

# Antimicrobial Activity in Chitosan-Treated Prosthetic Materials: A Systematic Review

## Keywords

Chitosan  
Anti-Infective Agents  
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Denture Rebasings

## Authors

Isis de Araújo Ferreira Muniz \*  
(DDS, MSc)

Débora e Silva Campos \*  
(DDS, MSc, PhD)

Isabella Lima Arrais Ribeiro §  
(DDS, MSc, PhD)

Wellington Francisco Rodrigues †  
(MSc, PhD)

Sabrina Daniela da Silva ^  
(DDS, MSc, PhD)

André Ulisses Dantas Batista \*  
(DDS, MSc, PhD)

Paulo Rogério Ferreti Bonan \*  
(DDS, MSc, PhD)

## Address for Correspondence

Isis de Araújo Ferreira Muniz \*  
Email: isismuniz13@hotmail.com

\* Universidade Federal da Paraíba, João Pessoa, Paraíba, Brasil

§ Universidade São Paulo, São Paulo, Brasil

† Universidade Federal do Triângulo Mineiro, Minas Gerais, Brasil

^ McGill University, Montreal, Quebec, Canada

## ABSTRACT

*Objective:* The antimicrobial effect of prosthetic materials treated with chitosan was systematically reviewed. *Methods:* The searches were carried out on PubMed/Medline, Scopus, ISI Web of Science, LILACS, Embase, and Open Grey with searches performed in March 2022. Selection of in vitro studies, data extraction and risk of bias analysis were performed following the PRISMA guidelines and registered at the Open Science Framework. The evaluated prosthetic materials corresponded to PMMA and tissue conditioner, treated with chitosan. *Results:* After evaluating the eligibility criteria, 11 articles were included for the qualitative synthesis. The evaluated prosthetic materials corresponded to PMMA (n=8) and tissue conditioner (n=3). All studies performed the incorporation of chitosan in the tissue conditioner (n=3). Regarding PMMA, the use of chitosan as a denture cleanser was the most used (n=3), followed by incorporation (n=2), multilayers coating onto PMMA (n=2) and denture adhesive for PMMA (n=1). Chitosan has antimicrobial activity and is effective in the treatment of prosthetic materials in most studies, but it depends on some factors, such as the treatment method, the type and characteristics of the chitosan, the microorganism evaluated, and its form of organization. *Conclusion:* Chitosan showed highly antimicrobial activity and was effective when used in prosthetic materials.

## INTRODUCTION

Poly(methyl methacrylate) (PMMA) acrylic resin is the material most used for the construction of denture bases due to its characteristics and properties such as aesthetics, low cost, and biocompatibility.<sup>1</sup> Over time, the wear of the denture base, combined with the alveolar bone resorption, can lead to the necessity of the use a tissue conditioner, a temporary soft material designed to improve the stability of ill-fitting prostheses, relining the denture and improving the distribution of masticatory force and healing of traumatized tissues.<sup>2-4</sup>

However, it is known that both PMMA acrylic resin and poly(ethyl methacrylate) (PEMA) present surface roughness and porosity as problems, which favors the accumulation of biofilm, as well as colonization by pathogens, especially *Candida albicans*.<sup>1,4,5</sup> This is a predisposing factor for denture stomatitis (DS), an inflammation of the tissues underlying the prosthesis, of multifactorial etiology, common in denture users.<sup>6-8</sup> Treatment of DS consists of more efficient oral hygiene strategies, with emphasis on denture cleaning procedures, removal of dentures before sleeping and rebase or replacement of worn prosthesis, in addition to the

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use of systemic or topical antifungal medication. However, the recurrent use of antifungals can cause resistance to these drugs, and it is associated with other side effects, like gastrointestinal problems, renal and liver toxicity, or interaction with other drugs.<sup>8,9</sup>

Among the possibilities for use in prosthetic materials, chitosan (CS) has been widely studied due to its antimicrobial properties, in addition to its biocompatibility, biodegradability, hydrophilicity, non-toxicity and low-cost.<sup>10-13</sup> Due to these properties and versatility, several *in vitro* studies have been developed with the aim of providing antimicrobial activity to prosthetic materials, acting in the prevention and treatment of denture stomatitis, which includes the incorporation of CS into commercial material,<sup>14</sup> the coating with membranes,<sup>15</sup> the application of adhesives<sup>9</sup> and even the immersion into CS antimicrobial solution.<sup>16</sup>

Despite the great diversity of antimicrobial substances, like CS, and the protocols and methodologies used in these *in vitro* studies, the review of the methods used for CS association to PMMA and PEMA prosthetic materials becomes relevant and necessary. This systematic review evaluated the antimicrobial effect of prosthetic materials treated with chitosan.

## MATERIALS AND METHODS

This systematic review followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA), 2020 version<sup>17</sup> and was registered at the Open Science Framework (protocol DOI#10.17605/OSF.IO/BZS9N at <https://osf.io/bzs9n>). The research question was: "What is the most effective protocol for antimicrobial activity in chitosan-treated prosthetic materials?". The search strategy was based on the acronym POT (P = population; O = outcome; T = type of study), in which P corresponded to prosthetic materials treated with chitosan (PMMA and conditioners), O to effectiveness of antimicrobial activity, and T to *in vitro* studies.

### SELECTION CRITERIA

The inclusion criteria were *in vitro* studies that evaluated the impact on the antimicrobial activity where chitosan was included in the PMMA or tissue conditioners, regardless of the form of presentation of CS. There were no restrictions on the year of publication or language. Studies that did not present direct application to dentures, when PMMA was not the denture-base material and when chitosan was associated with other substances were not included. In addition, case reports, case series, clinical studies, *in vivo* studies, editorials, letters to the editor, and literature reviews were not included.

### SEARCH STRATEGY

The databases used were PubMed/Medline, Scopus, ISI Web of Science, LILACS and Embase, and for gray literature, the Open Grey was consulted, with searches performed up to

March 2022. The following Medical Subject Headings (MeSH) or "text words" were: "Polymethyl Methacrylate", "PMMA", "Acrylic Resin", "Denture Bases", "Denture Relining", "Tissue conditioner", "Chitosan", "Anti-Bacterial Agents", "Antifungal Agents", "Antimicrobial Agent". Search for each database was performed by combining the terms with Boolean operators (AND, OR), as shown in Table 1.

### STUDY SELECTION

Articles were extracted from databases and imported into Mendeley Desktop reference management software (Mendeley Desktop, version 1.16.1, Mendeley Ltd., Elsevier Inc., NY, USA). Duplicates were identified and removed. Titles and abstracts were read by two independent examiners (IAFM, DSC). The articles that presented the eligibility criteria were read in full for data extraction. Any disagreement between the two reviewers was resolved by a third reviewer (PRFB) for final inclusion.

### DATA EXTRACTION

The data extraction was performed by the two reviewers (IAFM, DSC) and verified by a third author (PRFB) using a standardized collection form, containing the following information: authors, year of publication, sample size, prosthetic material, chitosan presentation, control, treatment method, strains, evaluation of antimicrobial activity, application, antimicrobial effect.

### RISK OF BIAS ASSESSMENT

The risk of bias was assessed by two independent reviewers (IAFM and DSC) using the Review Manager software (version 5.4, Cochrane Collaboration, Oxford, UK) through an adaptation of previous systematic reviews of *in vitro* studies<sup>18-20</sup> and the Cochrane Collaboration's tool.<sup>21</sup> The following items were analyzed: Comparable groups, Detailed information regarding measurements, Proper statistical analysis, Manufacturer's instructions, Single operator, and Blinded operator. The risk of bias was judged as "low", "high", or "unclear". For the parameters performed, the qualification was designated as low risk of bias. Otherwise, the item was classified as high risk of bias. In cases where the information is not described in the article, the corresponding authors were contacted by email for clarification. If there was no answer to the email, the parameter was classified as "unclear" risk. Based on the number of "Yes" assigned, the study was classified as high risk of bias (1 to 3), medium risk of bias (4 to 5) and low risk of bias (6 to 8), according to previous study of our group.<sup>22</sup>

### SUMMARY OF STUDIES

The information extracted from the eligible studies was evaluated and summarized in a qualitative and narrative way to define and generate the indicators of answers to the guiding question.

**Table 1. Review question, POT approach, search strategy in the different databases evaluated and the inclusion/exclusion criteria.**

<b>Review Question</b>	What is the most effective protocol for antimicrobial activity in chitosan-treated prosthetic materials?
<b>POT approach</b>	Population - P: prosthetic materials (PMMA and tissue conditioners) that have been treated with chitosan. Outcome - O: antimicrobial activity Type of study - T: Observational
<b>Database</b>	<b>Strategy</b>
<b>PubMed</b>	# 1 (((((((("Polymethyl Methacrylate"[MeSH Terms]) OR (PMMA[Title/Abstract])) OR (Polymethylmethacrylate[Title/Abstract]) OR ("Acrylic Resins"[MeSH Terms]) OR ("Acrylic Resin"[Title/Abstract]) OR ("Denture Bases"[MeSH Terms]) OR ("Denture Base"[Title/Abstract]) OR ("Tissue Conditioning, Dental"[MeSH Terms]) OR ("Tissue conditioner"[Title/Abstract]) OR ("Denture Rebasings"[MeSH Terms]) OR ("Denture Relining"[Title/Abstract]))))))) #2 (Chitosan[MeSH Terms]) #3 (((((((("Antifungal Agents"[MeSH Terms]) OR ("Antifungal Agent"[Title/Abstract]) OR (Antifungal[Title/Abstract]) OR ("Anti-Bacterial Agents"[MeSH Terms]) OR ("Anti Bacterial Agents"[Title/Abstract]) OR ("Antibacterial Agents"[Title/Abstract]) OR (Antibacterial[Title/Abstract]) OR (Antimicrobial[Title/Abstract]) OR ("Antimicrobial Agent"[Title/Abstract]))))))) #1 AND #2 AND #3
<b>Scopus</b>	#1 TITLE-ABS-KEY("Polymethyl Methacrylate" OR PMMA OR Polymethylmethacrylate OR "Acrylic Resins" OR "Acrylic Resin" OR "Denture Bases" OR "Denture Base" OR "Tissue Conditioning, Dental" OR "Tissue conditioner" OR "Denture Rebasings" OR "Denture Relining") #2 TITLE-ABS-KEY(Chitosan) #3 TITLE-ABS-KEY("Antifungal Agents" OR "Antifungal Agent" OR Antifungal OR "Anti-Bacterial Agents" OR "Anti Bacterial Agents" OR "Antibacterial Agents" OR Antibacterial OR Antimicrobial OR "Antimicrobial Agent") #1 AND #2 AND #3
<b>Web of Science</b>	#1 TS=("Polymethyl Methacrylate" OR PMMA OR Polymethylmethacrylate OR "Acrylic Resins" OR "Acrylic Resin" OR "Denture Bases" OR "Denture Base" OR "Tissue Conditioning, Dental" OR "Tissue conditioner" OR "Denture Rebasings" OR "Denture Relining") #2 TS=(Chitosan) #3 TS=("Antifungal Agents" OR "Antifungal Agent" OR Antifungal OR "Anti-Bacterial Agents" OR "Anti Bacterial Agents" OR "Antibacterial Agents" OR Antibacterial OR Antimicrobial OR "Antimicrobial Agent") #1 AND #2 AND #3
<b>Lilacs</b>	#1 ((mh:(("Polymethyl Methacrylate")) OR (tw:(PMMA)) OR (tw:(Polymethylmethacrylate)) OR (mh:(("Acrylic Resins")) OR (tw:(("Acrylic Resin")) OR (mh:(("Denture Bases")) OR (tw:(("Denture Base")) OR (mh:(("Tissue Conditioning, Dental")) OR (tw:(("Tissue conditioner")) OR (mh:(("Denture Rebasings")) OR (tw:(("Denture Relining"))))) #2 ((mh:(Chitosan))) #3 ((mh:(("Antifungal Agents")) OR (tw:(("Antifungal Agent")) OR (tw:(Antifungal)) OR (mh:(("Anti-Bacterial Agents")) OR (tw:(("Anti Bacterial Agents")) OR (tw:(("Antibacterial Agents")) OR (tw:(Antibacterial)) OR (tw:(Antimicrobial)) OR (tw:(("Antimicrobial Agent"))))) #1 AND #2 AND #3
<b>Open Grey</b>	#1 ("Polymethyl Methacrylate" OR "PMMA" OR "Polymethylmethacrylate" OR "Acrylic Resins" OR "Acrylic Resin" OR "Denture Bases" OR "Denture Base" OR "Tissue Conditioning, Dental" OR "Tissue conditioner" OR "Denture Rebasings" OR "Denture Relining") #2 ("Chitosan") #3 ("Antifungal Agents" OR "Antifungal Agent" OR "Antifungal" OR "Anti-Bacterial Agents" OR "Anti Bacterial Agents" OR "Antibacterial Agents" OR "Antibacterial" OR "Antimicrobial" OR "Antimicrobial Agent") #1 AND #2 AND #3
<b>EMBASE</b>	#1 ('Polymethyl Methacrylate':ab,ti OR PMMA:ab,ti OR Polymethylmethacrylate:ab,ti OR 'Acrylic Resins':ab,ti OR 'Acrylic Resin':ab,ti OR 'Denture Bases':ab,ti OR 'Denture Base':ab,ti OR 'Tissue Conditioning, Dental':ab,ti OR 'Tissue conditioner':ab,ti OR 'Denture Rebasings':ab,ti OR 'Denture Relining':ab,ti) #2 (Chitosan:ab,ti) #3 ('Antifungal Agents':ab,ti OR 'Antifungal Agent':ab,ti OR Antifungal:ab,ti OR 'Anti-Bacterial Agents':ab,ti OR 'Anti Bacterial Agents':ab,ti OR 'Antibacterial Agents':ab,ti OR 'Antibacterial':ab,ti OR Antimicrobial:ab,ti OR 'Antimicrobial Agent':ab,ti) #1 AND #2 AND #3
<b>Inclusion Criteria</b>	<ul style="list-style-type: none"> <li>• <i>in vitro</i>, <i>in vivo</i>, observational and preclinical studies</li> <li>• studies that evaluated antimicrobial activity of prosthetic materials (PMMA or tissue conditioners) treated with chitosan, regardless of presentation</li> </ul>
<b>Exclusion Criteria</b>	<ul style="list-style-type: none"> <li>• case reports</li> <li>• case series</li> <li>• editorials</li> <li>• letters to the editor</li> <li>• literature reviews</li> <li>• studies that have PMMA that does not constitute prosthetic material</li> <li>• studies that have no direct application in dental prosthesis</li> <li>• products containing chitosan associated with other substances</li> </ul>

## RESULTS

The process to extract and select studies according to PRISMA guidelines are presented in Figure 1. A total of 424 articles were identified in the evaluated databases, with 216 duplicates that were removed, the remaining 208 were screened for the title and abstract reading, and 25 were eligible for full-text reading. According to the inclusion criteria, 11 articles remained for the qualitative synthesis of this systematic review.

The detailed information extracted from the selected studies are described in the Table 2. The evaluated prosthetic materials corresponded to PMMA (n=8)<sup>14-16,23-27</sup> and tissue conditioner (n=3).<sup>28-30</sup> The 3 studies related to tissue conditioners incorporated the CS.<sup>28-30</sup> Regarding PMMA, the use of CS as a denture cleanser was the most used (n=3),<sup>16,23,27</sup> followed by incorporation (n=2),<sup>14,26</sup> multilayers coating onto PMMA (n=2)<sup>15,24</sup> and denture adhesive for PMMA (n=1).<sup>25</sup> Changes in the polymer chain during chitosan synthesis generate different types of this polymer influencing the antimicrobial activity. Among these types, oligomer CS, low and high molecular weight CS, trimethylchitosan, quaternized CS showed antimicrobial activity in the formulation used in the studies. While N-succinyl CS, CS hydrochloride, CS-

glutamate, and chitosan quaternary ammonium salt did not have antifungal property. Strains of microorganisms that were tested where CS was used included *C. albicans*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*.

The authors of the selected primary studies mentioned that the incorporation of chitosan into the tissue conditioner resulted in positive results in terms of antimicrobial activity in all studies, with a reducing or inhibitory effect on the colonies of the evaluated strains. Regarding the treatment carried out with PMMA, the denture cleaner reduced the count of CFU and biofilm, while one incorporation had a controversial result since one study did not show antimicrobial activity<sup>14</sup> and another study showed good antimicrobial effect for the strains analyzed.<sup>26</sup> For the multilayer coating, an antifungal effect against *C. albicans* was observed. The denture adhesive had antifungal activity when using high molecular weight chitosan, while N-succinyl chitosan was ineffective for *C. albicans*.

The risk of bias was evaluated (Figure 2) and most of the studies were classified as medium risk of bias (n=8) and the others as high risk of bias (n=3). The domains "comparable groups", "detailed information regarding measurements"

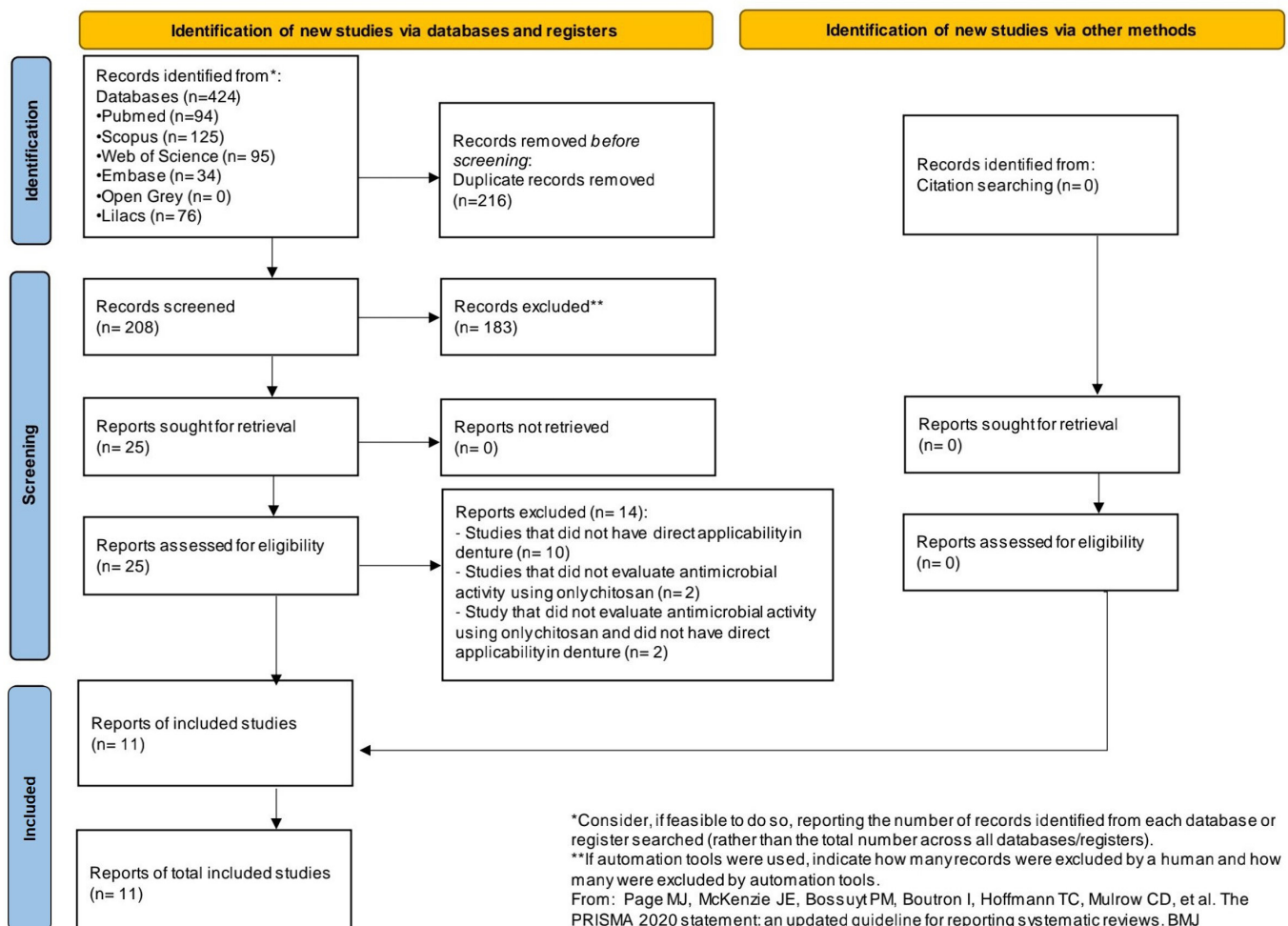


Figure 1: The flowchart of the process of selecting studies according to PRISMA guidelines.

**Table 2.** Data extraction of the included studies

Authors and year	Prosthetic Material	Application	Chitosan Presentation	Control	Treatment Method	Strains	Antimicrobial Activity Assay	Antimicrobial Effect
Curylofo et al., 2021 <sup>16</sup>	PMMA (Clássico, Artigos Odontológicos Clássico Ltda)	Denture cleanser	Low molecular weight chitosan (75-85% deacetylation)	Distilled water, Effervescent tablet (ET, Nitradine®-BonifAG) and negative control group without contamination and immersion	Solutions for immersion: Chitosan solution at 5 mg/mL (CH); Chitosan nanoparticle solution at 3.8 mg/mL (CN)	<i>Streptococcus mutans</i> , <i>Staphylococcus aureus</i> , <i>C. albicans</i> , or <i>C. glabrata</i>	Microdilution and biofilm	ET was the most effective solution for reducing biofilm; CH showed moderate action against <i>C. albicans</i> ; CH and CN significantly reduced the CFU count for <i>S. mutans</i> and <i>S. aureus</i>
Gondim et al., 2018 <sup>23</sup>	Acrylic resin (Vipi Wave; VIPi)	Denture cleanser	Chitosan Low molecular weight (LMW; MW = 107 kDa) 75-85% degree of deacetylation	Sodium hypochlorite	Chitosan nanoparticles (ChNPs) as a daily immersion of disinfection of denture base resin against <i>Candida spp.</i> biofilm.	<i>C. krusei</i> , <i>C. albicans</i> , or <i>C. tropicalis</i>	Microdilution, Time-kill assays, biofilm adhesion	The chitosan nanoparticles significantly reduced the CFU <i>Candida spp.</i> , inhibited <i>C. albicans</i> biofilm, and at 4 MIC showed complete inhibition in the time-kill assays. Also reduced <i>Candida</i> biofilm on resin.
Jiang et al., 2014 <sup>15</sup>	PMMA (Lucitone 199, Dentsply Intl)	Multilayers Coatings onto the Surfaces of the PMMA-Based Materials	Trimethylchitosan (TMC)/sodium alginate (SA)	Unmodified PMMA	TMC/SA layer-by layer self-assembly coatings PMMA surface.	<i>C. albicans</i>	Initial Fungal Adhesion XTT and Biofilm Formation	The anti-adhesion and biofilm controlling effects were stable for at least 4 weeks.
Jung et al., 2019 <sup>24</sup>	PMMA (Lucitone 199, Dentsply Intl)	Multilayers Coatings onto the Surfaces of the PMMA-Based Materials	Amphiphilic quaternary ammonium chitosan (CS612)	PMMA	CS612/sodium alginate (SA) layer-by layer self-assembly coatings PMMA surface.	<i>C. albicans</i>	Biofilm	CS612/SA LBL multilayers had fungal repelling effects and more layers further decreased fungal adhesion levels.
Lee et al., 2017 <sup>28</sup>	Poly(ethyl methacrylate) (PEMA) soft liner	Incorporation into PEMA	Chitosan (CS) or a Quaternized chitosan (QCS) (50 - 190 KDa, 75-85% deacetylated)	PEMA soft liner	CS or QCS powders (5.0, 7.5, and 10.0 wt%) were blended with the PEMA soft liner powder	<i>C. albicans</i>	Microdilution	Significantly fewer fungal colonies in tissue conditioners modified with CS or QCS, and none when the weight percentage of QCS exceeded 5%.
Mousavi et al., 2018 <sup>29</sup>	Tissue conditioner	Incorporation into tissue conditioner	Chitosan	Tissue conditioner without modification	Chitosan nanoparticles gel with mass percentages 0.675, 1.25, 2.5, 5, 10, and 20, were added to fluid tissue conditioner	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterococcus faecalis</i> , and <i>C. albicans</i>	Microdilution	Chitosan nanoparticles have an inhibitory effect on the growth of bacteria and pathogenic fungi.

**Table 2** Continued overleaf.....

Table 2. Data extraction of the included studies continued.

Namangkalakul et al., 2019 <sup>9</sup>	PMMA (Meliodent; Kulzer GmbH)	Denture adhesive	N-succinyl chitosan (NSC); High-molecular-weight water-soluble chitosan (HMWC; 150-200 kDa); Low-molecular-weight water-soluble chitosan (LMWC; 45 kDa)	Carboxymethyl cellulose (CMC)-coated and noncoated disks of PMMA	Each acrylic resin disk was coated with 15 mg of the Adhesive (chitosan gel with of NSC and HMWC that adhered to acrylic resin)	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. krusei</i> , <i>C. parapsilosis</i> , and <i>C. tropicalis</i> and 10 clinical isolates of <i>C. albicans</i> and 1 of <i>C. dubliniensis</i>	Microdilution	HMWC had the highest antifungal activity against most <i>Candida</i> species tested and <i>C. albicans</i> clinical isolates and NSC gel was ineffective to <i>C. albicans</i>
Saeed et al., 2019 <sup>30</sup>	Tissue Conditioner (GC)	Incorporation into Tissue conditioner	Chitosan oligosaccharide (COS) and low molecular weight chitosan (CS)	Tissue Conditioner without the addition of PMMA	Part of tissue conditioner powder was replaced either by CS or COS at concentrations, equivalent to 2 and 4 times MIC.	<i>C. albicans</i>	Microdilution	Tissue conditioners supplemented by chitosan or COS exhibited antifungal activity.
Song et al., 2016 <sup>26</sup>	PMMA (Heraeus Kulzer)	Incorporation into PMMA	Chitosan quaternary ammonium salt	PMMA without the addition of PMMA	Material 1: adding chitosan quaternary ammonium salt (0.50, 1.0, 2, and 3 mg/mL) directly to the PMMA (Powder) Material 2: adding chitosan quaternary ammonium salt (0.50, 1.0, 2, and 3 mg/mL) to the PMMA monomer	<i>Streptococcus mutans</i> and <i>C. albicans</i>	Film contact method	Material 1 exhibited better performance in both antimicrobial and antifungal tests as compared to Material 2. Material 2 did not have antifungal property.
Srimaneepong et al., 2021 <sup>27</sup>	PMMA (Rodex, SPD)	Denture cleanser	Oligomer chitosan (7-9 kDa, 30 kDa, 890 kDa, 1,700 kDa, and 2,100 kDa)	Commercial effervescent tablet (EFF; Polident), and 0.2% chlorhexidine gluconate	Chitosan solution for immersion	<i>C. albicans</i> , <i>C. krusei</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i> , and <i>C. glabrata</i>	Broth microdilution assays	Oligomer and 30 kDa chitosan, showed the best antifungal activity against all tested <i>Candida</i> species
Walczak et al., 2020 <sup>14</sup>	PMMA (Palapress pink, Hereus, Hanau).	Incorporation into PMMA	Chitosan-hydrochloride (CS-HCl) or chitosan-glutamate (CS-G)	PMMA	CS-HCl and CS-G in concentrations of 0.1%, 0.3%, 1% and 3% (for each salt and concentration) were added to PMMA resin	<i>C. albicans</i>	Biofilm assay	Did not result in antifungal, antibiofilm, or anti-adherent effect

and “proper statistical analysis” were present in all included studies, while the domain “manufacturer’s instructions” was not described in two studies (Figure 3). None of the selected studies mentioned the “blinded operator” domains and just one mentioned “single operator”.

## DISCUSSION

Due to the high rates of denture stomatitis, research has been carried out to develop prosthetic materials capable of preventing the proliferation of pathogenic microorganisms through the modification of these materials with antimicrobial substances. De Fátima Souto Maior *et al.* (2019)<sup>31</sup> incorporated terpinen-4-ol and cinnamaldehyde in tissue conditioner and obtained anti-*Candida* activity. Naoe *et al.* (2020)<sup>32</sup> evaluated a tissue conditioner containing cetylpyridinium chloride with montmorillonite (CPC-Mont), which showed good antimicrobial activity against *C. albicans* and *S. aureus*. Dos Santos *et al.* (2021)<sup>33</sup> incorporated diallyldimethylammonium chloride in acrylic resin and obtained an antimicrobial effect against *C. albicans*. In a systematic review, Adam and Khan (2022)<sup>34</sup> showed that the inclusion of silver nanoparticles in denture acrylic and denture liner reduced the growth of *C. albicans* in most of the included studies. Not only the modification of the prosthetic material has been evaluated, but also the modification of commercial adhesives used in dental prostheses, as shown by Almeida *et al.* (2017)<sup>35</sup> where they incorporated *Equisetum giganteum* and *Punica granatum* in a commercial adhesive showing antimicrobial results against *C. albicans* biofilm.

This systematic review showed antimicrobial activity of chitosan coated prosthetic materials. However, this result was dependent on the application, treatment method, and type of prosthetic material. The form of treatment of the prosthetic material can influence its antimicrobial activity. The incorporation method may require a higher concentration of the minimum inhibitory concentration (MIC) of the active compound because its action starts to be through indirect contact with the microorganism.<sup>30</sup> All studies included in this review that incorporated tissue conditioner had antimicrobial activity.<sup>28-30</sup> However, unanimity of antimicrobial effects was not observed in the incorporation into PMMA, as demonstrated in the study by Walczak *et al.* (2020)<sup>14</sup> in which the chitosan salts alone showed antifungal activity, but their incorporation into PMMA made them ineffective, in addition to increasing the surface roughness of the material, favoring

	Comparable groups	Detailed information regarding measurements	Proper statistical analysis	Manufacturer's instructions for denture materials	Single operator	Blinded operator
Curylofo <i>et al.</i> , 2021	+	+	+	+	?	?
Gondim <i>et al.</i> , 2018	+	+	+	+	+	?
Jiang <i>et al.</i> , 2014	+	+	+	+	?	?
Jung <i>et al.</i> , 2019	+	+	+	+	?	?
Lee <i>et al.</i> , 2017	+	+	+	+	?	?
Mousavi <i>et al.</i> , 2018	+	+	+	?	?	?
Namangkalakul <i>et al.</i> , 2019	+	+	+	+	?	?
Saeed <i>et al.</i> , 2019	+	+	+	+	?	?
Song <i>et al.</i> , 2016	+	+	+	+	?	?
Srimaneepong <i>et al.</i> , 2021	+	+	+	?	?	?
Walczak <i>et al.</i> , 2020	+	+	+	+	?	?

Figure 2: Classification of the included studies according to risk of bias.

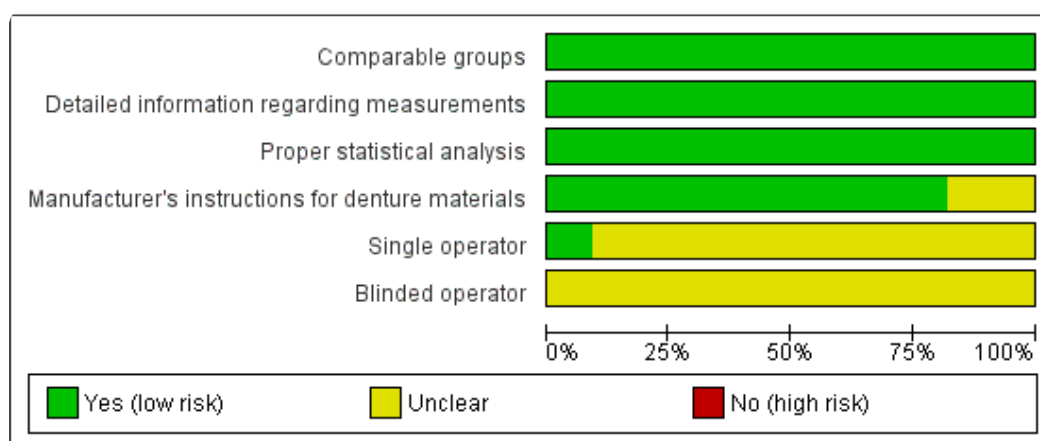


Figure 3: Distribution of risk of bias among the studies included according to pre-established criteria.

increased biofilm adhesion. Thus, the positive antimicrobial results obtained from incorporation into tissue conditioner can be justified based on the properties of relining prosthetic materials, since they are soft materials for temporary use and have greater porosity<sup>4</sup> which may allow the interaction of chitosan with the microorganism. In addition, relining materials, especially those based on acrylic resin, undergo the leaching process, releasing plasticizers and possibly the components incorporated into them, including chitosan. Sánchez-Aliaga *et al.* (2020)<sup>36</sup> expose in their study this leaching process, in which the antifungals incorporated into the tissue conditioner are released into the medium.

In addition to incorporation, other methods of treatment of prosthetic materials were evaluated, including the denture cleanser. The denture cleanser, developed by Srimaneepong *et al.* (2021)<sup>27</sup> from a low molecular weight chitosan solution, was able to reduce CFU count and biofilm. Curylofo *et al.* (2021)<sup>16</sup> using an immersion solution of low molecular weight chitosan and nanoparticles obtained an antifungal effect for the evaluated strains of *Streptococcus mutans*, *Staphylococcus aureus*, *C. albicans*. Gondim *et al.* (2018)<sup>23</sup> also used a solution of low molecular weight chitosan nanoparticles with action in the reduction and formation of *Candida ssp.* biofilm. Thus, it is possible to observe that the denture cleanser proved to be an effective and promising method for denture disinfection, acting in the reduction of *Candida ssp.* viability, possibly through a direct action of chitosan with the biofilm.

The studies showed that the action of chitosan nanoparticles and derivatives on the evaluated strains are dependent on factors such as concentration and contact time with the material, being directly proportional, as demonstrated by Mousavi *et al.* (2018),<sup>29</sup> who used chitosan nanoparticles, and Song *et al.* (2016),<sup>26</sup> who used salt of chitosan quaternary ammonium. Although nanoparticles have the greatest antifungal effect due to the larger surface area that allows greater interaction in the microorganism's membrane,<sup>23</sup> it was evidenced in the studies by Curylofo *et al.* (2021)<sup>16</sup> and Gondim *et al.* (2018)<sup>23</sup> that the solution immersion time was a determining factor for the action antifungal of the tested solutions, since both used chitosan nanoparticles solutions at the same concentration (3.8 mg/mL) against *Candida ssp.* and obtained different results, explained by the immersion time, which ranged from 15 minutes, with no antifungal effect<sup>16</sup> to eight hours, with a satisfactory effect.<sup>23</sup>

The PMMA coating represented another method to obtain an antimicrobial activity. Among them, a carboxymethylcellulose adhesive incorporated with different molecular weights of chitosan and a derivative, N-succinyl chitosan, was developed and showed different antifungal actions depending on the type of chitosan used.<sup>9</sup> Likewise, multilayer coatings made from chitosan derivatives, trimethylchitosan<sup>15</sup> and amphiphilic quaternary ammonium chitosan,<sup>24</sup> showed antibiofilm action for *Candida*, in which the greater the number of layers, the greater the antifungal effect, in addition to the use of chitosan in the outer layer potentiating the decrease in fungal adhesion.<sup>15,24</sup>

Despite the numerous advantages of chitosan, one of its limitations is its low solubility in water, being soluble only in an acidic medium.<sup>10</sup> However, one strategy to overcome this disadvantage is their chemical modification, producing derivatives with greater solubility and greater antimicrobial potential at neutral pH compared to unmodified chitosan.<sup>10,37</sup> This ability was observed in the studies included in this review, as demonstrated by Lee *et al.* (2017)<sup>28</sup> who used chitosan quaternary ammonium salt incorporated into PEMA in *C. albicans* strains, demonstrating that the quaternization of chitosan promoted an increase in the positive charge and, consequently, greater interaction with fungi, which have negative charges on their surface, inhibiting metabolic activity, resulting in greater activity compared to untreated chitosan. Saeed *et al.* (2019)<sup>30</sup> used chitosan oligosaccharide and also observed a greater antifungal activity compared to commercial chitosan, mainly against *Candida ssp.* Likewise, Srimaneepong *et al.* (2021)<sup>27</sup> showed greater action on the five *Candida* species tested for the oligomer and 30 kDa chitosan.

However, Namangkalakul *et al.* (2020)<sup>25</sup> presented a controversial result, since the N-succinyl chitosan gel was ineffective for *C. albicans*, although high molecular weight chitosan had the greatest fungicidal action when compared to low molecular weight chitosan as well. It is observed, then, that among the properties of chitosan, molecular weight is a relevant factor for antimicrobial activity, with a greater action at high molecular weight compared to low weight, as it has a greater solubility in water, and is effective in neutral pH, being more favorable for use in the oral environment.<sup>25</sup>

Prosthetic materials are easily adhered and colonized by microorganisms, especially *Candida ssp.* due to their hydrophobicity.<sup>15,23,38,39</sup> Thus, changing this characteristic through treatment with chitosan, makes the surface hydrophilic and reduces the adhesion of fungi<sup>15</sup> having antifungal action due to the cationic characteristic of chitosan that allows the interaction with the negative charge present on the membrane of these microorganisms,<sup>23,39</sup> obtaining a fungistatic action in greater proportion than that of a fungicide, in addition to presenting an antibacterial action, although its mechanism is not fully explained,<sup>25</sup> and its inhibitory action is greater than that of the bactericide.<sup>26</sup>

The antimicrobial action depends on some factors such as the type of strain, pathogen, planktonic form, and biofilm. Regarding the type of strain, the literature reports that the clinical strain is more resistant than the ATCC strain,<sup>40</sup> and among the studies included in this review, only one used clinical isolates of *Candida ssp.*<sup>25</sup> Among the fungal species evaluated by Namangkalakul *et al.* (2020)<sup>25</sup> and Curylofo *et al.* (2021),<sup>16</sup> *C. glabrata* showed greater resistance to the proposed treatments with chitosan, which were not effective at the concentrations tested. As for the form of organization, the microorganisms in the biofilm are more resistant to treatment with antimicrobials, such as chitosan, compared to the planktonic form, which is more sensitive, and lower concentrations show a greater antimicrobial effect.<sup>16,29</sup>

It is important to highlight that the objective of this study was to evaluate the biological properties of the treatment of prosthetic materials with chitosan, specifically the antimicrobial activity, but the physical-mechanical properties are also relevant and must be taken into account in laboratory tests of these treatments. There is an intrinsic limitation regarding the studies evaluated since *in vitro* studies are the basis of scientific evidence and cannot be directly extrapolated to clinical practice. Thus, there is a limitation of systematic reviews of *in vitro* studies. However, these reviews serve to guide the production of new laboratory research that is important for discoveries, being previous studies and the basis for the continuation of pre-clinical and clinical research.

## CONCLUSION

Nowadays, there is no effective protocol that guarantee antimicrobial properties to prosthetic materials in oral cavity. Most of the prosthetic materials showed antimicrobial activity when combined with chitosan. This is an open and promising field for further studies; however, it is necessary to understand the specific properties of each type of chitosan as well as the target microorganisms before any association with new materials.

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