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In vitro Inhibition of Biofilm Formation on Silicon Rubber Voice Prosthesis: A Systematic Review and Meta-Analysis

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Keywords
Biofilm · Silicone · Voice prosthesis · Inhibition

Abstract

Introduction: Biofilm formation on voice prostheses is the primary reason for their premature implant dysfunction. Multiple strategies have been proposed over the last decades to achieve inhibition of biofilm formation on these devices. The purpose of this study was to assess the results of the available in vitro biofilm inhibition modalities on silicone rubber voice prostheses. ***Methods:*** We conducted a systematic search in PubMed, Embase, and the Cochrane Central Register of Controlled Trials databases up to February 29, 2020. A total of 33 in vitro laboratory studies investigating the efficacy of different coating methods against *Candida*, *Staphylococcus*, *Streptococcus*, *Lactobacilli*, and *Rothia* biofilm growth on silicone rubber medical devices were included. Subgroup analysis linked to the type of prevention modality was carried out, and quality assessment was performed with the use of the modified CONSORT tool. ***Results:*** Data from 33 studies were included in qualitative analysis, of which 12 qualified for quantitative analysis. For yeast biofilm formation assessment, there was a statistically significant difference in favor of the intervention group (standardized mean difference [SMD] = –1.20; 95% confidence interval [CI] [–1.73, –0.66]; $p < 0.0001$). Subgroup analysis showed that combined methods (active and passive surface modification) are the most effective for biofilm inhibition in yeast (SMD = –2.53; 95% CI [–4.02, –1.03]; $p = 0.00001$). No statistically significant differences between intervention and control groups were shown for bacterial biofilm inhibition (SMD = –0.09; 95% CI [–0.68, 0.46]; $p = 0.65$), and the results from the subgroup analysis found no notable differences between the surface modification methods. After analyzing data on polymicrobial biofilms, a statistically significant difference in favor of prevention methods in comparison with the control group was detected (SMD = –2.59; 95% CI [–7.48, 2.31]; $p = 0.30$). ***Conclusions:*** The meta-analysis on biofilm inhibition demonstrated significant differences in favor of yeast biofilm inhibition compared to bacteria. A stronger inhibition with the application of passive or combined active and passive surface modification techniques was reported.

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Introduction

The insertion of a silicone rubber voice prosthesis into a surgically created tracheoesophageal fistula is the main strategy for voice rehabilitation in laryngectomized patients [1]. Currently, there are 2 different types of voice prostheses: the non-indwelling voice prosthesis, which is removable in order to be cleaned by the patients, and the indwelling voice prosthesis, which usually stays for an extended period in the tracheoesophageal fistula [2]. The material of choice for their production is silicone rubber because of its excellent mechanical and molding properties [2]. However, the hydrophobicity of silicone rubber surfaces [3] in combination with the continuous exposure to saliva, food, drinks, and oropharyngeal microflora [4] contributes to the rapid colonization of the prostheses by bacteria and yeasts [5] and leads to biofilm formation [6], which induces structural damage to the medical device. Leakage of esophageal contents (through and around the prosthesis valve) and increased airflow resistance are signs of biofilm formation, leading to frequent replacement of indwelling voice prostheses, limiting their clinical lifetime to 4–6 months [7]. Even though a replacement of a voice prosthesis potentially leads to problem-solving, narrowing or insufficiency of the tracheoesophageal fistula, local inflammation, and formation of granular tissue are possible complications of biofilm formation and may require additional surgical revision [8].

Among the different microorganisms that can easily colonize vocal implants, fungal species are the most commonly isolated, with a prevalence of 72.9% [9]. The predominant genera of yeasts found to form biofilms are *Candida* strains. In most cases, biofilms on silicone rubber prostheses extracted from patients are polymicrobial communities, in which different populations are present; in those mixed biofilms, *Staphylococcus*, *Streptococcus*, *Lactobacilli*, and *Rothia* species (both oral and cutaneous) are frequently isolated [10].

In the last decades, various biofilm methods have been described with the aim to prolong the lifespan of these devices. Antimycotic or antibiotic agents [11] are ineffective prevention of biofilm formation due to the risk of resistance [12]. It is known that in this environment, microorganisms undergo physiological and metabolic alterations that make them more resistant and recalcitrant to antimicrobials and consequently notoriously difficult to eradicate [13]. Thus, alternative approaches improving the antifouling properties of the silicone rubber material and preventing biofilm-associated biomaterial infections are urgently needed.

The initial step of biofilm formation involves adhesion of microorganisms to a surface [14] and depends not only on the nutrient environment, pH, and temperature but also on the physicochemical properties of the surface [15]. The modification of these properties of silicone voice prostheses could help in reducing biofilm formation and therefore improve the lifetime of these devices. The use of antimicrobial agents as coating substances has been indicated as the primary focus of researchers. The surface modification methods include among others metal coating techniques [16], coating with biosurfactants [17–21] or natural products such as essential oils [22], plasma surface treatment [23], metal nanoparticles coatings [24, 25], chitosan coatings [26], grafting hydrophilic monomers by laser [27], covalently coupled quaternary ammonium silane coatings [28], covalently coupled dimethylaminoethyl methacrylate and polyethylenimine coatings [29], parylene coatings [30] or polyacrylamide coatings [31]. However, surface modification has failed to significantly extend the lifespan of voice prostheses as of yet, predominantly because the active surface gets covered by a layer of proteins and dead cells, thus inhibiting its antifouling prosperities [32]. An alternative strategy could be the impregnation of silicon rubber with antimicrobials, allowing controlled release over time [29, 33]. In this systematic review and meta-analysis, we sought to explore the potential of the available in vitro methods inhibiting the biofilm formation on silicone rubber surfaces and discuss their future perspectives on elongating the survivorship of the implants.

Methods

For this systematic review and meta-analysis, we used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [34]. We utilized the following search terms: "biofilm," "silicone," and "inhibition."

Eligibility Criteria

We exclusively enrolled studies focused on biofilm inhibition methods on silicone rubber surfaces in vitro. Experimental studies conducted in vivo, on surfaces different from silicone rubber, or against preformed biofilms were excluded. Furthermore, we sought to include only studies involving both bacteria and yeasts from the oropharyngeal tract [9, 10].

Outcome Assessment

The outcome measured in the quantitative synthesis was the number of biofilm cells by colony-forming unit counting. Furthermore, outcomes including inhibition of the initial deposition rate, inhibition of microbial adhesion, cytotoxicity, minimum inhibitory concentration, and minimum fungicidal concentration were incorporated in the qualitative synthesis.

Literature Search

Two independent reviewers (A.T. and C.F.) performed a literature search for potentially relevant published and unpublished studies using electronic databases, clinical trial registries, conference abstract books, and reference lists of relevant studies. The databases of PubMed, Embase, and Cochrane Central Register of Controlled Trials were comprehensively searched with no language restrictions from January 1, 1997, up to February 29, 2020. In addition, the registries of ClinicalTrials.gov, Australian New Zealand Clinical Trials Registry (ANZCTR), and International Standard Randomized Controlled Trial Number (ISRCTN) were searched for completed unpublished studies up to the same date. The search strategy for PubMed included the use of the following terms: "silicone," "biofilm," and "inhibition." This strategy was adapted to each included electronic database, and no specific database filters were applied.

Study Selection

Two reviewers (A.T. and C.F.) identified potentially relevant records in a blinded fashion. Then, article deduplication took place, and the remainder of the articles was examined using title and abstract screening. Subsequently, the full text of the remaining articles was assessed for eligibility. Any discrepancies between the 2 investigators were discussed, and a consensus was reached.

Data Extraction

Two reviewers (A.T. and C.F.) independently abstracted data. We extracted information including the author, the year, and the country of publication, the microbial species investigated, the prevention method in the intervention groups, and the test material. Data extraction also included information about the applied in vitro model, time of biofilm incubation, infection-related outcome assessment, and infection-related outcomes.

We only presented the extracted data associated with the purpose of this systematic review. Thus, we considered data from the studies only referring to in vitro experiments investigating the biofilm inhibition methods on silicone surfaces with microbes related to oropharyngeal flora. In case, the biofilm inhibition was tested under both in vitro and in vivo conditions in the same study, data from in vitro experiments only were enrolled. Additionally, we sought to assess only the outcomes regarding the inhibition of biofilm formation and not the treatment of biofilms. Furthermore, if the same paper focused on additional materials other than silicone, we examined only the outcomes regarding silicone surfaces. Similarly, from studies that incorporated microbes both related or not to the oropharyngeal flora, we evaluated only the microbes of our interest.

Quality Assessment

The quality of the enrolled studies was independently assessed by 2 investigators (A.T. and C.F.) using the modified Consolidated Standards of Reporting Trials (CONSORT) risk of bias instrument [35], which represents an adapted version of the CONSORT risk of bias tool specially created for the bias assessment for in vitro studies. By and large, in the absence of similar tools for in vitro studies, this instrument was adapted to the current in vitro study. For the quality appraisal, the following sections were considered: Background and Objectives, Methods and Interventions, Outcomes, Sample size, Randomization, Allocation Concealment, Implementation, Blinding, Statistical Methods, Outcomes and Esti-

Table 1. Characteristics of the included studies: vibratory stimulus as a method for biofilm formation inhibition

Author [Ref]	Country	Prevention method	Species	Test material	Applied in vitro model	Infection-related outcome assessment	Outcome reporting
Wannemuehler et al. [62]	USA	Direct vibratory stimulus with 260 Hz frequency before and after meals for 5 d to the tracheoesophageal prostheses	*Candida albicans, Candida tropicalis, Streptococcus salivarius, Rothia dentocariosa, Staphylococcus aureus,* and *Staphylococcus epidermidis*	Provox 2 low-resistance, indwelling silicone voice prostheses of 22.5 French	Modified Robbins device, aerobic incubation with 5% CO_2 for 36 h	Quantification of antibacterial and antifungal activities by counting the CFU	(1) Statistically significant reduction of CFU for all TEP lengths; (2) overall reduction of 5.56-fold of CFU between vibrating and non-vibrating TEPs; and (3) greater CFU reduction for 8-mm length TEPs than for 6-mm length TEPs

TEP, tracheoesophageal prosthesis; CFU, colony-forming units.

Table 2. Characteristics of the included studies: passive surface modification methods (part 1)

Author [Ref]	Country	Prevention method	Species	Test material	Applied in vitro model	Infection-related outcome assessment	Outcome reporting
Janek et al. [18]	Poland	(1) Assessment of biosurfactant pseudofactin II anti-adhesive potential (50 µL of pseudofactin II; 0.035–0.5 mg/mL) and (2) pre- and post-adhesion inhibition of adhesion by pseudofactin II (100 µL of 0.035–0.5 mg/mL)	*Escherichia coli*, *Enterococcus faecalis*, *Enterococcus hirae*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Vibrio ordalii*, *Vibrio harveyi*, and *Candida albicans*	Silicone rubber out of urethral catheter, implants, and internal prostheses	96-well microliter plates, incubation at 37°C for 24 h	CLSM for the visualization of bacterial and *Candida* biofilms in the absence or presence of pseudofactin II (final concentration 0.25 mg/mL) in the culture medium	(1) Inhibition of bacterial adhesion by 36–90% and of *C. albicans* adhesion by 92–99% with pretreatment of a polystyrene surface with 0.5 mg/mL pseudofactin II; (2) total growth inhibition of *S. epidermis*, partial (18–37%) inhibition of other bacteria and 8–9% inhibition of *C. albicans* growth to silicone with the highest concentration tested (0.5 mg/mL)
Buijssen et al. [45]	The Netherlands	Modification of surface roughness on silicone rubber voice prostheses	*Candida tropicalis* GB 9/9, *Candida albicans* GBJ 13/4A, *Staphylococcus aureus* GB 2/1, *Staphylococcus epidermidis* GB 9/6, *Streptococcus salivarius* GB 24/9, and *Rothia dentocariosa* GBJ 52/2B	Groningen ultralow resistance voice prostheses	Modified Robbins device, incubation at 37°C for 72 h	CFU counting for the quantification of antibacterial and antifungal activities	40% reduction in the prevalence of bacteria and yeast in in vitro formed biofilms by decrease in surface roughness from 46 to 8 nm
Gottenbos et al. [48]	The Netherlands	Assay of the antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber in the absence or presence of adsorbed human plasma proteins	*Staphylococcus aureus* ATCC 12600, *Staphylococcus epidermidis* HBH2 102, *Escherichia coli* O2K2, and *Pseudomonas aeruginosa* AK1	Silicon rubber discs (8 mm diameter and 0.5 mm thick) out of implant grade silicone rubber (Medin, Groningen, The Netherlands) sheets	Parallel plate flow chamber, incubation at 37.1°C in ambient air for 24 h	(1) Application of live/dead fluorescent stain and CLSM for the determination of the viability of the adherent bacteria	Up to 90% reduction of adherent staphylococci
De Prijck et al. [29]	Belgium	Covalent bonding and quaternization of DMAEMA and PEI moieties to the surface of PDMS	*Candida albicans* SC5314	PDMS disks (diameter of 6.8 mm) cut out of medical-grade silicone rubber kit (Q7-4735; Dow Corning Corp., Midland, MI, USA)	Modified Robbins device, incubation at 37°C for 24 h	CFU counting for the quantification of antifungal activity	(1) Up to 92% reduction in *C. albicans* sessile cell counts with the quaternization of poly-DMAEMAq-modified PDMS; (2) no significant differences in cell count by the use of longer (C16 and C18) alkyl side chains compared to unmodified PDMS; (3) no increase in antibiofilm effect by combination of quaternizing agents compared to the use of single agents; (4) slight inhibition of *C. albicans* biofilm formation by immobilization of PEIq by covalent binding
Depan et al. [47]	USA	Incorporation of nanophase titania in silicone as an integral part of the silicone network structure through cross-link mechanism with aiming to reduction of bacterial adhesion to a minimum	*Staphylococcus aureus* strain (25923)	Addition of functionalized titania (2 wt% titania) and of the curing agent to silicone base for the preparation of silicone-titania hybrid network structure elastomer	24-well microliter plates, incubation at 37°C for 24 h	(1) Quantification of antibacterial activity by counting the CFU; (2) determination of MIC; (3) determination of MBC; (4) crystal violet assay for the evaluation of biofilm growth inhibition; and (5) analysis of zone inhibition for the determination of the time dependence of antimicrobial activity of nanocrystalline titania	(1) Reduction in the viability of *S. aureus* and its adherence on the surface of hybrid silicone by 93% with incorporation nanophase titania; (2) complete disintegration of biofilm after 6 h of incubation; and (3) increase in the observed zone of *S. aureus* inhibition with the increase in titania content from 2 to 5 wt% in silicone; (4) MIC = 3.92 µg/mL; and (5) MBC = 9.76 µg/mL
Contreras-Garcia et al. [46]	Spain	Grafting of DMAEMA to silicone rubber for enhancement of its antifouling features and preservation of its mechanical and biocompatibility properties	*C. albicans* SC5314 and *S. aureus* Mu50	Silicone rubber disks (1 mm thickness, 6.8 mm diameter), Deltalab (Barcelona, Spain)	24-well microtiter plates: (1) for *C. albicans* biofilm formation, incubation at 37.8°C for 48 h and (2) for *S. aureus* biofilm formation, incubation at 37.8°C for 24 h	(1) Balb/3T3 clone A31 cell line for cytocompatibility testing; (2) UV spectrophotometry at 328 nm for 48 h for the determination of the concentration of nalidixic acid in the medium; and (3) CFU counting for the evaluation of the growth inhibition from the surface of the films grafted with DMAEMA, before and after quaternization	(1) Significant reduction in *Candida albicans* biofilm formation but inadequate reduction in *S. aureus* biofilm formation by grafting DMAEMA; (2) reduction by more than 99% of *C. albicans* and *S. aureus* biofilm formation by quaternization of surfaces; and (3) cytocompatibility of grafted materials (fibroblast cell survival 70%)
Taylor et al. [52]	UK	Investigation of the effect of crosslinker levels on mechanical properties and colonization of silicone surfaces by *C. albicans*	*Candida albicans*	Maxillofacial silicone elastomer consisted of resin and crosslinker component supplied by Prestige (3 mm thick, 20×10 mm)	Orbital incubator shaker, incubation at 37°C for 6 weeks	CLSM for the determination of the colonization and ingrowth of *C. albicans*	Increased number of hyphae and blastopores observed into the elastomer by increase in the unbound polymer content

Table 2 (continued)

Author [Ref]	Country	Prevention method	Species	Test material	Applied in vitro model	Infection-related outcome assessment	Outcome reporting
Zhou et. al. [30]	China	Examination of the anti-adhesive activity of L199-parylene coatings against *C. albicans*	*C. albicans* ATCC 90028	Discs of silicone elastomer A-2186 (10 mm in diameter, 2 mm thick for cell adhesion assay; 15 mm in diameter, 1 mm thick for XTT reduction assay)	24-well microtiter plates, incubation at 37.8°C for 1.5 h	(1) Contact angle measurement for the assessment of the hydrophilic or hydrophobic characteristics of the surface; (2) assay of XTT reduction for the quantification of the viable cells in biofilm; (3) SEM for observation of morphology of *C. albicans* adhesion for 48 h; (4) CLSM in combination with fluorescent dyes FUN-1 and concanavalin A for illustration of *C. albicans* adhesion for 4 h	(1) Statistical difference between mean contact angles of silicone elastomer A-2186 before and after parylene coating ($p < 0.05$); (2) slight decrease in *C. albicans* adhesion to surfaces of silicone elastomer A-2186 before and after parylene coating (median [QL, QU] of 2.18 [1.9, 2.26] and 0.48 [0.42, 0.58], $p < 0.05$); and (3) significant decrease in XTT absorbance readings of A-2186-parylene compared to those of A-2186 ($p < 0.05$); (4) no significant difference in cell adhesion to the same specimens between 2 and 4 d ($p > 0.05$); and (5) reduction in *C. albicans* adhesion parylene coating on the surface of silicone elastomer A-2186 confirmed by both cell count and XTT reduction assay
Everaert et al. [23]	The Netherlands	Assessment of the effects of repeated argon plasma treatment of medical grade, hydrophobic silicone rubber on in vitro adhesion and growth of bacteria and *C. albicans*	*Streptococcus salivarius* GB 24/9, *Staphylococcus epidermidis* GB 9/6, *C. albicans* GB 1/2 and *Candida tropicalis* GB 9/9	Silastic/medical-grade silicone rubber plates 0.5 mm thick, 50×76 mm (for flow chamber studies) or discs 1×6.3 mm (for studies in the modified Robbins device) (Q7-4750; Dow Corning)	(1) Parallel plate flow chamber; and (2) modified Robbins device, incubation for 24 h at 37°C in ambient air	(1) Contact angle measurement for the assessment of the hydrophilic or hydrophobic characteristics of the surface; (2) assessment of initial deposition rate, j0; (3) calculation of the number of microorganisms adhering after 4 h; and (4) visualization of biofilms by SEM	(1) Reduced initial microbial adhesion over a 4-h time span to plasma-treated, hydrophilized silicone rubber compared to original, hydrophobic silicone rubber, both in the absence and presence of a salivary conditioning film on the biomaterial and (2) fewer *Candida* cells adhered on plasma-treated, hydrophilized silicone rubber as compared to on original, hydrophobic silicone rubber (growth studies over a time period of 14 d at 37°C in a modified Robbins device)

CLSM, confocal laser scanning microscopy; SEM, scanning electron microscopy; CFU, colony-forming units; PDMS, polydimethylsiloxane; PEI, polyethylenimine; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; DMAEMA, 2-(dimethylamino)ethyl methacrylate; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl amino)carbonyl]-2H-tetrazolium hydroxide; TMS, trimethylsilyl; MTT, 3-(4,5-dimethyliazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium.

mations, Limitations, and Funding and Protocol. The quality of reporting in vitro studies was assessed by checking whether the 14 checklist criteria were met in the papers selected. For each section, a judgment relating to the reporting was assigned by taking into consideration a prespecified binary question, that is, yes (reported)/no (not reported).

Statistical Analysis

Pair-wise meta-analysis of standardized mean differences (SMDs) was performed using the Review Manager (RevMan) Software (version 5.3) [36]. A random effects model was preferred in all cases, given the expected heterogeneity across trials. For continuous outcomes, random effects quantitative synthesis utilizing the effect size of SMD was conducted and 95% confidence intervals (CIs) were calculated.

Statistical heterogeneity issues were handled using the Cochran's Q statistic and quantified using the *I*-squared measure. A *p* value of <0.05 indicated statistical significance. We considered the following classification of statistical heterogeneity [37]:

- I^2 = 0–40%: not important heterogeneity
- I^2 = 30–60%: moderate heterogeneity
- I^2 = 50–90%: substantial heterogeneity
- I^2 = 75–100%: considerable heterogeneity

Cochrane guidelines were followed for the assessment of publication bias in the present meta-analysis [38].

Synthesis of the Results

The outcome measure in the quantitative synthesis was the number of biofilm cells by colony-forming unit counting, converted to $\log_{10}$ before the inclusion in the meta-analysis. Further outcomes, including inhibition of initial deposition rate, inhibition of microbial adhesion, and cytotoxicity were incorporated in the qualitative synthesis.

Clinical Interpretation of the Results

For the classification of effect sizes in this meta-analysis, the clinical interpretation of meta-analysis results was undertaken according to Cohen's classification. Thus, an SMD value of 0.2 demonstrated a small effect, a value of 0.5 represented a moderate effect, and a value of 0.8 showed a large effect [39].

Subgroup Analysis

We conducted a predetermined subgroup analysis, in which we classified the studies according to their strategy of action into 3 groups [40], adapting it on in vitro experimental studies on voice prostheses.

Passive Surface Modification

Passive surface modification methods include chemical and/or physical modifications of the surface layer of an existing biomaterial, resulting in a substantial change of its susceptibility to bacterial colonization [40]. For the assessment of passive surface modification methods, data from 18 studies were synthesized (Tables 2, 3). Various techniques were employed, including application of biosurfactants [18–20, 41–44]; modification of surface roughness [45]; application of anti-adhesive polymers such as 2-(dimethylamino)ethyl methacrylate (DMAEMA) [29, 46]; creation of nanopatterned surface through incorporation of nanophase titania in silicone [47]; application of coatings, such as quaternary ammonium silane coating [48, 49], L199-parylene coatings [30], or a nov-

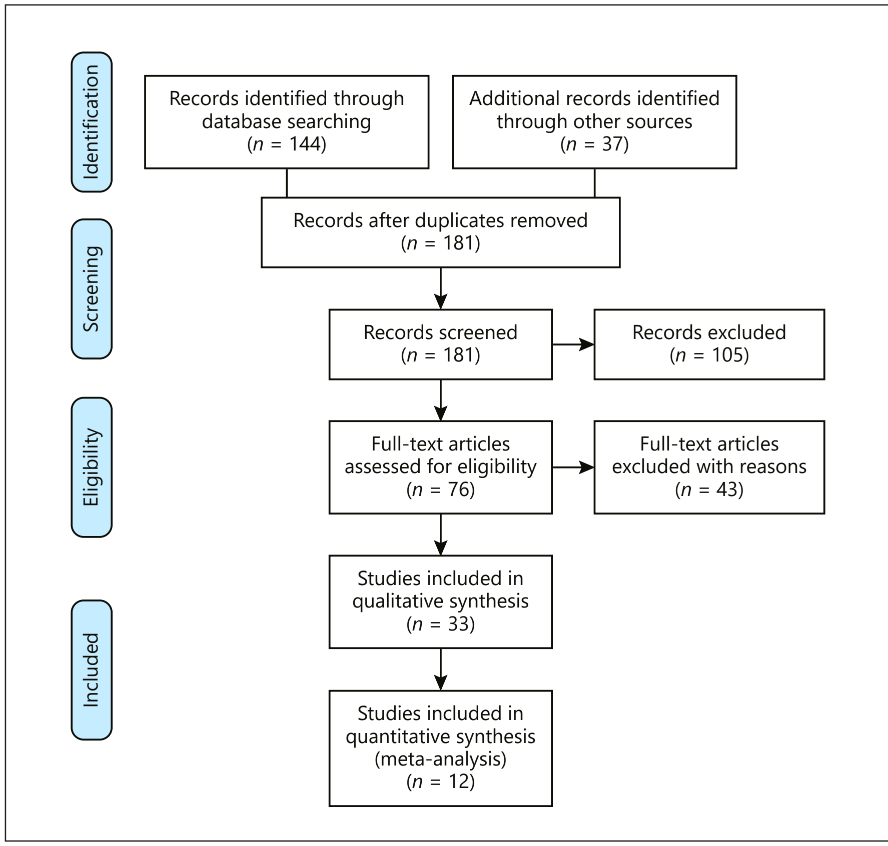

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Fig. 1. Flow diagram of the study selection procedure.

el nanoscale plasma coating [50]; and finally, creation of hydrophilic surfaces through repeated argon plasma treatment [23, 51] or addition of crosslinker in silicone elastomers [52].

Active Surface Modification

Active surface modification methods include pharmacologically activated coatings changing the implant from a passive, pharmacologically inert medical device to a drug agent [40]. For the assessment of active surface modification methods, data from 11 studies were synthesized (Table 4). Various techniques were employed, including application of linked antibiotics on silicone surfaces, such as of filastatin [53] and caspofungin [54]; application of nonantibiotic antimicrobial compounds such as of *Cymbopogon citratus* essential oil [22] and *Lactobacilli supernatant* [55]; application of chitosan derivatives [56, 57]; and finally, application of silver [24, 58] and selenium [25] nanoparticles or metal coatings [16, 59].

Combined Active and Passive Surface Modification

For the assessment of combined active and passive surface modification, data from 3 studies were synthesized (Table 5). The results of incorporation of 5 antimycotics in polydimethylsiloxane (PDMS) [60], immobilization of liposomal amphotericin B on PDMS [33], and the synergistic effect of a lipopeptide from *Bacillus subtilis* AC7 combined with the quorum-sensing molecule farnesol [61] were assessed against the formation of *Candida albicans* biofilms.

Results

The literature search yielded 181 potentially relevant studies. Duplicates were removed, and the remaining studies were screened according to the information provided in their title and abstract. We gained full access to 76 articles, and we assessed them for eligibility. We excluded in vivo studies, studies investigating treatment of preexisting biofilms, studies focusing on biofilm development on materials other than silicone, and studies investigating biofilms from microbes not related to oropharyngeal flora. After the exclusion of 43 records, we enrolled 33 published studies in the qualitative synthesis. Finally, we statistically pooled the results from 12 in vitro laboratory studies (Fig. 1).

Study Characteristics

In this systematic review, we considered 33 comparative studies for the qualitative analysis, of which 12 were eligible for inclusion in the quantitative analysis. The enrolled studies were published between 1997 and 2019. Three trials were conducted in the Italy [19, 20, 61], 1 in Poland [18], 2 in Austria [2, 55], 4 in Portugal [21, 33, 43,

Table 3. Characteristics of the included studies: passive surface modification methods (part 2)

Author [Ref]	Country	Prevention method	Species	Test material	Applied in vitro model	Infection-related outcome assessment	Outcome reporting
Xu et al. [50]	USA	Assay of a novel nanoscale plasma coating technology (TMS), using oxygen to coat the surfaces of silicone rubber for the inhibition of the formation of *Staphylococcus aureus* biofilms	*S. aureus* RN6390 and NRS234	Silicone rubber (PDMS) coupons of 5 mm×10 mm×1 mm or 5 mm×5 mm×1 mm (Bentec Medical Inc., Wakefield, MA)	24-well microtiter plates, incubation for 8 h at 37°C	(1) Contact angle measurement for the assessment of the hydrophilic or hydrophobic characteristics of the surface; (2) counting of CFU for measurement of the biofilm formation; (3) SEM for visualization of biofilms of *S. aureus*; (4) ELISA for measurement of protein adsorption on silicone coupons; and (5) CCL-1 fibroblasts (10,000 cells/well) for cytotoxicity assessment	(1) Significant reduction in *S. aureus* biofilm formation by coating PDMS with TMS alone (69.5±10.1%; $p < 0.001$); (2) maximal inhibition (92.7±0.95%; $p < 0.001$) by coating PDMS with TMS and oxygen at a 1:4 ratio (termed TMS/ O_2 1:4); (3) exhibition of a hydrophobic surface with contact angles over 105°(106°±5°) by bare PDMS substrates without plasma coating; (4) great reduction in PDMS surface hydrophobicity with TMS/O_2 plasma coating, generating a contact angle of 51°±3° measured at day 1 after plasma coating; and (5) slight increase in the cell viability ($p < 0.034$). with TMS/O_2 1:4 coating
Everaert et al. [64]	The Netherlands	Assessment of adhesion of yeasts and bacteria after oxidization of silicone rubber surfaces with argon plasma treatment and creation of organic layers by chemisorption of fluoro-alkyltrichlorosilanes	*Streptococcus salivarius* GB 24/9, *Staphylococcus epidermis* GB 9/6, *Candida albicans* GB 1/2, *Candida tropicalis* GB 9/9	Silastic implant silicone plates 0.5 mm thick, 50×76 mm^2 (MED + E12-4750, NuSil Silicone Technology, Antwerp, Belgium)	Parallel plate flow chamber, incubation for 24 h at 37°C in ambient air	(1) Initial deposition rate j0; (2) microbial adhesion numbers in a stationary end point 4 h; and (3) contact angle measurement for the assessment of the hydrophilic or hydrophobic characteristics of the surface	(1) Fewer microorganisms after 4 h on Ar-SR-CF3 and Ar-SR-C8F17 surfaces than on untreated silicone rubber both in the absence and presence of a salivary conditioning film; (2) increase in the hydrophobicity of the silicone rubber surfaces to advancing water contact angles of 125±5° and 140±5° for Ar-SR-CF3 and Ar-SR-C8F17 surfaces, respectively, by increasing the chemisorption of fluoro-alkyltrichlorosilanes ; and (3) greatest reduction in microbial adhesion by silicone rubber surfaces with chemisorbed, long fluorocarbon chains (Ar-SR-C8F17)
Busscher et al. [41]	The Netherlands	Assessment of adhesion of 2 *C. albicans* and 2 *C. tropicalis* strains of biosurfactant *Streptococcus thermophilus* B on silicone rubber with and without a salivary conditioning film	Two *C. albicans* and 2 *C. tropicalis* strains	Silastic medical-grade silicone rubber 0.4-mm-thick plates (50×76 mm) (Q7-4750; NuSil, Anglet, France)	Parallel plate flow chamber, incubation for 16 h in ambient air at 37.8°C	(1) Initial deposition rate of j0 and (2) determination of the number of adhering microorganisms after 4 h	(1) Decrease in *C. albicans* GB 1/2 adhesion on silicone rubber surfaces covered by 1–2% *S. thermophilus* B or by preabsorption of biosurfactants to silicone rubber prior to yeasts adhesion; (2) lower effect of preadsorbed biosurfactant layer against *C. tropicalis* GB 9/9 adhesion
Oosterhof et al. [49]	The Netherlands	Evaluation of the effects of the application of 2 quaternary ammonium silanes coatings, either through chemical bonding or through spaying, on the formation of biofilms on silicone rubber tracheoesophageal shunt prostheses	*C. tropicalis* GB 9/9, *C. albicans* GBJ 13/4A, *S. aureus* GB 2/1, *Staphylococcus epidermidis* GB 9/6, *S. salivarius* GB 24/9, and *Rothia dentocariosa* GBJ 52/2B	"Ultralow resistance" silicone rubber Groningen button tracheoesophageal shunt prostheses	Modified Robbins device, incubation for 3 days at 37°C	(1) Quantification of CFU for the assessment of biofilm formation; (2) CLSM for the imaging of biofilm formation; and (3) determination of in vitro cytotoxicity using L929 mouse fibroblast cell culture	Significant reduction by all coated prostheses in the numbers of viable yeast (up to 16%) and bacteria (up to 36%) compared with those for silicone rubber controls, as confirmed using CLSM after live/dead staining of the biofilms
Ceresa et al. [19]	Italy	Production of sophorolipids and evaluation of their antimicrobial properties in medical-grade silicone discs. Three different products were obtained: SLA (acidic congeners), SL18 (lactonic congeners), and SLV (mixture of acidic and lactonic congeners)	*S. aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 10145, and *C. albicans* IHEM 2894	Medical-grade silicone elastomeric discs (10 mm in diameter and 1.5 mm in thickness)	24-well culture tissue plates, incubation for 24 h at 37°C	(1) SEM for the evaluation of the effect of SLA, SL18 and SLV on cells of *C. albicans* IHEM 2894, *S. aureus* ATCC 6538, and *P. aeruginosa* ATCC 10145; (2) crystal violet (0.2%) assay for evaluation of the anti-adhesion and anti-biofilm activity of SL-coated discs after 1.5 and 24 h	(1) Reduction in *S. aureus* biofilm formation by 75% using SLA 0.8% w/v on pre-coated silicone discs; (2) inhibition of *C. albicans* attachment between 45 and 56% after 1.5 h; (3) inhibition of cell attachment by 68–70% when using SLA 0.8% w/v after 24 h incubation; (4) 90–95% reduction in *S. aureus* and *C. albicans* biofilm formation and adherence to surfaces at concentrations between 0.025 and 0.1% w/v in co-incubation experiments using SLA 0.05% w/v; and (5) 75% inhibition on the cell attachment using SL18 (0.8% w/v) after incubation of *S. aureus* ATCC 6538 cells for 24 h
Rodrigues et al. [42]	Portugal	Assessment of microbial adhesion of 4 bacterial and 2 yeast strains to silicone rubber before and after conditioning with a biosurfactant obtained from the probiotic bacterium *Streptococcus thermophilus* A	*S. epidermidis* GB 9/6, *S. salivarius* GB 24/9, *S. aureus* GB 2/1, *R. dentocariosa* GBJ 52/2B, *C. albicans* GBJ 13/4A, and *C. tropicalis* GB 9/9	Silicone rubber plates	Parallel plate flow chamber, incubation at 37°C in ambient air for 24 h	(1) Contact angle measurements for the characterization of silicone rubber with and without an adsorbed biosurfactant layer; (2) CLSM images of polymicrobial biofilm with or without the adsorbed biosurfactant layer; (3) determination of initial deposition rate j0; and (4) determination of the number of adhering microorganisms after 4 h	(1) 89–97% and 67–70% reduction of cell adherence to silicone rubber treated with biosurfactant for bacterial and fungal cells, respectively; (2) $7{\times}10^6$ and $15{\times}10^6$ cm^{-2} adhering microorganisms to silicone rubber for all the microorganisms; and (3) lowest value of adhering microorganisms ($1{\times}10^6$ cm^{-2}) for *C. albicans* GBJ 13/4A
Rodrigues et al. [43]	Portugal/ The Netherlands	Biosurfactants obtained from the probiotic bacteria *Lactococcus lactis* 53 and *S. thermophilus* A against the formation of biofilms on voice prosthesis	*S. epidermidis* GB 9/6, *S. salivarius* GB 24/9, *S. aureus* GB 2/1, *R. dentocariosa* GBJ 52/2B, *C. albicans* GBJ 13/4A, and *C. tropicalis* GB 9/9	"Low-resistance" Groningen button voice prostheses	Modified Robbins device, incubation at 37°C in ambient air for 24 h	(1) CLSM images of polymicrobial biofilm and (2) CFU counting for the quantification of antibacterial and antifungal activity	(1) Decrease in amount of bacteria in the biofilm to 4 and 13% and decrease in the amount of fungal organisms to 15 and 26% of the control from *L. lactis* 53 and *S. thermophilus,* respectively; (2) significant decrease in airflow resistance, 16 and 22 cm $H_2O \times s \times L^{-1}$ with the application of biosurfactants obtained from *L. lactis* 53 and *S. thermophilus,* respectively, compared with the values observed for the control

Table 3 (continued)

Author [Ref]	Country	Prevention method	Species	Test material	Applied in vitro model	Infection-related outcome assessment	Outcome reporting
Cochis et al. [20]	Italy	Assessment of the in vitro *C. albicans* anti-biofilm activity of biosurfactants, obtained from endophytes selected from *Robinia pseudoacacia* (AC5 and AC7) and *Nerium oleander* (OC5) at concentrations 312.5, 156.3, and 78.1 μg/mL	*C. albicans* CA2894	2 mm thick and 1 cm diameter of silicon-based elastomeric discs (Silastic Q7-4735; Dow Corning)	96-well microliter plates, incubation for 48 h at 37°C	(1) CFU counting for the quantification of the number of biofilm cells; (2) XTT assay for assessment of cell viability; and (3) MTT assay for the evaluation of biosurfactant cytotoxicity	(1) Greater reduction ($p < 0.01$) in biofilm cell number and viability by precoating with biosurfactant than chlorhexidine; (2) high anti-adhesion activity and low cytotoxicity of the biosurfactants at low concentrations (78.12 and 156.12 μg/mL); (3) significant reduction of the presence of viable *C. albicans* cells by the biosurfactant from *Nerium oleander* (OC5): 78.1 μg/mL as measured with XTT assay and 78.1 μg/mL with the CFU count method; (4) greater reduction in biofilm cell number by OC5 ($p < 0 .01$) than 0.3% CHX starting at concentration of 312.5 μg/mL for XTT values ($p < 0 .01$) and at 156.3 μg/mL for CFU count method ($p < 0.01$); and (5) effective reduction of biofilm cell number by AC5 compared to CHX at 78.125 μg/mL for CFU number and XTT values ($p < 0 .01$)
Rodrigues et al. [44]	Portugal	Assessment of the effects and extent of adhesion of 4 bacterial and 2 yeast strains to silicone rubber with and without an adsorbed rhamnolipid biosurfactant layer obtained from *P. aeruginosa* DS10-129	*S. salivarius* GB 24/9, *C. tropicalis* GB 9/9, *S. aureus* GB 2/1, and *S. epidermidis* GB 9/6	Silicon rubber plates	(1) Adhesion assay in 96-well plate and (2) adhesion experiments in the parallel-plate flow chamber, incubation for 24 h in ambient air at 37°C	(1) CLSM images of biofilm with or without the adsorbed rhamnolipid; (2) determination of the initial deposition rate j0; and (3) determination of the number of adhering microorganisms after 4 h	(1) Reduction in the initial deposition rates and in the bacterial cells adhesion after 4 h for all microorganisms tested; (2) reduction up to 66% of adhesion rate for *S. salivarius* GB 24/9 and *C. tropicalis* GB 9/9; and (3) reduction up to 48% of the number of cells adhering after 4 h on silicone rubber conditioned with biosurfactant for *S. epidermidis* GB 9/6, *S. salivarius* GB 24/9, *S. aureus* GB 2/1, and *C. tropicalis* GB 9/9

CLSM, confocal laser scanning microscopy; SEM, scanning electron microscopy; CFU, colony-forming units; PDMS, polydimethylsiloxane; PEI, polyethylenimine; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; DMAEMA, 2-(dimethylamino)ethyl methacrylate; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl amino)carbonyl]-2H-tetrazolium hydroxide; TMS, trimethylsilyl; MTT, 3-(4,5-dimethyliazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium.

44], 5 in the USA [47, 50, 53, 58, 62], 1 in Slovenia [57], 2 in the UK [24, 52], 1 in Germany [16], 1 in Spain [46], 1 in France [54], 2 in Belgium [29, 63], 1 in Australia [25], 8 in the Netherlands [23, 41, 44, 45, 48, 49, 59, 64], and 1 in China [30]. The biofilms were incubated in a parallel plate flow chamber (a modified Robbins device) or in microtiter plates. The microbial strains investigated were associated with the oropharyngeal flora, including *Candida*, *Staphylococcus*, *Streptococcus*, *Lactobacilli*, and *Rothia* species. The infection-related outcomes were the colony-forming unit counting, the inhibition of initial deposition rate, the microbial adhesion, the airflow resistance, and the cytotoxicity (Tables 1–5).

Quality Assessment

The results of the quality assessment of individual studies are shown in Tables 6 and 7. For all the enrolled studies, there were no available registration protocols. Additionally, sample size calculation methods, determination of randomization, allocation concealment mechanisms, and details about implementation, blinding, and statistical methods for any study were not reported. On the contrary, background and objectives, determination of methods and interventions, presentation of outcomes and estimations, and the presentation of the results for each study were provided. A structured summary in the abstract, the trial limitations in discussion, and information about the sources of funding are provided in most of the studies.

Qualitative Synthesis of the Results

For the purpose of the current study, the minimum biofilm inhibition concentration was defined as the concentration of a substance inhibited biofilm growth by 80% as a minimum when compared with controls [65].

Passive Surface Modification

Especially effective proved to be the grafting of DMAEMA or the covalent bonding and quaternization of DMAEMA and polyethylenimine moieties to the surface of PDMS, leading to reduction of *C. albicans* and *Staphylococcus aureus* biofilm formation by 99 and 92% respectively [29, 46]. Equally effective results achieved the incorporation of nanophase titania in silicone, leading to reduction in the biofilm formation of *S. aureus* by 93% on it [47]. Furthermore, coating PDMS with trimethylsilyl and oxygen at a 1:4 ratio achieved a reduction in *S. aureus* biofilm by 92.7% [50]. Finally, as far as biosurfactants concern, the application of pseudofactin II on silicone rubber surfaces achieved a total growth inhibition of *Staphylococcus epidermis*, but only partial (18–37%) inhi-

Table 4. Characteristics of the included studies: active surface modification methods

Author [Ref]	Country	Prevention method	Species	Test material	Applied in vitro model	Infection-related outcome assessment	Outcome reporting
Lara et al. [58]	USA	AgNPs (1–3 nm in diameter) against *Candida auris* biofilm formation	*C. auris* 0390	Silicone elastomer sheets (Bentec Medical) (1 cm diameter)	24-well microtiter plates, incubation in an orbital shaker at 37°C (100 rpm) for 24 h	(1) SEM for visualization of the inhibition of biofilm formation and (2) XTT reduction assay for the calculation of the extent of biofilm inhibition compared to the untreated elastomer	More than 50% inhibition of *C. auris* biofilm formation with concentrations of AgNPs from 2.3 to 0.28 ppm
Vargas-Blanco et al. [53]	USA	Application of filastatin on silicone surfaces to prevent the adhesion of *C. albicans*	*C. albicans* SC5314	Silicone coupons (1.2 cm diameter) made out of a mix of PDMS	96-well microtiter plates, incubation for 4 h at 37°C, without agitation and light exposure	(1) AFM for measurement of the adhesion force of *C. albicans* cells attached to surfaces treated with filastatin; (2) electrochemical impedance spectroscopy for the quantification of the effect of filastatin under microfluidic flow conditions; and (3) live-dead assay (Syto9® and PI) for the determination of the effect of viability on the cells	(1) 62.7% decrease in adhesion of *C. albicans* on silicone surfaces with chemisorbed filastatin; (2) 87.27% decrease in cell attachment compared to DMSO solvent controls by incubation with *C. albicans* cells in presence of 50 μM filastatin; and (3) 6.5-fold decrease in adhesion of *C. albicans* compared to untreated silicone coupons ($p < 0.001$) by incorporation of 25 μM filastatin into the composition of silicone coupons
Cocuaud et al. [54]	France	Antimetabolic activity of caspofungin against *Candida albicans* and *Candida parapsilosis* biofilms	Seven strains (92, 109, 163, 165, 182, 240, and 444) of *C. albicans*	Calibrated sections of 5 m cut out of 100% silicone catheters (2 mm inside and 3.2 mm outside diameters) obtained from A-M systems (USA)	96-well microtiter plates, incubation for 2, 24, or 48 h	XTT assay for assessment of metabolic activity of yeast included in biofilm	(1) No modification of the metabolic activity of *C. albicans* by caspofungin used at MIC and (2) significant reduction of the metabolism ($p < 0.001$) of 25% (biofilms of 48 h) to 50% (biofilms of 2 h) of the *C. parapsilosis* yeasts at the same concentration of caspofungin
Meran et al. [24]	UK	Coating of silicone facial prostheses with 5 and 50 mg L^{-1} dispersions of either Ag NPs or $AgNO_3$ for assessment of their biocompatibility and antifungal properties	*C. albicans* (NCPF-3179)	Silicone discs (37 mm in diameter) synthesized out of platinum-catalyzed, vinyl-terminated poly (dimethyl siloxane) elastomer (A-2186; Factor II, Lakeside, AZ)	6-well microtiter plates, fibroblast cells grown on the silicone elastomer for 96 h and inoculated with *C. albicans* in the last 24 h	(1) SEM and energy dispersive X-ray spectroscopy for coating characterization; (2) human dermal fibroblasts (Hs68) for examination of the biocompatibility; and (3) measurement of LDH activity, protein content and tissue electrolytes for assessment of the fibroblast viability	(1) No effects on the LDH activity of fibroblast cell homogenates and (2) decrease in ethanol production 43.2±25 in controls to 3.6 μmol mL^{-1} in all the silver treatments
Tran et al. [25]	Australia	Assessment of the effect of coating with selenium nanoparticles on inhibition of bacterial growth	*Staphylococcus aureus* (ATCC, 25923)	Silicone discs (6 mm in diameter and 2 mm in height) cut from silicone tracheostomy tubes (Bivona, number 60PFS30)	24-well microtiter plates, incubation for 8 h in a standard bacteria culture incubator (37°C, 95% humidified air, 5% CO2 environment, non-shaking)	(1) SEM visualization of bacteria on the uncoated and coated substrates and (2) crystal violet assays for bacteria quantification	Significant inhibition of bacterial growth on the Se-coated substrates compared to their uncoated counterparts
Tan et al. [26]	Austria	Assessment of the long-term antibiofilm activity of carboxymethyl chitosan on mixed biofilm	*C. albicans*, *Candida tropicalis*, *Lactobacillus gasseri*, *Streptococcus salivarius*, *Rothia dentocariosa*, and *Staphylococcus epidermidis*	Medical-grade silicone plates (Websinger GmbH, Vienna, Austria) (diameter: 8 mm and thickness: 3 mm)	12-well microliter plates daily reseeding, incubation on an orbital shaker at 155 rpm for 24 h	(1) Digital microscopy camera for monitoring of biofilm growth kinetics surveillance of biofilm growth kinetics for assessment of the biofilm surface coverage and (2) SEM for analysis of biofilm architecture	Less than 4% surface coverage on the CM-chitosan-treated plates in the testing period ($p < 0.05$) in comparison with a maximum of 23% biofilm surface in control group
Tan et al. [55]	Austria	Assessment of the inhibition activity of lactobacilli supernatant against fungal-bacterial multispecies biofilms on silicone surface	*C. albicans*, *C. tropicalis*, *S. salivarius*, *R. dentocariosa*, and *S. epidermidis*	3-mm-diameter medical-grade silicone platelets (Websinger, Austria)	96-well microtiter plates, incubation at 37°C for 48 h	(1) SEM and CLSM for biofilm characterization and (2) CCK-8 method for assessment of cell viability inside the biofilm	(1) Reduction higher than >90% of adhesion (90 min) of mixed fungal and bacterial species by lactobacilli supernatant; (2) reduction up to 72.23 and 58.36% of mixed biofilm formation and the metabolic activity of the biofilms; (3) reduction up to 58.36% in the cell viability in the biofilms after 24 h of culture with supernatant; and (4) inhibition of *Candida* yeast-to-hypha transition
Dijk et al. [59]	The Netherlands	Assessment of the activity of colloidal palladium/tin solution against biofilm formation	*C. albicans*, *C. tropicalis*, *R. dentocariosa*, *Streptococcus sobrinus*, *S. salivarius*, *S. epidermidis*, *Stomatococcus mucilaginosus*, and *Streptococcus mitis*	"Low-resistance"' Groningen button voice prostheses (Medin Instruments and Supplies, Groningen, the Netherlands)	Modified Robbins device, incubation at 37°C under aerobic conditions for 4 days	(1) SEM for the determination of the biofilm formation; (2) CFU per unit valve area for determination of the number of antibacterial and antifungal activities; and (3) cytotoxicity assay with the culture of human skin fibroblasts PK 84 in RPMI 1640 medium	(1) Statistically significant decrease in the number of bacteria ($p < 0.1$) and the number of yeast ($p < 0.001$); (2) similar fibroblast proliferation inhibition index between the treated silicone rubber (20±18%) samples and untreated silicone rubber (2±4%); and (3) no cytotoxicity observed

Table 4 (continued)

Author [Ref]	Country	Prevention method	Species	Test material	Applied in vitro model	Infection-related outcome assessment	Outcome reporting
Sahal et al. [22]	The Netherlands	Investigation of the antifungal and biofilm inhibitory effects of *Cymbopogon citratus*, *Cuminum cyminum*, *Citrus limon*, and *Cinnamomum verum* essential oils on biofilm formed by 3 *C. tropicalis* isolates	*C. tropicalis* T26, *C. tropicalis* U71, and *C. tropicalis* V89	Silicone rubber (M511 Maxillofacial Silicone System; Technovent Ltd., South Wales, UK) for the preparation of 60×60×1.5 mm sheets	24-well microliter plates, incubation for 7 d at 37°C under aerobic conditions	(1) Crystal violet staining for determination of biofilm formation; (2) spectrophotometer by measuring the absorbance at 560 nm for the quantification of the biofilm; (3) contact angle measurement for the assessment of the hydrophilic or hydrophobic characteristics of the surface; and (4) determination of the MIC and MFC of lemongrass oil and the other 3 essential oils	(1) Statistically significant ($p = 0.001$) effect of all essential oils against biofilm formation of different *C. tropicalis* strains; (2) significantly different biofilm inhibition effect of sub-MICs of *C. verum* and *C. citratus* oils in comparison with *C. cyminum* and *C. limon* oils against all tested *C. tropicalis* strains; (3) more significant biofilm inhibition of *C. tropicalis* U71 than other *C. tropicalis* strains; (4) significantly less inhibitory activity of *C. verum* oil against biofilm formation of *C. tropicalis* T26 strain, whereas *C. citratus* oil was significantly more inhibitive against biofilm formation of *C. tropicalis* U71 and *C. tropicalis* V89 strains; (5) no significant difference in biofilm inhibition between different concentrations of the essential oils ($p = 0.980$); and (6) statistically significant biofilm inhibitory effects of 0, 2, 4, and 8% *C. citratus* oil-coated silicone rubber surfaces ($p = 0.001$)
Ajdnik et al. [57]	Slovenia	Encapsulation of co-amoxiclav drug mixture into chitosan nanoparticles. Adsorption of them onto O_2 plasma-activated tympanostomy silicone tubes	*S. aureus* strain (DSM 799)	PDMS as a representative silicone material	24-well microliter plates, incubation on agar plates at 37°C after 24 h and after 1 month	(1) SEM for the evaluation of surface morphology; (2) XPS spectra for the assessment of the surface of the functionalized silicone material; and (3) CFU determination for the assessment of antimicrobial activity	(1) Decrease in bacterial growth up to 93.98% by chitosan nanoparticles with encapsulated drug, as well as by pre-activated O_2 plasma sample with absorbed chitosan nanoparticles with embedded drug and (2) after 1 month, 99.75% decrease in *S. aureus* growth by PDMSCN-CoAM and 67.37% decrease by PDMSPA5, CN-CoAM
Arweiler-Harbeck et al. [16]	Germany	Application of a solid film of gold or titanium metal (layer thickness <100 nm) on silicone voice prostheses by applying an anodic vacuum arc coating	*C. albicans*	Provox voice prostheses	Vacuum chamber, incubation for 1 week	SEM for the assessment of infiltration of *C. albicans* on silicone rubber	(1) Inhibition of growth of *Candida* colonies on homogeneous (gold-coated, titanium-coated) surfaces and (2) restricted inhibition of *C. albicans* colonization on inhomogeneous (aluminum-coated) surfaces

AgNPs, silver nanoparticles; SEM, scanning electron microscopy; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl amino)carbonyl]-2H-tetrazolium hydroxide; AMF, atomic force microscopy; DMSO, dimethyl sulfoxide; MIC, minimum inhibitory concentration; $AgNO_3$, silver nitrate; CCK-8, cell counting kit-8; CFU, colony-forming units; MFC, minimum fungicidal concentration; PDMS, polydimethylsiloxane; XPS, X-ray photoelectron spectroscopy.

Table 5. Characteristics of the included studies: combined active and passive surface modification

Author [Ref]	Country	Prevention method	Species	Test material	Applied in vitro model	Infection-related outcome assessment	Outcome reporting
De Prijck et al. [60]	Belgium	Incorporation of 5 antimycotics in PDMS, either by admixture or by solvent-based impregnation for the prevention of biofilm formation by *Candida albicans*	*C. albicans* SC5314 (ATCC MYA-2876)	Medical-grade PDMS kit (Q7-4735; Dow Corning Corp., Midland, MI, USA) of 10 mm diameter for use in the microtiter plate, 8 mm diameter for the commercial modified Robbins device and 6.8 mm for the custom-made miniaturized modified Robbins device	(1) Microtiter plates and (2) modified Robbins device, incubation at 37°C for 24 h	(1) Calculation of number of colonies and (2) calculation of CFU for all disks	(1) No significant inhibition of biofilm formation in the MRD ($p > 0.05$); (2) strong inhibitory effect on *C. albicans* biofilms in the microtiter plate ($p < 0.05$); (3) low reduction with TMS-nystatin (<1 log unit; significant difference in biofilm biomass only for the highest concentration, $p < 0.05$); (4) reduction higher than 1 log unit ($p < 0.05$) for miconazole, TTO, and zinc pyrithione, with either absent or at best marginal concentration-dependent effect; and (5) higher decrease in biomass in the MTP (approximately 4 log units) than in the MRD (<2 log units) for zinc pyrithione-loaded disks
Alves et al. [33]	Portugal	Immobilization of LAmB on PDMS surfaces for prevention of *C. albicans* colonization	*C. albicans* SC 5314	PDMS (kit Sylgard 184; Dow Corning, USA) cut into circle pieces with (0.9 cm diameter and with 0.3 cm thickness)	48-well microtiter plates, PDMS (kit Sylgard 184; Dow Corning, USA) cut into circle pieces with (0.9 cm diameter and 0.3 cm thickness)	(1) SEM for analysis of surface morphology of materials; (2) contact angle measurement for assessment of the hydrophilic or hydrophobic characteristics of the surface; (3) fibroblast cells 3T3 obtained from ATCC for cytotoxicity evaluation; (4) fluorescence microscopy for the assessment of the ability of modified surfaces to interfere with *C. albicans* adhesion and/or viability on the PDMS surfaces; and (5) CFU counting for enumeration of the yeast cells adhered to the surfaces for the investigation of the potential of the modified surfaces to impair biofilm formation	(1) Approximately 3 log CFU reduction of fungal attachment by LAmB immobilization; (2) no impartment of the surfaces of PDMS with antifungal features with simple adsorption of LAmB; and (3) less roughness and more hydrophilic features with functionalization of surfaces with LAmB
Ceresa et al. [61]	Italy	The synergistic effect of a lipopeptide from AC7BS combined with the quorum-sensing molecule farnesol against the formation of *C. albicans* biofilms	*C. albicans* IHEM 2894, *C. albicans* 40-DSM 29204, and *C. albicans* 42-DSM 29205	Silicone discs (15 mm diameter, 1.5 mm thickness; TECNOEXTR S.r.l., Italy)	12-well microliter plates, incubation at 37°C for 24 and 48 h	(1) Viable count method for the evaluation of anti-adhesive and anti-biofilm properties of AC7BS, farnesol, and their combination after 1.5, 24, and 48 h; (2) SEM and CLSM for fungal biofilm characterization; and (3) LDH assay for evaluation of cytotoxicity on human cell lines using normal lung fibroblasts (MRC5), according to TOX7 operative procedures	(1) Reduction of *C. albicans* adhesion up to 74% at 1.5 h; (2) reduction of biofilm growth up to 93% at 24 h and 60% at 48 h; (3) SEM and CLSM for the confirmation of the synergistic anti-adhesive and anti-biofilm activity; and (4) no cytotoxicity on eukaryotic cells after exposures to AC7BS concentrations up to 0.5 mg mL^{-1}

PDMS, polydimethylsiloxane; MRD, modified Robbins device; MTP, microtiter plate; SEM, scanning electron microscopy; CLSM, confocal laser scanning microscopy; LAmB, liposomal amphotericin B; CFU, colony-forming units; TMS-nystatin, trimethylsilyl-nystatin.

Table 6. Quality assessment of the included studies (part 1)

	Cocuaud et al. [53]	Vargas-Blanco et al. [48]	Contreras-García [52]	Ajdnik et al. [50]	Lara et al. [47]	Xu et al. [46]	Depan et al. [49]	De Prijck et al. [29]	Busscher et al. [60]	Ceresa et al. [19]	Janek et al. [18]	Rodrigues et al. [44]	Taylor et al. [51]	Cochis et al. [20]	Dijk et al. [59]	Everaert et al. [23]	Buijssen et al. [55]
Abstract	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	Yes
Background	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Objectives	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Methods intervention	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Outcomes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sample size	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Randomization: sequence generation	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Allocation concealment mechanism	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Implementation	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Blinding	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Statistical methods	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Outcomes and estimation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Limitations	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Funding	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Protocol	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No

Table 7. Quality assessment of the included studies (part 2)

	Meran et al. [24]	Wannemuehler et al. [62]	Gottenbos et al. [48]	Tan et al. [55]	Rodriguez et al. [42]	Zhou et al. [30]	Tran et al. [25]	Alves et al. [33]	Arweiler-Harbeck et al. [16]	Contreras-García et al. [46]	Everaert et al. [64]	Ceresa et al. [61]	Rodrigues et al. [43]	Tan et al. [26]	De Prijck et al. [60]	Sahal et al. [22]	Oosterhof et al. [49]
Abstract	No	Yes	No	No	No	Yes	No	No	No	No	No	No	No	Yes	No	Yes	No
Background	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Objectives	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Methods intervention	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Outcomes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sample size	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Randomization: sequence generation	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Allocation concealment mechanism	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Implementation	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Blinding	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Statistical methods	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Outcomes and estimation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Limitations	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Funding	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Protocol	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. mean difference IV, random, 95% CI
2.1.1 Active surface modification								
Dijk 2000	2.903	3.342	10	3.623	2.301	10	6.8%	–0.24 [–1.12, 0.64]
Subtotal (95% CI)			**10**			**10**	**6.8%**	**–0.24 [–1.12, 0.64]**
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.54$ ($p = 0.59$)								
2.1.2 Passive surface modification								
Buijssen 2017	5.722	5.407	4	5.944	2.301	4	5.3%	–0.05 [–1.43, 1.34]
Cochis 2012 AC5	4.518	2	12	6.353	1.659	12	6.9%	–0.96 [–1.82, –0.11]
Cochis 2012 AC7	4.835	1.147	12	6.353	1.659	12	6.8%	–1.03 [–1.89, –0.17]
Cochis 2012 CHX	5.576	1.659	12	6.353	1.659	12	7.0%	–0.45 [–1.26, 0.36]
Cochis 2012 OC5	4.624	1,218	12	6.353	1.659	12	7.0%	–0.00 [–0.80, 0.80]
De Prijck 2010 polyDMAEMAq	5.69	0.58	8	6.79	0.48	8	5.7%	–1.95 [–3.21, –0.70]
Oosterhof 2006 Biocidal	4.784	4.756	4	5.58	2.301	4	5.3%	–0.19 [–1.58, 1.21]
Oosterhof 2006 QAS	4.659	4.625	4	5.58	2.301	4	5.3%	–0.22 [–1.61, 1.17]
Rodriquez 2004 L.lactis	4.322	2.201	3	5.146	2.301	3	4.7%	–0.29 [–1.92, 1.33]
Rodriquez 2004 S.thermo	4.561	2.248	3	5.146	2.301	3	4.7%	–0.21 [–1.82, 1.41]
Zhou 2010	6.683	0.232	8	7.338	0.166	8	4.9%	–3.07 [–4.64, –1.50]
Subtotal (95% CI)			**82**			**82**	**63.6%**	**–0.73 [–1.21, –0.25]**
Heterogeneity: $\tau^2 = 0.30$; $\chi^2 = 19.26$, df = 10 ($p = 0.04$); $I^2 = 48\%$								
Test for overall effect: $Z = 2.97$ ($p = 0.003$)								
2.1.3 Active/passive surface modification								
Alves LAmB 2019	1.333	1.6	9	5.333	0.467	9	5.0%	–3.23 [–4.74, –1.72]
Ceresa 2018	7.937	0.126	18	8.0696	0.676	18	7.4%	–0.27 [–0.92, 0.39]
De Prijck 2009 Micon	4.02	0.46	9	6.06	0.61	9	4.7%	–3.60 [–5.22, –1.98]
De Prijck 2009 nyst	5.05	0.22	7	6.06	0.61	7	5.3%	–2.06 [–3.44, –0.68]
De Prijck 2009 TTO	4.05	0.37	3	6.06	0.61	3	1.9%	–3.19 [–6.66, 0.28]
De Prijck 2009 Zinc	4.48	0.08	11	6.06	0.61	11	5.3%	–3.49 [–4.91, –2.08]
Subtotal (95% CI)			**57**			**57**	**29.6%**	**–2.53 [–4.02, –1.03]**
Heterogeneity: $\tau^2 = 2.74$; $\chi^2 = 34.23$, df = 5 ($p < 0.00001$); $I^2 = 85\%$								
Test for overall effect: $Z = 3.32$ ($p = 0.0009$)								
Total (95% CI)			**149**			**149**	**100.0%**	**–1.20 [–1.73, –0.66]**
Heterogeneity: $\tau^2 = 0.90$; $\chi^2 = 63.69$, df = 17 ($p < 0.00001$); $I^2 = 73\%$								
Test for overall effect: $Z = 4.37$ ($p < 0.0001$)								
Test for subgroup differences: $\chi^2 = 6.74$. df = 2 ($p = 0.03$), $I^2 = 70.3\%$								

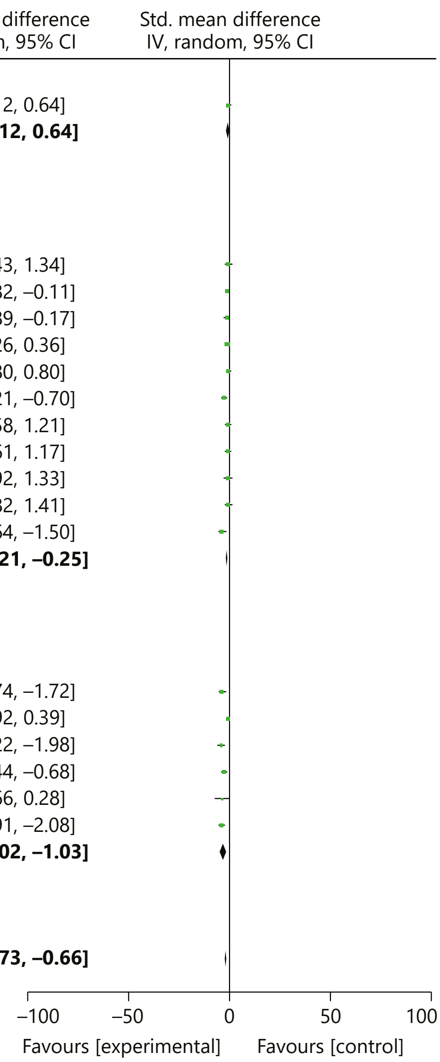

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Fig. 2. Forest plot of SMDs for the assessment of methods of yeast biofilm inhibition. SMD, standardized mean difference; IV, inverse variance; SD, standard deviation; CI, confidence interval.

bition of other bacteria and 8–9% inhibition of *C. albicans* growth to silicone with the highest concentration tested (0.5 mg/mL) [18]. Biosurfactants obtained from the probiotic bacteria *Lactococcus lactis* 53 and *Streptococcus thermophilus A* decreased the amount of bacteria in the biofilm to 4 and 13% and the amount of fungal organisms to 15 and 26% of the control, respectively [43]. Additionally, the application of sophorolipids on silicone surfaces at concentrations between 0.025 and 0.1% w/v in co-incubation experiments using SLA 0.05% w/v led to 90–95% reduction of *S. aureus* and *C. albicans* biofilm formation [19] (Tables 2, 3).

Active Surface Modification

Only few methods of active surface modification managed to inhibit biofilm growth by 80%. With the exception of encapsulation of co-amoxiclav drug mixture into chitosan nanoparticles, which led to decrease in bacterial growth up to 93.98%, the rest of the methods exhibited weaker antibiofilm activity [57]. The application of lactobacilli supernatant against fungal-bacterial multispecies biofilms on silicone surface achieved a reduction up to 72.23% of mixed biofilm formation. Moreover, the application of silver nanoparticles against *Candida auris* biofilm formation [55] and the application of caspofungin

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. mean difference IV, random, 95% CI
1.1.1 Passive surface modification								
Ajdnik 2019 PDMS + CN	4.621	6.792	3	4.751	5.123	3		Not estimable
Ajdnik 2019 PDMS + CN-CoAM	3.593	6.792	3	4.751	5.123	3		Not estimable
Ajdnik 2019 PDMS + PA1, CN	4.407	6.792	3	4.751	5.123	3		Not estimable
Ajdnik 2019 PDMS + PA1, CN-CoAM	3.439	6.792	3	4.751	5.123	3		Not estimable
Ajdnik 2019 PDMS + PA5, CN	5.519	6.792	3	4.751	5.123	3		Not estimable
Ajdnik 2019 PDMS + PA5, CN-CoAM	3.286	6.792	3	4.751	5.123	3		Not estimable
Dijk 2000	4.978	4.623	10	5.196	4.23	10	50.3%	–0.05 [–0.92, 0.83]
Subtotal (95% CI)			**10**			**10**	**50.3%**	**–0.05 [–0.92, –0.83]**
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.11$ ($p = 0.92$)								
1.1.2 Passive surface modification								
Buijssen 2017	7.079	6.792	4	7.301	5.123	4	20.1%	–0.03 [–1.42, 1.35]
Depan 2013 Silicone – 5wt% Titania	5.507	5.037	6	5.984	5.123	6		Not estimable
Oosterhof 2006 Biocidal	5.754	5.827	4	6.322	4.23	4		Not estimable
Oosterhof 2006 QAS	5.879	5.526	4	6.322	4.23	4		Not estimable
Rodriquez 2004 L.lactis	5.428	2.738	3	6.826	5.123	3	14.7%	–0.27 [–1.89, 1.35]
Rodriquez 2004 S.thermo	5.94	3.028	3	6.826	5.123	3	14.9%	–0.17 [–1.78, 1.44]
Subtotal (95% CI)			**10**			**10**	**49.7%**	**–0.14 [–1.03, 0.74]**
Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.05$, df = 2 ($p = 0.98$); $I^2 = 0\%$								
Test for overall effect: $Z = 0.32$ ($p = 0.75$)								
Total (95% CI)			**20**			**20**	**100.0%**	**–1.10 [–0.72, 0.53]**
Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.07$, df = 3 ($p = 0.99$); $I^2 = 0\%$								
Test for overall effect: $Z = 0.30$ ($p = 0.76$)								
Test for subgroup differences: $\chi^2 = 0.02$. df = 1 ($p = 0.88$), $I^2 = 0\%$								

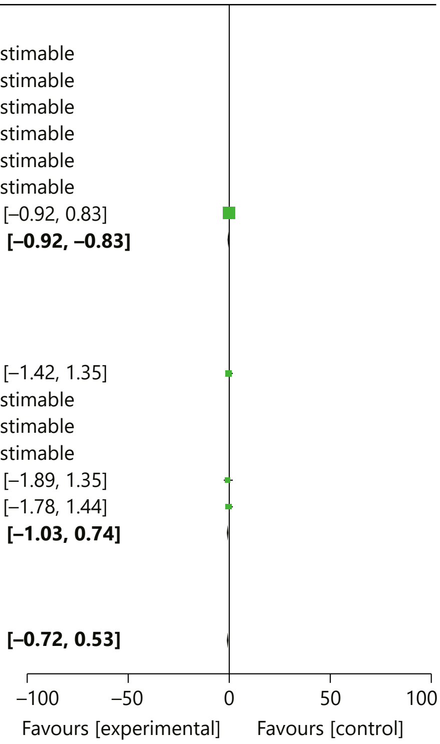

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Fig. 3. Forest plot of SMDs for the assessment of methods of bacterial biofilm inhibition. SMD, standardized mean difference; IV, inverse variance; SD, standard deviation; CI, confidence interval.

against *C. albicans* and *Candida parapsilosis* biofilms [54] led to reduction of biofilm formation up to 50% (Table 4).

Combined Active and Passive Surface Modification

Lipopeptide from *B. subtilis* AC7 combined with the quorum-sensing molecule farnesol [61] achieved an effective reduction of fungal biofilm growth up to 93% (Table 5).

Quantitative Synthesis of the Results

Assessment of Methods of Yeast Biofilm Inhibition

For the assessment of yeast biofilm inhibition methods, data from 10 studies investigating 18 different methods were synthesized and based on the surface modification method (active, passive, and active/passive) examined in each study, classified the studies into 3 subgroups. Statistical pooling was possible for 298 disks. There was a statistically significant difference in favor of the intervention group (SMD −1.20; 95% CI [−1.73 to −0.66]; $p < 0.0001$). In these analyses, heterogeneity was considered substantial ($I^2 = 73\%$, $p < 0.00001$). The larger effect size (SMD = −3.60, 95% CI [−5.22, −1.98]) was in favor of the prevention method with impregnation of miconazole in the study conducted by De Prijck et al. [60]. On the other hand, the smallest effect size (SMD = −0.00, 95% CI [−0.80, 0.80]) was found in the study conducted by Cochis et al. [20] with the precoating with biosurfactants obtained from endophyte biofilms selected from *Robinia pseudoacacia* and from *Nerium oleander*. In the subgroup analysis, we found out that combined active and passive surface modification methods are the most effective in yeast biofilm formation inhibition (SMD = −2.53, 95% CI [−4.02, −1.03], $p = 0.0009$) (Fig. 2).

Assessment of Methods of Bacterial Biofilm Inhibition

For the assessment of bacterial biofilm inhibition methods, data from 6 studies investigating 13 different methods were synthesized. Statistical pooling was possible for 104 disks, and based on the surface modification

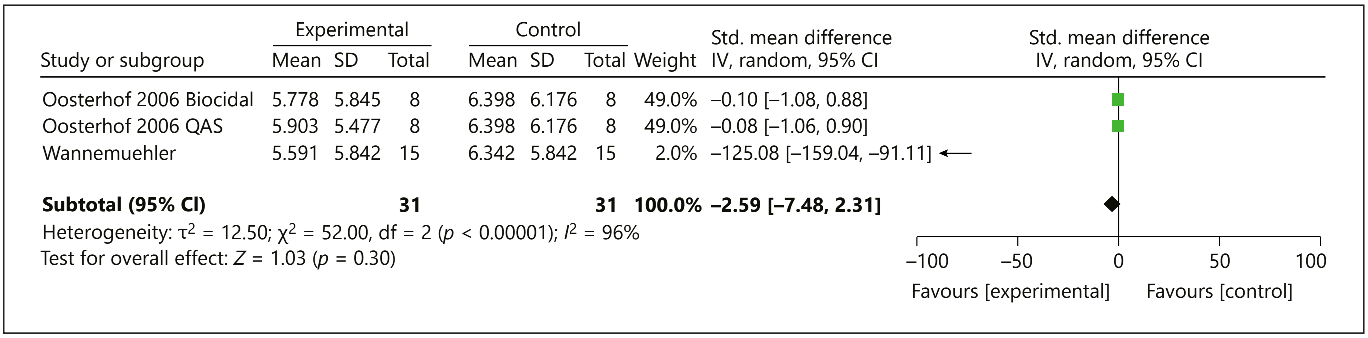

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Fig. 4. Forest plot of SMDs for the assessment of methods of mixed biofilm inhibition. SMD, standardized mean difference; IV, inverse variance; SD, standard deviation; CI, confidence interval.

method, 2 subgroups were created. There was a statistically insignificant difference in favor of prevention methods in comparison with the control group (SMD = −0.09, 95% CI [−1.07, 0.89], $p = 1.00$). In these analyses, heterogeneity was considered not important ($I^2 = 0\%$, $p = 1.00$). The larger effect size (SMD = −0.27, 95% CI [−1.89, 1.35]) was in favor of the precoating method with biosurfactants obtained from *L. lactis*. The lower effect size (SMD = −0.02, 95% CI [−1.62, 1.58]) was found in the study conducted by Ajdnik et al. [57], with the use of chitosan and sodium tripolyphosphate nanoparticles only. For this analysis, we detected no significant heterogeneity levels ($I^2 = 0\%$). From the subgroup analysis, there were no notable differences between the different surface modification methods (Fig. 3).

Assessment of Methods of Mixed Biofilm Inhibition

There was a statistically significant difference in favor of prevention methods in comparison with the control group (SMD = −2.59, 95% CI [−7.48, 2.31], $p = 0.30$). For this analysis, we detected substantial heterogeneity levels ($I^2 = 96\%$, $p < 0.00001$) (Fig. 4).

Discussion

With a view to reduce microorganism colonization and potentially improve device survivorship, researchers have exerted substantial effort to improve the antifouling properties of silicone rubber voice prostheses over the last decades. Given the high failure rates of silicone rubber voice prostheses due to microbial biofilm development and the paucity of human literature in this field, we felt that a systematic review of in vitro evidence was warranted. Therefore, we sought to explore the efficacy of the applied in vitro prevention methods and to compare their effect on biofilm inhibition where it was possible.

In the present systematic review, we summarized data from 33 published in vitro studies, 12 of which qualified for meta-analysis. To reliably evaluate the efficacy of the presented methods, the cutoff value of 80% was deemed to be a meaningful inhibition threshold when compared with controls [65]. A significant proportion of studies looking at the prevention potential of the passive and combined active and passive surface modification methods reached the above inhibition rate. On the contrary, only one of the included studies utilizing active surface modification methods achieved an equally strong antibiofilm activity [57]. Those results were verified with the overall meta-analysis findings and especially with subgroup analysis results. Consequently, we reached the conclusion that active surface modifications have a limited effect on the inhibition of biofilm formation, whereas the antibacterial ability of passive and especially combined active and passive surface modifications is sufficient enough to allow for predictable biofilm inhibition. Therefore, we advocate that future experimental studies should focus on passive and combined active and passive surface modification methods in order to improve the antifouling properties of silicone rubber.

Furthermore, fungal species are the most commonly isolated microorganisms of voice prostheses, with a prevalence of 72.9% [9]. However, in clinical practice, biofilms isolated from those devices are polymicrobial communities [10]. From the assessment of the efficacy of novel prophylactic techniques against microbial biofilms, the quantitative synthesis proved that the majority of those techniques were especially effective against the formation of yeast and mixed microbial biofilms. Taking into consideration these facts, those results show great promise for application on silicone rubber voice prostheses and must be considered as ideal candidates for the elongation of the lifetime of those devices. The limited existing in vivo studies on laryngectomized humans [66] or rats [67] confirm this hypothesis, since they have demonstrated that coating in human patients resulted in a significant reduction of biofilm formation on silicone rubber voice prostheses.

Strengths and Limitations of the Present Systematic Review

The present study described novel biofilm inhibition methods that have been tested over the last decades and summarized their in vitro influence on biofilm formation on silicone rubber surfaces, which could predict the clinical behavior of those techniques on voice prostheses in vivo. It is worthy of note that sample size was adequate enough to allow us to test our hypothesis. We performed a broad search, and we attempted to maintain low levels of clinical diversity [68] by applying stringent inclusion criteria for meta-analysis to decrease heterogeneity of the data.

However, in the included in vitro studies, many domains were deemed to be at a medium or high risk of bias, since they are not reported correctly in the studies. This finding seems to be usual in systematic reviews of laboratorial studies [69]. The main reason in the current study is the lack of information about sample size calculation methods, randomization, sequence generation, allocation concealment, implementation, and blinding and should be carefully considered in future in vitro studies. On top of that, considering the methodological variability among studies, heterogeneity was unavoidable. As a result, pooled results showed that there were significant statistical heterogeneity levels, especially in the assessment of yeast biofilm inhibition methods. Thus, the intervention effect in this case was significantly affected by the factors that varied across the included studies, and we suggest that the results of the present systematic review be interpreted with caution. To address heterogeneity issues and selection bias, we suggest that future authors utilize a randomized controlled clinical trial study design and consider not only comprehensive registration protocols but also core outcome sets [70].

Another source of potential bias identified in the current study is the lack of reporting of quantifiable data. For transparency and clarity in reporting, we advocate that authors report their results in a more comprehensive and statistically sound manner. To elaborate not only mean/median values but also measures of variations need to be presented to allow for reliable conclusions to be drawn.

Implications for Future Research

In the current study, we sought to compare the effects of prevention methods on biofilm formation on silicone rubber surfaces exclusively in vitro. Studies executed in vivo on humans [66, 71–73] or animals [67] were excluded. Thus, the in vivo efficacy and long-term effects of these techniques both on host's cells and on bacterial resistance need to be further investigated before clinical applications and market introduction. Many studies have demonstrated that body fluids, proteins, enzymes, electrolytes, and lipids in vivo can potentially corrode biomaterials including silicone elastomers, leading to the roughness of the surface of biomaterials, thus making it more easily for bioactive compounds to attach to the surfaces of the corroded biomaterials [74]. Consequently, high-quality clinical studies examining the in vivo efficacy of the tested prevention methods are warranted for their further introduction in the clinical practice and for the extrapolation of our findings to human biology. Our systematic review could be used to better guide these clinical trials.

Conclusion

Great progress has been made in the last decades in the development of novel techniques aiming to protect silicone rubber voice prostheses from bacterial and fungal colonization. The current systematic review and meta-analysis provides an overview of the existing in vitro experimental studies exploring the inhibition of biofilm formation on silicone rubber surfaces. Various prevention methods present efficacy in vitro, and their possible application in clinical practice shows excellent promise. More specifically, the results of the qualitative and quantitative syntheses support evidence that the prevention of yeast and mixed microbial biofilm formation is especially active on silicone rubber surfaces. Should the efficacy and safety of those methods get tested and also approved in vivo, those techniques could extend the lifespan of silicone rubber prostheses, improving the quality of life of laryngectomized patients by reducing the frequent replacements of voice prostheses and decreasing healthcare costs. Finally, since passive and combined active and passive surface modification methods seem to be especially effective for the inhibition of yeast biofilm formation on silicone surfaces, we indicate that future studies should aim toward this direction, given the high prevalence of *Candida* strains on the colonization of silicone rubber prostheses.

Statement of Ethics

The paper is exempt from Ethical Committee approval because it does not involve experiments with humans and animals.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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Author Contributions

A.T. is the primary author. The supervisors of this study are E.P., L.D., and C.S., and this systematic review was conducted under their close guidance. K.T. contributed with his knowledge on meta-analysis to the quantitative synthesis as well as conducted a major review on the paper. C.F. assisted the primary author with the data extraction and quality assessment. Finally, G.M. contributed to this study with his guidance in specific scientific issues. All authors read and approved the final manuscript.

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