Effect of Chitosan-Based Tissue Conditioners on Candida Albicans: A Systematic Review

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Abstract

Chitosan had been studied as a free molecule and associated with other materials as denture materials, it improved a notable antibacterial and antifungal effect. Nevertheless, it's still confusing if it could be effective in the treatment of denture stomatitis induced by candida albicans. This systematic review was conducted to answer the question: Does chitosan combined with tissue conditioners have an in-vitro antifungal activity against candida albicans?

The MEDLINE/central (PubMed), Scopus, Science Direct, and Web of science were screened up to 16/01/2023 for relevant papers exploring the antifungal effect of chitosan-based tissue conditioners. The main investigated outcomes were: Sample size, study design, type of chitosan, synthesis of chitosan, control group, characterization of chitosan, proportions of chitosan, and tissue conditioners, bacterial species, microorganisms culture, bacterial growth rate measurement, antifungal efficacy, assessment of the disease, and Results. The risk of bias assessment was assessed based on the judgment model suggested by Sarkis-Onofre and adapted to the context of chitosan.

A total of 5574 publications were found. After screening titles, abstracts, and duplicates six studies were retained based on inclusion criteria predefined. However, only four studies were included in the current systematic review after screening the full text. Chitosan and its derivatives yield good results in association with tissue conditioners against candida albicans.

Chitosan-based tissue conditioners showed significant antifungal activity against candida albicans. However, further investigations should be done to assess its potential therapeutic for treating denture stomatitis.

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Introduction

Denture stomatitis (DS) is a pathology affecting almost 65% of removable denture wearers[1]. It is characterized by inflammation, erythema, and hyperplasia of the oral mucosa [2]. Its localization is mostly in the palatal mucosa beneath dentures[3].

DS is considered a multifactorial pathology, the factors contributing in its development are poor denture and oral hygiene, low flow of saliva, and oral mucous trauma due to ill-fitting dentures [4]. Candida albicans are the main cause of denture stomatitis [5]. It is an innocuous commensal of the oral microbial communities. Candida albicans became virulent and generate candidiasis when the host defense system is altered by systemic pathologies like immunodeficiency [6].

Tissue conditioners are resilient materials that are used for conditioning the denture-bearing mucosa [7] and improving its recovery from trauma, or residual ridge resorption [8]. It is formed from a mixture of polymer powder and liquid plasticizer [3]. It has been reported that soft liners can be profoundly penetrated by candida albicans. In fact, these materials lead to greater levels of growth and colonization of candida than hard denture base resin [9]. This microbial growth results from the adherence of microbial cells and from adhesive interactions between candida species and oral bacteria (streptococci) [8].

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To overcome these problems, antifungal agents have been incorporated in tissue conditioners to inhibit the growth of C. albicans [10], for example, nystatin, azole group derivatives, and chlorhexidine. Recently, the incorporation of other antifungal agents such as metallic oxide powders, natural and herbal oils [11], and silver nanoparticles into tissue conditioners have been also investigated in terms of effectiveness [7].

Among these agents, chitosan was studied as an effective antifungal and antibacterial material. It is derived from chitin which is the second most abundant biopolymer on earth [12, 13]. It's obtained from chitin by alkaline hydrolysis with an inorganic base [14], and it is a biodegradable, biocompatible, and bioactive compound that has shown several biological properties such as antitumor [15,16], immune-enhancing [17], antimicrobial, antioxidant, and antifungal activities [18]. It is also used as a food supplement for the reduction of plasma cholesterol and triglycerides [19]. Furthermore, chitosan has pharmaceutical [18], and biomedical applications in food technology, textile industry, and other fields of dentistry application [20].

Chitosan has a large field of use in dentistry. The modification of oral hygiene products like toothpaste [21], mouthwashes, and varnishes was used to enhance the control of biofilm and protection of enamel from adherence bacteria [22,9,23,24,26]. In the treatment of endodontic infections by using drug delivery [27], intracanal irrigant, or sealants [28]. He also induces pulp connective tissue formation and dentin formation due to hemostatic and antimicrobial properties [29]. Numerous studies have shown that chitosan is effective in the treatment of periodontitis, and in periodontal tissue regeneration due to its biodegradability [30]. Chitosan thermosensitive and antibacterial hydrogel was used as a sealant and lubricant at the interface between the implant and the abutment. In fact, it decreases dental implant complications [31].

Besides, researchers reported, in many studies the effectiveness of chitosan in the treatment of denture stomatitis by modification of denture adhesives [5], and tissue conditioners, or administrated as antifungal drug delivery.

Exploratory research was carried out to look for studies evaluating the effect of chitosan and/or its derivatives combined to tissue conditioners on candida albicans. We found one clinical trial [32], and six in vitro studies, which have only been compared with a placebo (tissue conditioner Chitosan free). As a result, there are no studies, according to the present research, which compares chitosan combined with tissue conditioners with tissue conditioners associated with other antifungal agents.

The main aim of conducting this systematic review is to evaluate the scientific evidence regarding the antimicrobial efficacy of chitosan incorporated on a tissue conditioner as well as its clinical applicability.

Materials and methods

Focused question:

Does chitosan combined with tissue conditioners have in vitro antifungal activity against candida albicans?

Eligibility criteria:

The focus question was formulated as follows based on the population, intervention, comparison, outcome, and study design (PICOS) structure.

(P): Population: the tissue conditioners in discs were considered as the population of our study. It is a materiel used as denture lining. (the whole placed in a fungal suspension containing candida albicans).

(I): Intervention: the intervention was Chitosan and/or its derivatives as biodegradable, biocompatible material to the tissue conditioners.

(C): Comparison: the comparison was effected with placebo (tissue conditioners chitosan free).

(O) Outcome: antifungal effect on candida albicans.

The present systematic review was performed based on the preferred reporting items for systematics review and meta-analysis (PRISMA) recommendations, and the protocol was registered in Prospero by the number CRD42021260396, available on the website: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021260396

Search strategy:

In vitro studies that compared the efficacy of chitosan based tissue conditioners and control group were selected through the following databases: The MEDLINE/central (PubMed), Scopus, Science Direct and Web of Science were screened until 16/01/2023 for relevant manuscripts, and related to the focused question without restriction on date of publication. Authors of unavailable reports were contacted to get the

full-text articles. The structured search strategy used in The MEDLINE/central (PubMed) was: (chitosan OR chitosan hydrochloride OR chitosan glutamate OR chitosan nanoparticles OR quaternized chitosan) AND (tissue conditioners OR resilient denture liners OR conditioners OR tissue conditioning). A hand search was performed to screen reference lists to complete data collection.

The following question was adapted adequately for Science direct database; (chitosan OR chitosan hydrochloride OR chitosan glutamate OR chitosan nanoparticles OR quaternized chitosan) AND (tissue conditioners OR tissue conditioning) AND (candida albicans OR stomatitis).

For Scopus and Web of Science: (chitosan OR chitosan hydrochloride OR chitosan glutamate OR chitosan nanoparticles OR quaternized chitosan) AND (tissue conditioners OR tissue conditioning)

The literature search results were de-duplicated using Zotero 5.0

Inclusion criteria:

The following specific inclusion criteria were used to select papers: (I) topic: antifungal activity of chitosan or its derivatives based tissue conditioners against candida albicans— (II) study design: controlled in vitro studies comparing outcomes with a control solution using a statistical test. (III) Language: Arabic, French, English, and Spanish.

Exclusion criteria: Papers excluded were all publications with languages other than 'Arabic, French, English, or Spanish, case reports, reviews of literature, and studies that assessed chitosan alone without tissue conditioners.

Data selection:

Titles and abstracts of selected manuscripts from this search were screened in duplicate and independently by two reviewers (J.E. and W.E.) to select pertinent studies respecting inclusion criteria. Full-text of potentially eligible studies were then collected based on screening the title and abstract. These reports were reviewed and retrieved by two reviewers (J.E., W.E.). Disagreements regarding eligibility were determined through consensus. If any doubt persisted, the opinion of a third reviewer was retained (A.B.). The final reports that fulfilled the selection criteria were used for data extraction.

Assessment of heterogeneity

The items used to assess in vitro studies were: Sample size, type of chitosan, synthesis of chitosan, control group, characterization of chitosan, proportions of chitosan and tissue conditioners, bacterial species, microorganisms culture, bacterial growth rate measurement, antifungal efficacy, assessment of disease, and Results.

Assessment of risk of bias (ROB)

The characteristics of included studies, their protocols and outcomes were extracted by two reviewers (J.E. and W.E.) independently that assessed the methodological quality of each study, Discrepancies were resolved by discussion to reach a consensus, the RoB assessment of the included in vitro studies was based on the judgment model proposed by Sarkis-Onofre et al [33] for the assessment of RoB in vitro trials. This model was adapted, in the current review to the context of chitosan. The seven items were chosen for risk of bias evaluation: microbial plates randomization, blinding of outcome assessment, sample size calculation, independent assessment, chitosan type, bacterial used for microbial test, method of antimicrobial screening.

The overall appraisal of the study was including if up to 6 or 7 items were fulfilled, it was considered as low risk of bias. Four or five items was considered as medium risk of bias. Finally, papers that reported one to three items were assessed as having a high risk of bias.

Results

Search

Databases PubMed Central, Medline, Science direct, Scopus, and web of science were conducted to a total of 5525 publications. After screening titles, abstracts, and duplicates, six studies were retained. However, only four studies were included in the current systematic review, as shown on the flow chart. (figure1)

Study characteristics:

Location

The geographical locations for the included papers are: Iran [34], Pakistan [35,36], and Taiwan [37].

Sample characteristics

Among the four studies three evaluated the growth of candida albicans [37,35,36], and only one evaluated the growth of four types of pathogens: staphylococcus aureus, pseudomonas

aeruginosa, enterococcus feacalis and candida albicans[34].

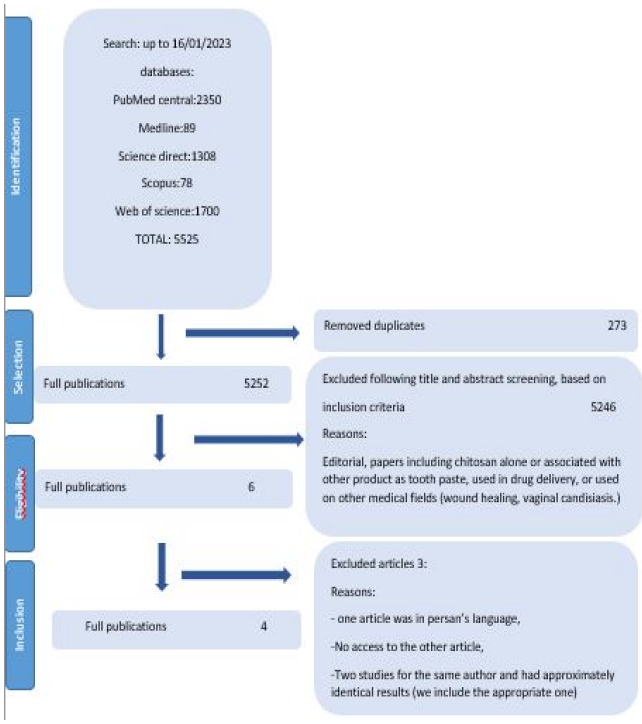

Figure1. Flow chart of the systematic review.

The type of chitosan included in tissue conditioners were different too. Mousavi (2020) used chitosan nanoparticles, Asfia Saeed et al (2018) studied the oligosaccharide chitosan, Hsin Lin Lee (2017) evaluated quaternized chitosan, and Hina Ashraf (2022) assesed the chitosan and chitosan loaded essential oils in tissue conditioners. More details are presented on Table 1.

Tested microorganisms

Candida albicans were cultured onto saboroud dextrose agar (SDB) for 24 hours and incubated at 37 ± 2°C for 24 h. The number of colony-forming units was then counted [35].

Four bacterial species were incubated for 24 hours at 37°C and were cultured on the sterile plates for the second study [34].

Candida albicans were used as test microorganisms to evaluate the antifungal activity of TC. It was suspended in SDB broth incubated at 37 °C for 24 hours. Cell concentration of the suspension was 2 x 10^6 CFU which was determined by plating serial dilution on agar plates [37]. The MIC method was used to determine the degree of bacterial growth inhibition, for two studies [34,35] and the weight percentage for the third one [37].

Quality assessment and RoB

Selected papers were published between (2017-2022), analysis of risk of bias was based on the judgment model of Sarkis-Onofre et al adapted to the context of chitosan. Among the four included in vitro studies three showed a medium risk of Bias [34,35,37], and one showed high risk of bias [36]. Bias most observed were microbial plates randomization, and blinding of outcomes assessement. A recap of RoB evaluation of the included in vitro studies is reported in Table.

Discussion

Our Search was focused to studies evaluating the association of chitosan to tissue conditioners and their antifungal effect against candida albicans. This systematic review, to our knowledge, provides the first synthesis of information considering the effect of chitosan associated with tissue conditioners on candida albicans. It includes Four in-vitro studies screened from five databases reviewed and retrieved with two reviewers independently.

The studies included in this review showed that combination of chitosan or its derivatives with tissue conditioners had an antifungal effect on candida albicans.

In fact, chitosan has been chemically modified to produce a variety of derivatives, among its derivatives, quaternized chitosan QCS which has shown a notable antifungal effect. Indeed, in 7,5 wt % or 10 wt % fewer colonies was revealed in QCS than commercial chitosan [37]. Chitosan is insoluble in most solvents, the modification through quaternization increases its positive charge thus enhanced its antifungal ability [38].

Oligosaccharide chitosan (COS) or low molecular weight of chitosan (COS) optimized the antifungal activity more than commercial chitosan, the minimum inhibitory concentration (MIC) of commercial chitosan and COS was 0.625 mg/ml and 0.312 mg/ml respectively [35]. The results were similar to the effect of chitosan based antifungal denture adhesive where they found that the average of minimum fungicidal concentration of low and high-molecular-weight water soluble chitosan ranged between 0.625 to 2.5 mg/ml [5]. In effect, COS had more ability to disrupt the metabolic activity and cell wall [39]. and it reduces the viability of single biofilms of C. albicans formed on acrylic resin [40].

Nanoparticulated chitosan was studied by several studies, Gondim et al found that at 120.4ug/ml led to a reduction greater than 70% in the number of CFUs from mixed candida SPP [41]. In our review, Ag, Zno and chitosan nanoparticles combined with tissue conditioners in complete prostheses had a significant reduction in opacity compared with that of the control group at the concentration of 0.625%. At concentrations of 2.5, 5, 10 and 20%, the nanoparticles completely inhibited Candida albicans [34]. These results have been confirmed in a clinical trial including 40 patients [32].

Chitosan was used as drug delivery vehicle for essential oils in tissue conditioner to synergize its antibacterial effect. Tissue conditioner loaded with blank chitosan nanoparticles exhibited significantly better antifungal properties than chitosan –free tissue conditioner, however, tissue conditioners containing chitosan nanoparticles loaded with essential oils showed better antifungal potential than blank chitosan nanoparticles [36]

To sum up, the minimum inhibitory concentration of chitosan nanoparticles and commercial chitosan was similar (0.625 mg/ml). Low molecular chitosan or oligosaccharide chitosan was more effective (MIC= 0.312 mg/ml), quaternized chitosan and chitosan effectiveness was expressed with a weight unit (7.5wt % and 10 wt. %), the addition of others molecules as essential oils or nanoparticles may synergize the effectiveness of chitosan on candida albicans.

The comparison between studies was difficult because of differences concerning the type of chitosan studied and the methodology carried out., in addition, according to the literature, there was a lack of studies evaluating chitosan and its derivatives added to tissue conditioners.

The majority of studies investigated other antifungals, such as Nystatin with tissue conditioners, and assessed their role in treating denture-induced stomatitis [42], and they proved good results.

The meta-analysis of this systematic review was not possible due to the high heterogeneity between the data (variability in unit measurements, doses, and molecules studied) of the included studies. Only qualitative analysis was performed.

Three studies were judged as medium risk of bias and only one as high risk of bias considering the domains: microbial plate randomization, blinding of outcome assessment, sample size calculation, independent assessment, chitosan type, bacterial used for the microbial test, and method of antimicrobial screening.

Certain limitations should be considered. First, the studies were tested on microbial suspension, which doesn't really mimic tissue conditioners in a saliva environment except for one study that immersed in artificial saliva. In addition, the effect of chitosan-based tissue conditioners on candida albicans strain alone is not the same as in biofilm strain.

There was no study that compared the efficacy of chitosan combined with TCs with other antifungals added to TCS. Indeed, studies had compared chitosan and its derivatives adding to tissue conditioners with chitosan tissue conditioners free. It represents then a limit for these studies.

We suggest that clinical trials are needed to assess chitosan and its derivatives as potential therapeutic agents and its eventual efficacy in healing denture stomatitis.

Conclusions

In general, the results showed a good efficacy of chitosan-based on tissue conditioners. As observed chitosan nanoparticles had complete inhibition at 5% on all microorganisms and a MIC at 0.625 %. It's similar to commercial chitosan and in comparison with low molecular chitosan, the minimal inhibition concentration was more effective 0.312 mg/ml. It was difficult to come out with conclusions due to the lack and the heterogeneity of studies besides the numerous biases in the reviewed studies that limited the strength of evidence.

Thus, more in vitro studies should be carried out to study other parameters as bond strength to acrylic resin denture base and evaluation of cytotoxicity in the human gingival epithelium. Moreover, a comparison between different derivatives of chitosan and their concentrations should be investigated, and then clinical trials should test the effect of chitosan-based tissue conditioners against candida albicans in the oral cavity.

Declaration of Interest

The authors declare that they have no conflict of interest.

First author/year	Study design	Sample size	Type of chitosan	synthesis of chitosan	Group control	Caracterisation of chitosan	Proportion of chitosan and tissue conditioners	bacterial species:	Microorganisms culture	Bacterial growth rate measurement	Antifungal efficacy	Assessment of disease MIC(wt %)	results
Hsin-Lin Lee (2017)	In vitro study	Samples: N=7 concentrations added to 24 well	Quaternized chitosan QCS	QCS was obtained by grafting 2-[(acryloyloxy ethyl]trimethyl ammonium chloride (AETMAC)	Soft liner chitosan Free :100(wt%) N=1	Transform infrared spectrophotometry (FTIR)	3 types: -GC soft liner -GC soft liner with chitosan -GC soft liner with QCS -CS or QCS powder (5.0, 7.5 and 10wt %) + poly (ethyl methacrylate powder) soft liner -the film cuted into a disc shape (10mm in diameter and 1 mm in thickness.	Candida albicans	- CA was suspended in SDB broth -incubated at 37° for 24 h, -transferred to 50 ml of SDB broth and cultured in a shaking incubator (100 rpm) at 370C.	- the subsequent CFU were counted visually	-The optical density of the fungal suspension was measured at 600 nm in Metertech SP-830+ spectrophotometer	7.5wt% and 10%: no colonies 5wt%: higher number of colonies in QCS	Compared with TC control group significantly fewer colonies were observed in the CS or QCS groups Tc with QCS were effective against CA; p< 0.05
Asfia Saeed (2018)	In vitro study	N=3 discs 96 well	Oligosaccharide COS chitosan	Water soluble COS was synthesized via hydrolysis under reflux condition	Tissue conditioner without chitosan N=1	Infrared spectroscopy Powder X-ray diffraction analysis	GC tissue conditioner without chitosan GC tissue conditioner -TC was replaced either by chitosan or COS at concentration equivalent to 2, 4 times MIC	Candida albicans	Dextrose Agar (SDA), Discs were seeded with 100µl of prepared c. albicans cell , incubated at 37 ±2°C for 24 h	The numbers of colony forming unit were counted visually	Immersion in a glass bottles containing 2 ml of sterile artificial saliva and stored in incubator at 37 % for 1,3,5,7 days Optical density was recorded spectrophotometricall y at 530nm	COS:0.3125 mg/ml Pure commercial Chitosan:0.625mg/ml	Tissue conditioners supplemented by chitosan or COS exhibited antifungal activity Control group: no inhibition of CA Significant difference p< 0.05
Seyyed Amine Mousavi (2020)	In vitro study	N=6 X 28 groupes = 168 samples	Chitosan nanoparticle	Optical sequestration method **-chitosan:** Chitosan+ citric acid+ distilled water+homogénéisation+ ultrasound for 5 h , tripolyphosphate, mechanical stirrer 3h, chitosan nanoparticles were prepared by a freezdrying device.	Tissue conditioner chitosan free N=6	Ag, Zno, and chitosan nanoparticles was approved by three scaning electron microscopy(SEM)image s, Xray diffraction spectrum (FT-IR)	0,25% silver 0,25% zinc oxide 0,5% chitosan Mass percentage: 0.625,1.25, 1.5, and 2.5, and 5,10 and 20 Homogenized with tissue conditioners liquid and placed in acrylic molds with dimensions: 8, 6, 2 mm	Staphylococcus aureus (ATCC 6538) Candida albicans(ATCC 10231) Pseudomonas aeruginosa (ATCC9027) Enterococcus faecalis (ATCC29212	Brain heart Broth and blood agar,culture mediums Lyophilized powder +brain heart broth, incubation for 24 h at 370, strain was cultured on the sterile plates, suspension of 0,5 Mc Farland was prepared and diluted to concentration 1,5×10 la puissance 5 CFU+tissue conditioners and Ag, Zno and chitosan+ Incubation 24 and 48h at 37°C.	Epoch spectrophotometre Bio tek instruments Inc highland Park The growth rate was recorded on turbidity at 600mm	Mass concentration: 0,625% of the macro particles combination: the opacity 1,55 and 83,1 mg/l at 24h and 48h Concentration 1,25% opacity: 0,23 and 0,71 at 24 and 48h Concentration of 2,5,5,10 and 20% the opacity was 0 at 24h and 48h	0.625% reduced opacity compared to control group Complete growth inhibition was achieved at a concentration of 2.5%	Addition of AG, Zno, chitosan nanoparticles to tissue conditioners in complete dentures, reduced fungal and bacterial growth P<0.05
Hina Ashraf (2022)	In vitro study	N=6 PEMA+ONP1 PEMA+ONP2 PEMA+LNP1 PEMA+LNP2 PEMA +BNP PEMA	Chitosan nanoparticles loaded with essential oils	CSNPs loaded EO were made by ionic gelation method 0.075g of chitosan powder+1% (w/w) acetic acid (agitation for 30min) 200µl and/ or 250 ml of oregano EO was dissolved separately in 1 ml sodium tripolyphosphate Chitosan nanoparticle loaded lemongrass: idem	Poly-ethyl methacrylate-based tissue conditioner	Scaning-electron microscope at resolution of 10 kv and 5,000-50,000 x	TC: powder+ liquid ratio of 2.2 g to 1.8 ml Liquid of TC + solution of CSNP loaded with EO were mixed with TC powder Teflon molds(10x1mm²)+ rectangular stainless-steel molds(100mmx20mmx 10mm)	Candida albicans		Colony forming units were counted	MIC was recorded by visually inspecting changes in turbidity and by comparing them with positive and negative controls	MIC of CSNP loaded Oregano EO 200 ± 0.007µL/ML MIC of CSNP loaded Lemograss EO: 156±0.006 µL/ML	There was negligible candida growth on the plate for all EO loaded TC C growth decrease to zero on the 10^4 dilution One way ANOVA showed statistically significant difference in candida colonization of all tissue conditioner disc p<0.01

Table 1. An overview of the characteristics of included in vitro studies.
Assessment of disease are expressed with wt %(weight percentage) and MIC (minimum inhibitory concentration), GC: tissue conditioner, QCS: quaternized chitosan, CS: chitosan, COS: oligosaccharide chitosan, CA: candida albicans, CFU: colony forming unit, PEMA: Poly-ethyl methacrylate, CSNPs: chitosan nanoparticles , ONP : oregano oil loaded CSNPs , EO: essential oils, LNP: lemongrass oil loaded CSNPs, BNP. : blank CSNP.

First author, year	Microbial plates randomization 1)	Blinding of outcome assessment 2)	Sample size calculation 3)	Independent assessment 4)	Chitosan type 5)	Bacterial used for microbial test 6)	Method of antimicrobial screening 7)	Overall appraisal
Hsin-Lin Lee 2017	N	N	Y	Y	Y	Y	Y	Medium
ASFIA Saeed 2018	N	N	Y	N	Y	Y	Y	Medium
Mousavi 2020	Y	N	Y	N	Y	Y	Y	Medium
Ashraf Hina 2022	Y	N	N	N	Y	Y	Y	High

Table 2. Summary of RoB evaluation of included in vitro studies.

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