the depletion of the components of the outer layer. Furthermore, reduced antimicrobial efficacy may be caused by the internal entrapment of CHX-SNPs in the polymeric network formed after the photopolymerization process. Therefore, in the absence of surface CHX-SNPs, *S. mutans* was able to adhere to the surface of the experimental material and form a biofilm within 72 h. This possibility will be need to be confirmed by quantification of the CHX released from the material studied, together with determination of the influence of pH on this process (work in progress). Another hypothesis is that over time,

the biofilm becomes more structured and thicker, so that the upper layers do not have contact with the restorative material, limiting the antibiofilm action of CHX.

The investigation of the influence of SNPs loaded with CHX, synthesized by the LbL method, in experimental resin composites provided useful advances in the application of CHX drug delivery using these materials. However, further studies are needed to evaluate the long-term antimicrobial efficacy against cariogenic bacteria adhesion and biofilm formation on the surface of the restorative material. Furthermore, information is required concerning the long-term influence of CHX-SNPs on the mechanical properties of experimental composites. It would also be desirable to perform other mechanical analyses, in addition to those employed here. Finally, more experiments should be carried out to determine the ideal concentrations of NPs in resin composites.

## Conclusions

The incorporation of CHX-SNPs in the experimental composites showed interesting and promising results. The findings suggested that the NPs exhibited desirable antimicrobial potential against the main bacteria related to oral biofilm formation, while also acting as fillers, improving the mechanical properties of the nanomaterial. Further modifications should be investigated in future studies. In general, the best overall results were obtained with addition of the CHX-SNPs at 30%. This initial study provides a gateway to the synthesis of experimental composites with improved performance using CHX-SNPs.

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## Conflict of interest

The authors declare no conflict of interest.

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## 3 DISCUSSÃO

No campo odontológico, a nanotecnologia constitui uma abordagem revolucionária e promissora para o aprimoramento físico-químico dos materiais restauradores e para o diagnóstico, prevenção e tratamento de complicações e patologias orais (Alkahtani et al., 2018; Patel et al., 2020). Dentre os materiais empregados para estas finalidades, as nanopartículas de sílica (SNPs) destacam-se por apresentar propriedades únicas como biocompatibilidade, grande área superficial com possibilidade de modificações, tamanho e forma ajustáveis, simples síntese e capacidade de nanocarrear e liberar fármacos de forma controlada e sustentada (Z. Li, 2020). Portanto, as SNPs tem sido extensivamente estudada e empregadas nas diferentes especialidades odontológicas, como dentística restauradora, endodontia, implantologia, ortodontia, periodontia e prótese como reforço ou sistema de entrega de substâncias.

Na tentativa de compreender o efeito e melhorar as propriedades gerais de materiais e formulações odontológicas, diferentes tipos e concentrações de SNPs, associadas ou não a outras substâncias, foram estudadas. Os resultados revelam que as propriedades físico-químicas e ópticas de materiais como o PMMA, resina composta, adesivos e cimentos odontológicos são influenciadas de modo divergente pela adição de diferentes concentrações de SNPs.

De modo geral, em baixas concentrações (0,05 a 1%) de SNPs, há um aprimoramento eficiente das propriedades gerais do PMMA, adesivos e cimentos odontológicos, como dureza (Balos et al. 2014), resistência à flexão (Azad et al., 2018; Jiangkongkho et al., 2018; Alzayyat et al., 2021; Mazloom-Jalali et al., 2020), tenacidade a fratura (Balos et al. 2014; Topouzi et al., 2017; Jiangkongkho et al., 2018; Mazloom-Jalali et al., 2020), módulo de elasticidade (Jiangkongkho et al., 2018; Alzayyat et al., 2021), translucidez (Alzayyat et al., 2022; Gad et al., 2021), rugosidade (Azad et al., 2018; Gad et al. 2020), resistência adesiva à microtração (μTBS) (Mazloom-Jalali et al., 2020; Akram et al., 2021) e resistência de união ao microcisalhamento (μSBS) (Rezvani et al., 2019). As investigações indicam uma tendência à diminuição destas propriedades com o aumento das concentrações de SNPs, provavelmente pela formação de nanoaglomerados que comprometem as propriedades mecânicas dos materiais.

Por outro lado, compósitos resinosos com concentrações intermediárias (4 a 10%) e altas (30 e 40%) de SNPs exibiram melhores propriedades gerais sobre baixas concentrações, como tenacidade a fratura (Hosseinalipour et al., 2010), resistência a flexão (Hosseinalipour et al., 2010; Liu et al., 2014; Zhang et al., 2014; Bai et al., 2020; Marovic et al., 2021), dureza (Alrahlah et al., 2020; Bai et al., 2020; Marovic et al., 2021), grau de conversão (Alrahlah et

al., 2020; Marovic et al., 2021) e módulo de elasticidade (Zhang et al., 2014; Alrahlah et al., 2020; Liu et al., 2014; Marovic et al., 2013; Bai et al., 2020; Marovic et al., 2021), mas reduzem a translucidez (Kim et al., 207).

A incorporação de diferentes concentrações de SNPs nestes materiais relaciona-se as próprias características destes materiais bem como suas aplicações clínicas. Altas concentrações de partículas de carga tendem a aumentar a viscosidade do PMMA, adesivos e cimentos odontológicos, limitando a adaptação dimensional da estrutura dental e do contorno gengival e o escoamento nos substratos dentinários e peças protéticas, respectivamente. Já para as resinas compostas, uma certa viscosidade facilita sua aplicação na cavidade oral, além de facilitar o molde no formato do dente, justificando a incorporação de um teor maior de partículas de carga inorgânica.

Resultados promissores também foram obtidos com o emprego de SNPs em outros materiais e formulações, intra ou extraoral, para o nanocarreamento de substâncias antimicrobianas (Ahn et al., 2009; Zhang et al., 2014; Lee et al., 2016; Stewart et al., 2018; Jung, Kim, et al., 2019; Bai et al., 2020; Akram et al., 2021; Alzayyat et al., 2022; Sungkhaphan et al., 2021), osteogênicas (Götz et al., 2008), anti-inflamatórias (Li et al., 2017), agentes de oclusão tubular com atividade remineralizante (Chiang et al., 2010; Tian et al., 2014; Yu et al., 2016; Jung, Kim, et al., 2019) e agentes de revestimento de próteses de PMMA (Yoshizaki et al., 2017; Cheng et al., 2019; Choi et al., 2020).

Considerando o dinamismo e a complexidade do microbioma oral, diversos nichos para o crescimento microbiano com características físico-químicas e biológicas específicas são encontrados na cavidade oral, como gengiva e sulco gengival, língua, bochecha, lábios, palato duro e mole e os dentes, além de restaurações, obturações, aparelhos ortodônticos e próteses fixas (Samaranayake & Matsubara, 2017; Mosaddad et al., 2019; Pedersen et al., 2018). Estas estruturas fornecem um habitat estável e ideal para a adesão e estabelecimento do biofilme quanto ao fornecimento de nutrientes, oxigênio e pH, especialmente as não descamativas (Sharma et al., 2018; Marsh, 2018). O microbioma oral pode ser encontrado em sua fase *planctônica*, disperso na saliva ou aderido às superfícies na forma de *biofilme* (Samaranayake & Matsubara, 2017).

É possível caracterizar os biofilmes como um fator de virulência associado a diversas infecções e doenças orais, incluindo cárie dentária, gengivite, periodontite e peri-implantite. Estas complicações surgem quando a relação simbiótica, comensal e mútua é interrompida e as espécies patogênicas predominam. Este fenômeno é conhecido como disbiose bucal e pode

estar relacionado a fatores intrínsecos e/ou extrínsecos aos hospedeiros (Deo e Deshmukh, 2019; Cornejo-Ulloa et al., 2019; Mosaddad et al., 2019).

Associado a isto, apesar dos constantes aprimoramentos, as resinas compostas ainda apresentam limitações físico-químicas que limitam seu desempenho clínico a longo prazo (Veloso et al., 2019; Bai et al., 2020). Visando superar estas limitações e considerando o efeito reforçador das SNPs, bem como a atividade antimicrobiana de amplo espectro da CHX, desenvolveu-se uma resina composta contento nanopartículas de sílica multicamadas carregadas com clorexidina (CHX-SNPs) em diferentes concentrações e seus desempenhos gerais foram avaliados. A adição de CHX-SNPs aos compósitos experimentais resultou em um material com propriedades antimicrobianas aumentadas em relação ao grupo controle (sem nanopartículas) e ao grupo com nanopartículas de sílica (SNPs), de modo concentração-dependente. Quanto as propriedades físico-químicas, não interferiu significativamente no grau de conversão apesar de aumentar os valores para contração volumétrica pós-gel.

Com a formação de zonas de inibição ao redor dos espécimes com CHX-SNPs, estipula-se que a inibição do crescimento das bactérias cariogênicas testadas (*S. mutans*, *S. gordonii* e *S. mitis*) se deva exclusivamente a difusão da CHX pelo meio ágar, excluindo assim, a atribuição da atividade antimicrobiana à monômeros citotóxicos residuais. Com base neste resultado, confirma-se a deposição efetiva de polieletrólitos e CHX na superfície de SNPs funcionalizados pelo sistema LbL e a capacidade de nanocarrear este fármaco, conforme descrito por Al Thaher et al. (2021).

Associado ao fato de que as nanopartículas de sílica apresentam a capacidade de transportar e liberar substâncias de modo sustentado e controlado e, considerando a síntese multicamadas (LbL) das CHX-SNPs, os resultados da atividade antibiofilme dos nanocompósitos experimentais contra *S. mutans* foi inesperado, visto que a efetividade das CHX-SNPs foi mais significativa em 24 h do que em 72 h. Para este experimento, era esperado que os nanocompósitos com CHX-SNPs apresentassem perfis de inibição da formação do biofilme semelhante nos dois tempos. Para este achado, pressupõe-se que a liberação de CHX tenha sido reduzida ao longo das 72 h, devido ao esgotamento das nanopartículas da camada externa e ao aprisionamento interno de CHX-SNPs na rede polimérica densamente reticulada formada após a polimerização, que limitou o seu deslocamento para a superfície dos compósitos, permitindo uma maior adesão superficial de *S. mutans* em 72 h da formação do biofilme. Outra hipótese é que com o passar do tempo o biofilme se torna mais estruturado e espesso, onde as camadas superiores não têm contato com o material restaurador, limitando a ação do antibiofilme CHX.

Em relação as propriedades físico-químicas, a adição de SNPs ou CHX-SNPs não afetou a polimerização dos compósitos resinosos. Isto foi confirmado pela ausência de diferença estatística entre os grupos analisados para o grau de conversão, apesar de ser sugerido que um aumento no teor de nanopartículas de carga induza um menor grau de conversão devido à alta viscosidade e redução da mobilidade dos macroradicais (Karabela & Sideridou, 2011). Este resultado, bem como a ausência de formação de halos de inibição para os grupos SNPs e controle, respaldam a atividade antimicrobiana dos grupos com CHX, reduzindo e/ou excluindo a ocorrência de monômeros residuais com potencial antimicrobiano citotóxico (Marovic et al., 2013).

O grau de conversão relaciona-se diretamente as propriedades mecânicas e desempenho clínico apresentados pelos compósitos resinosos (Cunha et al., 2018). Neste contexto e, apesar de não haver diferença estatísticas entre os grupos analisados para esta variável, maiores valores de contração volumétrica pós-gel e resistência a flexão (após 24 h da fotopolimerização) foram observados para o grupo CHX-SNPs. O módulo de elasticidade foi significativamente maior para CHX-SNPs a 30% nos dois períodos analisados quando comparados com o controle.

Para a contração volumétrica pós-gel, os grupos SNPs e controle apresentaram valores baixos e sem diferença significante entre si o que condiz com a afirmação de Al Sunbul et al. (2016), que partículas de carga inorgânica reduz a contração de polimerização em compósitos resinosos. Para tanto, os maiores valores desta variável para os grupos com CHX pode ser justificado pelo aumento das interações intermoleculares entre os monômeros durante a fotopolimerização dos nanocompósitos causado pelas moléculas de CHX, acelerando esta reação e, consequentemente, resultando em maior deformação volumétrica.

A composição química influencia diretamente as propriedades físico-químicas dos compósitos resinosos. A resistência a flexão e o módulo de elasticidade indicam a resistência do material restaurador à deformação e, para prática clínica, é relevante avalia-las considerando as tensões variadas geradas pela mastigação, tanto no dente quanto nas restaurações (Mallmann et al., 2010). Após 24 h da fotopolimerização, os compósitos com CHX-SNPs 30% apresentaram maiores valores para estas variáveis, indicando uma maior resistência à deformação antes da fratura coesiva. Estatisticamente, o teor de partículas de carga não foi significativo para a resistência a flexão na análise intragrupo com CHX. Por outro lado, influenciou significativamente o módulo de elasticidade para os grupos com sem e CHX, em relação ao controle, condizendo com a afirmação de que o alto módulo reflete proporcionalmente o alto teor de partículas de carga inorgânica no material restaurador (Labella et al., 1999; Cunha, et al., 2018).

Na prática clínica, é inviável utilizar compósitos com baixo módulo de elasticidade, pois usualmente, estes materiais podem não ter a recuperação dimensional completa para suportar a carga mastigatória. Mas vale ressaltar que o alto módulo de elasticidade tende a gerar restaurações mais rígidas e aumentar o estresse de polimerização na interface dente/compósito, o que também não é desejável (Cunha, et al., 2018).

O armazenamento dos corpos de prova por 60 dias em água destilada influenciou significativamente os resultados para alguns grupos, apresentando aumento ou redução destas propriedades, a depender do grupo. Pressupõe-se que estes resultados se relacionam a sorção de água no nanocompósito, o que gera plastificação, enfraquecimento da rede polimérica tridimensional e, a longo prazo, degradação hidrolítica da matriz orgânica. Esta degradação aumenta a solubilidade e sorção de mais fluídos e, consequentemente, reduz ainda mais as propriedades físico-químicas da resina (Ferracane, 2006; Nagano et al., 2018; Rummani et al., 2021). A extração de monômeros residuais e/ou existência de reação pós-cura também influenciam a viscoelasticidade dos compósitos. Para o módulo de elasticidade, apenas o grupo controle e 10% de NPs foram influenciados pelo tempo de armazenamento, portanto, especula-se que esses resultados possam estar relacionados ao baixo teor de nanopartículas incorporadas ao material resinoso. Por outro lado, para a resistência à flexão, não foi possível fazer essa correlação, pois o grupo com 30% de CHX-SNPs sofreu influência do tempo de armazenamento.

Por fim, a rugosidade superficial aumentou proporcionalmente ao aumento do teor de nanopartículas, para os grupos com SNPs e CHX-SNPs, o que já era esperado. Mas nenhum grupo apresentou resultados acima do limite crítico de 0,2 µm (Yahya et al., 2020), o que permite concluir que a rugosidade superficial não influencia a adesão e acúmulo bacteriano na superfície do compósito experimental.

O desenvolvimento e caracterização das propriedades físico-químicas e atividade antimicrobiana de SNPs carregadas com CHX e sintetizadas por LbL em compósitos resinosos experimentais exibiu resultados promissores, mas mais estudos são necessários para compreender realmente a eficácia destas nanopartículas à longo prazo no material restaurador, considerando outros experimentos que avaliem as propriedades físico-químicas e a adição de outras bactérias cariogênicas envolvidas na formação do biofilme oral. Por fim, estudos devem ser realizados para determinar a concentração ideal das NPs em compósitos resinosos.

Ainda, devido a diversidade de SNPs, concentrações e associação a outras substâncias, houve dificuldade em analisar e comparar o efeito das NPs em resinas compostas e nas diferentes aplicações odontológicas. Há uma ausência de informação na literatura sobre o

mecanismo pelo qual as SNPs influenciaram as propriedades gerais e apresentaram efeito biológico quando associadas a materiais ou formulações odontológicas.

## 4 CONCLUSÃO

Em conclusão, os estudos apontam o uso promissor das SNPs na prática odontológica, tanto para o aprimoramento geral de materiais e formulações quanto para o nanocarreamento de substâncias bioativas. Nossos achados mostram que a adição de SNPs carregadas com CHX em uma resina composta experimental forneceu ao material atividade antimicrobiana aumentada em relação ao grupo controle e SNPs, contra bactérias cariogênicas. Por outro lado, aumentou a contração volumétrica pós-gel. De modo geral, melhores resultados gerais foram obtidos com a adição de CHX-SNPs à 30%.

Considerando que este estudo é a porta de entrada para a síntese de compósitos experimentais com CHX-SNPs preparadas pelo método LbL, mais estudos são necessários para compreender os efeitos físico-químicos e biológicos das CHX-SNPs em compósitos experimentais, especialmente a longo prazo, levando em conta as variações da cavidade oral, como força mastigatória, presença de biofilmes multiespécies e flutuações de pH e a estepossível citotoxicidade do material.

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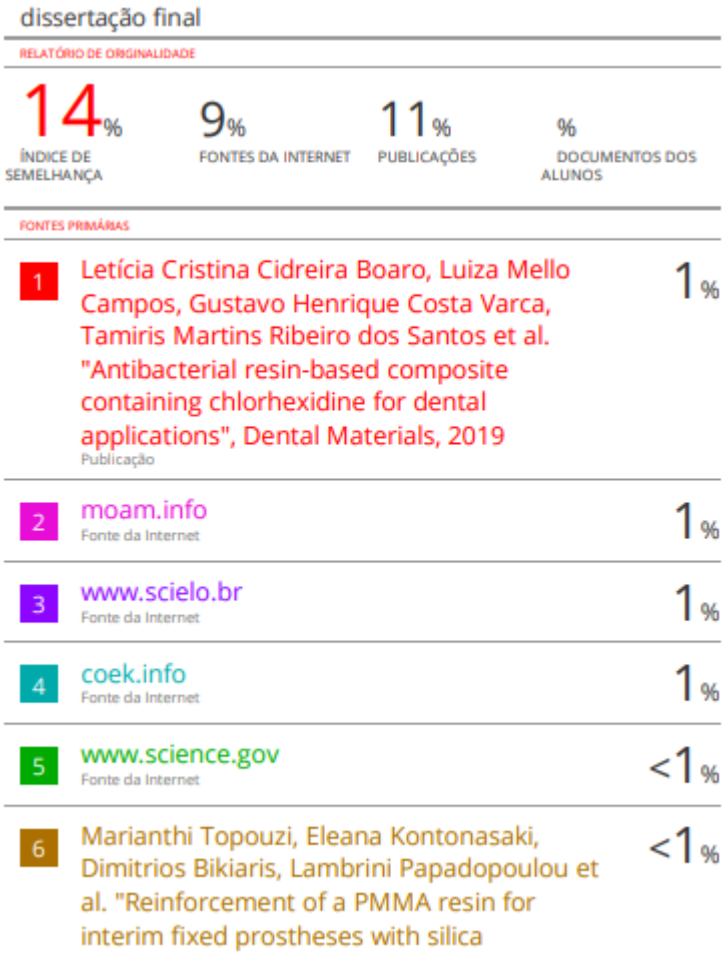

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