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Adhesion of *Candida Albicans* to digital versus conventional acrylic resins: a systematic review and meta-analysis

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Abstract

Background The present systematic review and meta-analysis investigated the available evidence about the adherence of *Candida Albicans* to the digitally-fabricated acrylic resins (both milled and 3D-printed) compared to the conventional heat-polymerized acrylic resins.

Methods This study followed the guidelines of the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA). A comprehensive search of online databases/search tools (Web of Science, Scopus, PubMed, Ovid, and Google Scholar) was conducted for all relevant studies published up until May 29, 2023. Only in-vitro studies comparing the adherence of *Candida albicans* to the digital and conventional acrylic resins were included. The quantitative analyses were performed using RevMan v5.3 software.

Results Fourteen studies were included, 11 of which were meta-analyzed based on Colony Forming Unit (CFU) and Optical Density (OD) outcome measures. The pooled data revealed significantly lower candida colonization on the milled digitally-fabricated compared to the heat-polymerized conventionally-fabricated acrylic resin materials (MD = -0.36; 95%CI = -0.69, -0.03; $P=0.03$ and MD = -0.04; 95%CI = -0.06, -0.01; $P=0.0008$; as measured by CFU and OD respectively). However, no differences were found in the adhesion of *Candida albicans* between the 3D-printed digitally-fabricated compared to the heat-polymerized conventionally-fabricated acrylic resin materials (CFU: $P=0.11$, and OD: $P=0.20$).

Conclusion The available evidence suggests that candida is less likely to adhere to the milled digitally-fabricated acrylic resins compared to the conventional ones.

Keywords Candida Albicans, Digital denture, CAD-CAM, Acrylic resin, Prosthodontics

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Background

The rapid and huge progress in all aspects of technology imposes a growing interest in using computer-aided design and computer-aided manufacturing (CAD/CAM) for fabricating removable digital dentures [1]. According to the Glossary of Digital Dental Terms, “digital denture” refers to a dental prosthesis fabricated through automation using CAD/CAM and computer-aided engineering [2]. Digital dentures can be fabricated using subtractive manufacturing, whereby the bulk denture resin is removed according to the designated denture in a milling machine [3]. In contrast, additive manufacturing involves incrementing the resin as layers with photopolymerization depending on the type of 3D printer, either by stereolithography or digital light processing (DLP) [4, 5]. Despite the differences in fabrication techniques, the chemical composition of the resins used is almost similar [6].

Studies have reported that the application of milling and 3D printing in the fabrication of dentures results in excellent surface adaptation, comparable strength to the conventional ones, and good clinical outcomes [7], with high levels of patient satisfaction [8]. According to the dental literature, the milled resin exhibits more stability and less polymerization shrinkage compared to the 3D-printed resin [9]. The milled acrylic resin is made of pre-polymerized blocks which minimize or even lack the after-processing shrinkage, while in the 3D-printed resin, the polymerization process may be less controllable, which can lead to variations in shrinkage. In addition, the surface properties of the milled dentures are superior to those of the 3D-printed dentures [10]. However, several factors such as the type of a printer, build angulation, and a layer thickness may affect the surface and mechanical properties [11].

Denture-related stomatitis (DS) is a primary concern when it comes to removable prostheses. The prevalence of DS among denture wearers ranges from 15 to 70% [12]. It is more common among the elderly population, and can, interestingly, increase the risk of systemic infection [13]. DS is a multifactorial disease with the predominantly associated factors are, among others, poor denture hygiene, continuous night-time denture wearing, and *Candida* infection [12]. DS can develop faster than previously reported, even with new dentures; continued denture wearing and poor cleaning of dentures revealed a considerable impact on DS onset, with *Candida albicans* (*C. albicans*) as the most identified kind of yeast [14]. Indeed, *Candida* adhesion and proliferation on the surface of the acrylic denture base can lead to inflammation of the oral tissue, especially of the denture-fitting surface [15, 16]. This issue is controversial; however, recent

studies suggested that the adherence of multiple species of microbes on the denture surface leads to the pathogenesis of denture stomatitis [17–19].

Candida albicans is the key pathogen implicated in the development of denture stomatitis [20, 21]. In this context, several studies have reported lower *Candida* adhesion to milled dentures compared to the conventional ones [18, 22, 23], with inconsistent results regarding the 3D-printed dentures [19, 24–26]. Other studies suggested that *C. albicans* has almost similar adherence on the 3D-printed and the milled denture surfaces [11, 18]. However, it is worth to note that the milled dentures have less adherence affinity, thus reducing the risk of DS occurrence [27], while the adherence affinity is high on the 3D-printed dentures [28], especially if the print orientation is not optimized, thus increasing the risk of DS [11]. Collectively, although the digital dentures have shown promising results, the long-term outcomes and clinical performance are still lacking. Moreover, since the digital dentures are new to the field, there has been no concrete evidence on the extent of *Candida* adherence on their surfaces so far [22, 23, 27–36]. Therefore, this systematic review and meta-analysis aimed to assess the available evidence regarding *Candida* adherence to the digitally-fabricated acrylic resins (both milled and 3D-printed) in comparison to conventional heat-polymerized acrylic resins.

Methodology

The registration and focused question

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [37]. The protocol of this systematic review was registered in the PROSPERO registry (ID: CRD42023390907). The focused research question is, “Does digital acrylic resin (milled and 3D-printed) have higher fungal adherence affinity than conventional heat-polymerized acrylic resins?”

Eligibility criteria

The inclusion criteria were as follows: a) Controlled in-vitro studies that compared candidal colonization on the digital (milled or 3D-printed) acrylic with the conventional heat-polymerized acrylic resins, and b) Articles published in English with no time limits. The exclusion criteria were: review articles, editorials, commentaries, abstracts, case reports, uncontrolled studies, in-vivo studies, and studies published in a language other than English. The PICOS framework was used to formulate the research question as follows: Population (P): digital

acrylic resin (milled or 3D-printed); Intervention (I): exposure to candidal culture; Comparator (C): conventional heat-polymerized acrylic resin; Outcome (O): candida growth; and study design (S): a controlled in-vitro study.

Search strategy and information sources

Two investigators conducted an independent, yet meticulous and thorough search of multiple online databases/search engines (Web of Science, Scopus, PubMed, Ovid, and Google Scholar) for all relevant studies published up until May 29, 2023. Different combinations of the following keywords were used with the aid of the Boolean operators (AND, OR): (“CAD-CAM denture” OR “CAD/CAM denture” OR “digital denture” OR “3d printed denture” OR “printed denture” OR “printed resin” OR “milled denture” OR “milled resin” OR “conventional heat-polymerized acrylic resin” OR “conventional resins” OR “conventional denture” OR “heat-polymerized acrylic resin” OR “heat-polymerized acrylic denture”) AND (“antimicrobial” OR “adhesion” OR “antifungal” OR “candida” OR “colonization”). Table 1 in Supplementary file 1 provides a detailed description of the search strategy in the different databases/search engines.

Screening and selection process

The studies retrieved were exported to the EndNote program, and the duplicates, if any, were removed. Two investigators independently screened the remaining studies, based on the title and abstract, to identify the relevant articles. The full-texts of the potentially relevant studies were retrieved and assessed for inclusion. In addition, a manual search of the reference lists of the included studies was performed to identify any additional relevant studies.

Data extraction

Two reviewers extracted the following data independently: the name of the author(s), publication year, type of acrylic resins used, number of samples per group, dimensions of the specimen, type, and number of microbial isolates, time, temperature, and concentration of candidal exposure, and the measurement method of efficiency. Concerning Wei et al. study [36], the numerical data were extracted from their figures using a semi-automated online tool called “WebPlotDigitizer”, available at <https://apps.automeris.io/wpd/> (Supplementary Fig. 1). Concerning Koujan et al. study [27], the standard errors of the means were converted to standard deviations following the formula: $SE = SD/\sqrt{n}$. With regard to Linder study [34], the differences between the groups were not reported completely; so that the mean, SD, and N were used for pairwise comparisons between the digital and conventional groups using GraphPad Prism 9.5.0

(GraphPad Software, San Diego, CA, USA) to obtain clear comparison results (Supplementary file 2). The extracted data were then tabulated for further analysis.

Risk of bias assessment

Two reviewers independently and thoroughly assessed the risk-of-bias of the included studies utilizing the QUIN tool. Discrepancies, if any, were resolved by group discussion. The QUIN tool consists of 12 criteria recommended for assessing the risk-of-bias of in-vitro studies conducted in dentistry [38]. According to the nature of the included studies, two criteria, namely “Detailed explanation of sampling technique” and “Randomization”, were excluded as being inapplicable. Each criterion is given a score of 2 points if adequately specified, 1 point if inadequately specified, and 0 points if not specified. The inapplicable criteria were excluded from the calculation. The criteria individual scores were then added to obtain a total score for a given in-vitro study. This total score was recalculated out of 100% according to the following formula:

$$\text{Final score} = \frac{\text{Total score} \times 100}{2 \times \text{number of criteria applicable}}$$

The included studies were qualified as having “low risk of bias”, “medium risk of bias”, or “high risk of bias” if they scored >70%, from 50 to 70%, or <50%, respectively.

Statistical analysis

The quantitative analyses were conducted using Review Manager (RevMan) v5.3 software program for Windows. Only studies that used CFU or OD values as outcome measures were included in the analysis. The pooled mean differences (MD) were calculated as a summary estimate between the digital and the conventional resins. According to the type of outcome measure, two separate meta-analyses were conducted; one for the CFU outcomes, and the other for OD outcomes. Additionally, subgroup analysis was utilized for the 3D-printed and milled resins groups separately. The summary estimates were reported along with their corresponding 95% confidence intervals (CIs). Heterogeneity among the studies was evaluated using the χ^2 test I^2 statistic. A fixed-effect model was applied for insignificant heterogeneity ($I^2 \leq 50\%$), while a random-effect model was used for significant heterogeneity ($I^2 > 50\%$). In addition, StataMP-64 was used for Egger’s test to quantitatively identify the publication bias of the included studies. The significance level was set at a P -value <0.05.

Results

Study selection

Figure 1 depicts the search strategy employed following the PRISMA guidelines. The online search yielded a total

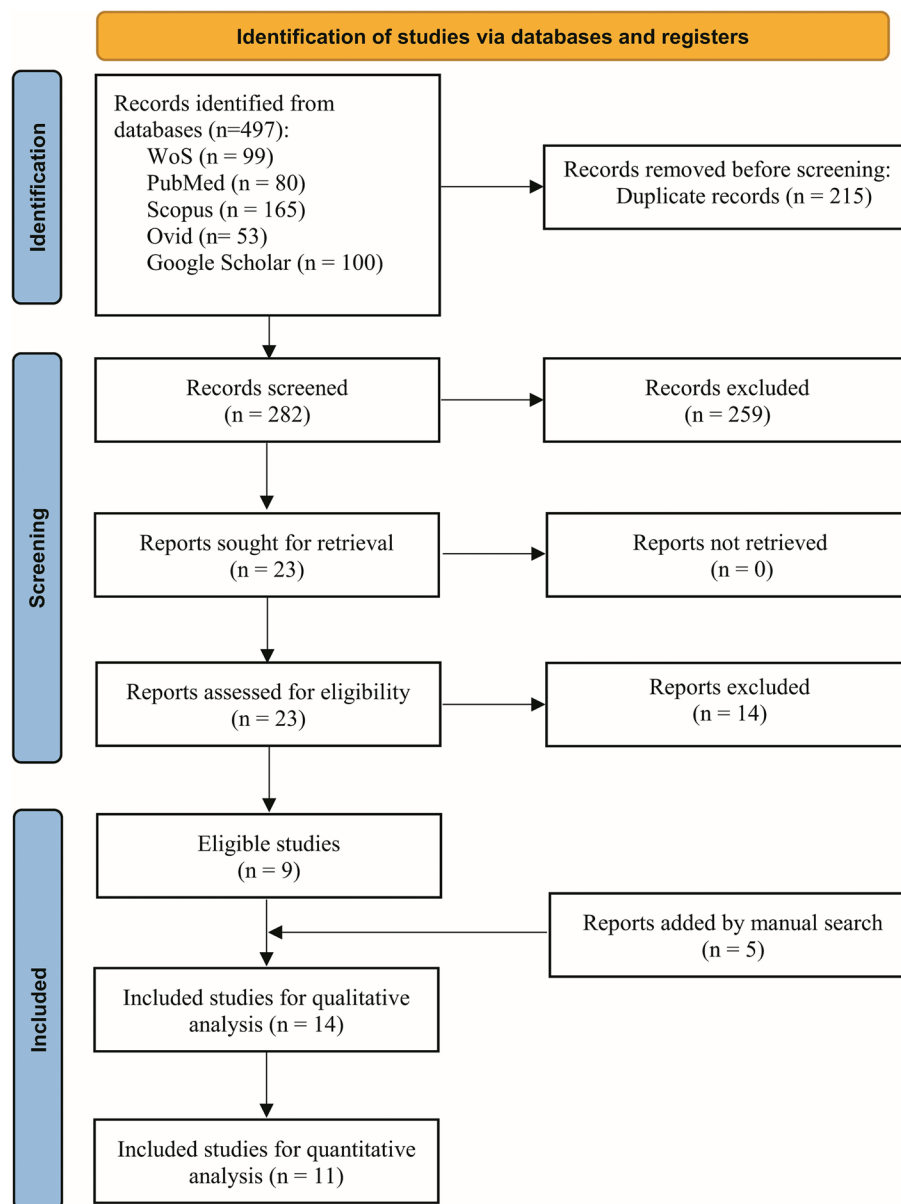


Fig. 1 Flow chart of the search strategy

of 497 studies; 215 of which were removed owing to being duplicates. Among the remaining 282 studies, 259 were excluded based on screening their titles and abstracts. The full-texts remaining and potentially relevant 23 studies were retrieved and assessed for eligibility. Of those, 14 studies were excluded for various reasons detailed in the Supplementary file 1: Table 2. A manual search yielded an additional 5 articles meeting the inclusion criteria. As a result, 14 studies were included for qualitative analysis, and 11 of which were included in the quantitative analysis.

Characteristics of the included studies

Table 1 presents the general characteristics of the included studies. There were 14 studies, including 35 independent comparison groups, published between 2014 and 2023. Five studies [22, 23, 33, 35, 36] used only milled acrylic resin, two studies [32, 40] used only 3D-printed acrylic resin, while 7 studies [27–31, 34, 39] used both milled and 3D-printed acrylic resins. The sample sizes varied from 4 to 15 bars/discs with different dimensions. Regarding the microbial isolate, 2 studies [22, 33] used four strains of *C. albicans*, while the other 12 studies [23, 27–32, 34–36, 39, 40] used only one strain.

Table 1 Characteristics of the included studies

Author, Year (Country)	Digital acrylic resin	Conventional acrylic resin	Sample size (dimensions in mm)	Microbial isolate (number of strains)	Measurement unit	Time of exposure Temperature (Concentration)	Polish/non-polish Saliva coated Stationary/dynamic	Main outcome
Makke, 2014 (USA) [35]	Milled (AvaDent)	Eclipse Light-cured Lucitone FRS flexible SR Ivocap High Impact Nature-CRYL Pour DC Acrylic S.P. Clear Ivocap	15 bars (5 × 15 × 2 mm)	<i>Candida albicans</i> (1)	Adhesion percent	1 hrs at 30 °C (1 × 10 ⁶ cells/mL)	Non-polish Saliva coated Stationary	A significant lower candida colonization in CAD-CAM group (P < 0.05).
Al-Fouzan et al., 2017 (KSA) [22]	Milled (VivaDent)	Heat-polymerized AR	10 discs (10 × 3 mm)	<i>Candida albicans</i> (4)	CFU/mL	90 min at 37 °C (1 × 10 ⁷ cells/mL)	Polished NR Dynamic	A significant lower Candida in the milled group (P < 0.05).
Murat et al., 2019 (Turkey) [23]	Milled (M-P-M) Milled (Polident) Milled (AvaDent)	Heat-polymerized AR	10 discs (10 × 2 mm)	<i>Candida albicans</i> (1)	cell count per field	2 hrs at 37 °C (NA)	Finish at 800-grit-saliva coated/non-coated Dynamic	A significant lower Candida in the milled group (P < 0.05).
Jung, 2020 (USA) [31]	Milled (AvaDent) 3D printed (Dentca) DLP printed (Lucitone)	Heat-polymerized AR	12 bars (10 × 10 × 2 mm)	<i>Candida albicans</i> (1)	CFU/mL	24 hrs at 37 °C (1 × 10 ⁶ cells/mL)	Non-polish Non-coated Stationary	No significant differences between the two groups.
Meirowitz et al., 2021 (Israel) [28]	Milled (Vira Vionic) 3D-printed (Detax)	Heat-polymerized AR Chemically-polymerized AR	6 discs (12 × 2 mm)	<i>Candida albicans</i> (1)	Microbial cell count	4 hrs at 37 °C (1 × 10 ⁶ cells/mL)	Polished Mucin coated Stationary	Significantly lower colonization in milled resin than conventional. 3D showed the highest colonization.
Freitas et al., 2023 (Brazil) [30]	Milled (AvaDent) 3D-printed (Yler)	Heat-polymerized AR Microwave-polymerized AR	9 discs (10 × 3 mm)	<i>Candida albicans</i> (1)	log CFU/mL	48 hrs at 37 °C (1 × 10 ⁶ cells/mL)	Polished Non-coated Stationary	Milled digital showed lower colonization than conventional denture (P < 0.05). Yet, 3D dentures showed higher colonization than conventional.
Koujian et al., 2022 (USA) [27]	Milled (AvaDent) 3D-printed (Dentca)	Heat-polymerized AR	10 bars (10 × 10 × 2 mm)	<i>Candida albicans</i> (1)	OD value	16 hrs at 37 °C (NA)	Polish Non-coated Shaker-dynamic	No significant differences between the milled and conventional, but significantly higher colonization in 3D digital.

Table 1 (continued)

Author, Year (Country)	Digital acrylic resin	Conventional acrylic resin	Sample size (dimensions in mm)	Microbial isolate (number of strains)	Measurement unit	Time of exposure Temperature (Concentration)	Polish/non-polish Saliva coated Stationary/dynamic	Main outcome
Larjani et al., 2022 (Iran) [33]	Milled (Glazed) Milled (High polished)	Heat-polymerized AR Chemically-polymerized AR	14 discs (10 × 1 mm)	<i>Candida albicans</i> (4)	OD value	48 hrs at 37°C (1 × 10 ⁵ cells/mL)	Polished Non-coated Stationary	No significant differences between the glazed milled and conventional group. However, significantly lower in polished milled compared to the conventional.
Linder, 2022 (USA) [34]	Milled (Ivotion) 3D-printed (Dentca) DLP printed (Lucitone) DLP printed (Envision)	Heat-polymerized AR (Injected) Heat-polymerized AR (Compressed)	6 discs (10 × 2 mm)	<i>Candida albicans</i> (1)	log CFU/mL	48 hrs at 37°C (NA)	Polished Non-coated Stationary	No significant differences between 3D group and conventional group. Yet, significantly lower DLP group than the conventional.
Wei et al., 2022 (China) [36]	Milled (Organic)	Heat-polymerized AR (Compressed)	4 discs (10 × 2 mm)	<i>Candida albicans</i> (1) <i>Staphylococcus aureus</i> (1) <i>Streptococcus mutans</i> (1)	log CFU/ml OD value	24 hrs at 37°C (1 × 10 ⁷ cells/mL)	Polished Saliva coated Stationary	A significant lower candida colonization in the milled group (P < 0.05).
Alfouzan et al., 2023 (KSA) [29]	Milled (IvoBase) 3D-printed (Next-Dent)	Heat-polymerized AR	10 discs (10 × 3 mm)	<i>Candida albicans</i> (1) <i>Streptococcus mutans</i> (1)	CFU/mL	72 hrs at 37°C (1 × 10 ⁻³ cells/mL)	Polished Non-coated Shaker -dynamic	No significant differences between the two groups.
Khattar et al., 2023 (KSA) [32]	3D-printed (Next-Dent)	Heat-polymerized AR	10 discs (15 × 2 mm)	<i>Candida albicans</i> (1)	CFU/mL	24 hrs at 37°C (NR)	Polished Non-coated Stationary	No significant differences between the two groups.
Osman et al., 2023 [39]	3D-printed (Next-Dent) Milled (Opera)	Heat-polymerized AR	9 specimens Not specific shape	<i>Candida albicans</i> (1)	OD value	24 hr. at 37°C (1 × 10 ⁶ cells/mL)	Non-polished Non-coated Dynamic-shaker 150 rpm	Milled digital showed lower colonization than conventional denture (P < 0.05). Yet, 3D-printed resin showed higher colonization than conventional.

Table 1 (continued)

Author, Year (Country)	Digital acrylic resin	Conventional acrylic resin	Sample size (dimensions in mm)	Microbial isolate (number of strains)	Measurement unit	Time of exposure Temperature (Concentration)	Polish/non-polish Saliva coated Stationary/dynamic	Main outcome
Teixeira et al., 2023 [40]	3D-printed (Cosmos)	Heat-polymerized AR	9 discs (9 × 1 mm)	<i>Candida albicans</i> (1) <i>Candida Glabrata</i> (1) <i>Streptococcus mutans</i> (1)	CFU/mL	90 min at 37°C (1 × 10 ⁶ cells/mL)	Polished Non-coated Dynamic-shaker 750 rpm	3D-printed resin showed higher colonization than conventional.

The measurement methods for the outcomes also varied across the included studies: 7 studies [22, 29–32, 34, 40] used CFU/ml (or log CFU/ml), 3 studies [27, 33, 39] used OD, and one study [36] used both CFU/ml and OD. Meanwhile, one study each used adhesion percent [35], cell count per field [23], and microbial cell count [28]. The numerical data which extracted from the included studies and utilized for the meta-analyses are shown in Table 2.

Qualitative results

The included 14 studies revealed variable results. Four studies [22, 23, 35, 36] showed significantly lower candida colonization on the digital dentures. Three studies [29, 31, 32] reported comparable results. Three studies [28, 30, 39] showed lower candida colonization on the milled digital dentures, but significantly higher candida colonization on the 3D-printed group as compared to the conventional dentures. Two studies [27, 40] reported higher candida colonization in the 3D-printed group than in the conventional group, but one of them reported

Table 2 The extracted means and SDs of the fungal colonization in different output units (CFU/ml or OD value), and the main conclusions of the studies subjected to quantitative analysis

Study	Digital acrylic resin		Conventional acrylic resin		Conclusion
	Type	Mean ± SD	Type	Mean ± SD	
Al-Fouzani et al., 2017 [22]	Milled (VivaDent)	$1.1 \times 10^3 \pm 6.0 \times 10^2$	HPAR	$2.3 \times 10^3 \pm 8.4 \times 10^2$	Dig. < Conv.
	Milled (VivaDent)	$2.1 \times 10^3 \pm 8.7 \times 10^2$	HPAR	$5.4 \times 10^3 \pm 1.6 \times 10^2$	Dig. < Conv.
	Milled (VivaDent)	$1.2 \times 10^3 \pm 8.8 \times 10^2$	HPAR	$2.0 \times 10^3 \pm 9.7 \times 10^2$	Dig. < Conv.
	Milled (VivaDent)	$1.5 \times 10^3 \pm 7.2 \times 10^2$	HPAR	$2.4 \times 10^3 \pm 1.1 \times 10^3$	Dig. = Conv.
Jung 2020 [31]	Milled (AvaDent)	100.92 ± 62.80	HPAR	109.75 ± 52.32	Dig. = Conv.
	3D-printed (Dentca)	81.70 ± 47.17			
	3D-printed (Lucitone)	65.58 ± 34.81			
Freitas et al., 2023 [30]	Milled (AvaDent)	3.74 ± 0.57	HPAR	5.12 ± 1.01	Dig. < Conv.
	3D-printed (Yllor)	5.77 ± 0.36			Dig. > Conv.
Linder 2022 [34]	Milled (Ivotion)	4.85 ± 0.19	HPAR (Injected)	4.90 ± 0.05	Dig. = Conv.
	3D-printed (Dentca)	4.91 ± 0.13			Dig. = Conv.
	3D-printed (Lucitone)	4.28 ± 0.13			Dig. < Conv.
	3D-printed (Envision)	4.25 ± 0.39			Dig. < Conv.
	Milled (Ivotion)	4.85 ± 0.19	HPAR (Compressed)	4.84 ± 0.04	Dig. = Conv.
	3D-printed (Dentca)	4.91 ± 0.13			Dig. = Conv.
	3D-printed (Lucitone)	4.28 ± 0.13			Dig. < Conv.
	3D-printed (Envision)	4.25 ± 0.39			Dig. < Conv.
Wei et al., 2022 [36]	Milled (Organic)	6.36 ± 0.22	HPAR	7.06 ± 0.13	Dig. < Conv.
	Milled (Organic)	0.04 ± 0.04	HPAR	0.21 ± 0.04	Dig. < Conv.
Alfouzani et al., 2023 [29]	Milled (IvoBase)	$7.7 \times 10^3 \pm 5.8 \times 10^3$	HPAR	$14.3 \times 10^3 \pm 13.1 \times 10^3$	Dig. = Conv.
	3D-printed (NextDent)	$5.0 \times 10^3 \pm 5.8 \times 10^3$			
Khattar et al., 2023 [32]	3D-printed (NextDent)	$123.3 \times 10^4 \pm 48.9 \times 10^4$	HPAR	$86 \times 10^4 \pm 45.31 \times 10^4$	Dig. = Conv.
Koujan et al., 2022 [27]	Milled (AvaDent)	0.90 ± 0.14	HPAR	1.04 ± 0.15	Dig. = Conv.
	3D-printed (Lucitone)	1.79 ± 0.13			Dig. > Conv.
Larijani et al., 2022 [33]	Milled (Glazed)	0.12 ± 0.02	HPAR	0.15 ± 0.02	Dig. = Conv.
	Milled (Glazed)	0.13 ± 0.01	HPAR	0.13 ± 0.02	
	Milled (Glazed)	0.12 ± 0.02	HPAR	0.13 ± 0.02	
	Milled (Glazed)	0.14 ± 0.02	HPAR	0.12 ± 0.01	
	Milled (High polished)	0.08 ± 0.01	HPAR	0.15 ± 0.02	Dig. < Conv.
	Milled (High polished)	0.09 ± 0.02	HPAR	0.13 ± 0.02	
	Milled (High polished)	0.12 ± 0.02	HPAR	0.13 ± 0.02	
	Milled (High polished)	0.07 ± 0.02	HPAR	0.12 ± 0.01	
Osman et al., 2023 [39]	Milled (Opera)	0.05 ± 0.004	HPAR	0.10 ± 0.02	Dig. < Conv.
	3D-printed (NextDent)	0.22 ± 0.02			Dig. > Conv.
Teixeira et al., 2023 [40]	3D-printed (Cosmos)	4.96 ± 0.43	HPAR	4.47 ± 0.60	Dig. > Conv.

no significant differences between the milled digital and the conventional groups. One study [33] reported lower candida colonization in the polished milled digital group compared to the conventional group, but no difference was noted between the glazed milled and the conventional group. One study [34] found no significant differences between the milled group and the conventional group, but it revealed significantly lower candida colonization in the 3D-printed groups compared to the conventional (Tables 1 and 2).

Meta-analysis results

Figure 2 shows the meta-analysis model of studies that used CFU/ml as a measurement method of the outcome. There were six studies with 12 independent comparison groups that used the 3D-printed acrylic resins and six studies with 10 independent comparison groups that used the milled acrylic resins. The pooled data regarding comparing the 3D-printed versus the heat-polymerized acrylic resins revealed lower but non-significant candida colonization on the former compared to the latter (MD = -0.21; 95%CI = -0.47, 0.05; P = 0.11). However, the pooled data regarding comparing the milled vs. heat-polymerized acrylic resins revealed significant lower

candida colonization on the former compared to the latter (MD = -0.36; 95%CI = -0.69, -0.03; P = 0.03). Owing to the high heterogeneity amongst the studies (I² = 89%; P < 0.00001), the random-effect model was used.

Figure 3 shows the meta-analysis model of studies that used OD value as a measurement method of the outcome. There were only two studies with two independent comparison groups that used the 3D-printed acrylic resin. The results of these studies revealed insignificant but higher candida colonization on the 3D-printed acrylic resin compared to the heat-polymerized acrylic resin (MD = 0.40; 95%CI = -0.21, 1.02; P = 0.11). There were four studies with 11 independent comparison groups that used the milled acrylic resin. The pooled data revealed a significantly lower candida colonization in the milled resin group compared to the heat-polymerized acrylic resin group (MD = -0.04; 95%CI = -0.06, -0.01; P = 0.0008). Owing to the high heterogeneity amongst the studies (I² = 97%; P < 0.00001), the random-effect model was used.

Publication Bias

As shown in Fig. 4, the qualitative (Fig. 4A) and quantitative (Fig. 4B) analyses revealed no publication bias among the studies that used CFU/ml (P = 0.910, Egger’s

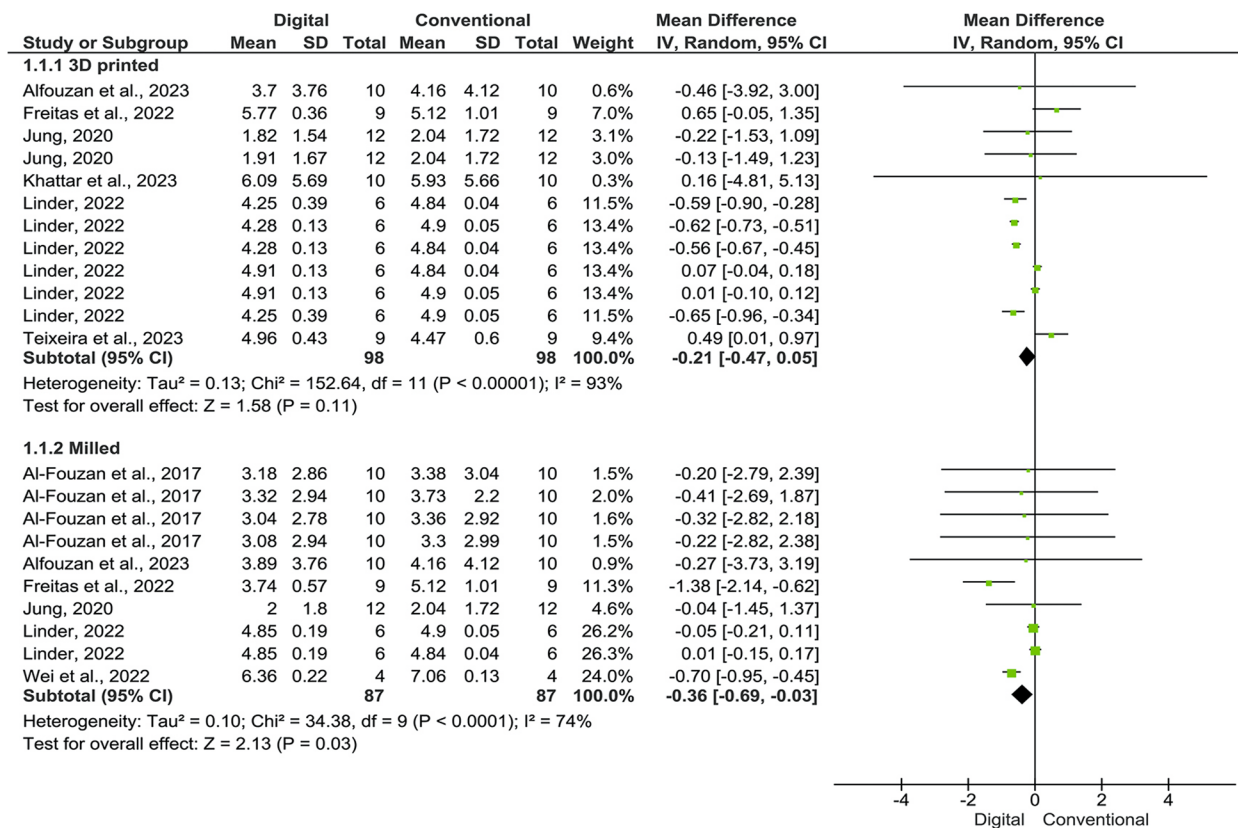


Fig. 2 Forest plot of the pooled data for the included studies that used log CFU/ml as measurement unit

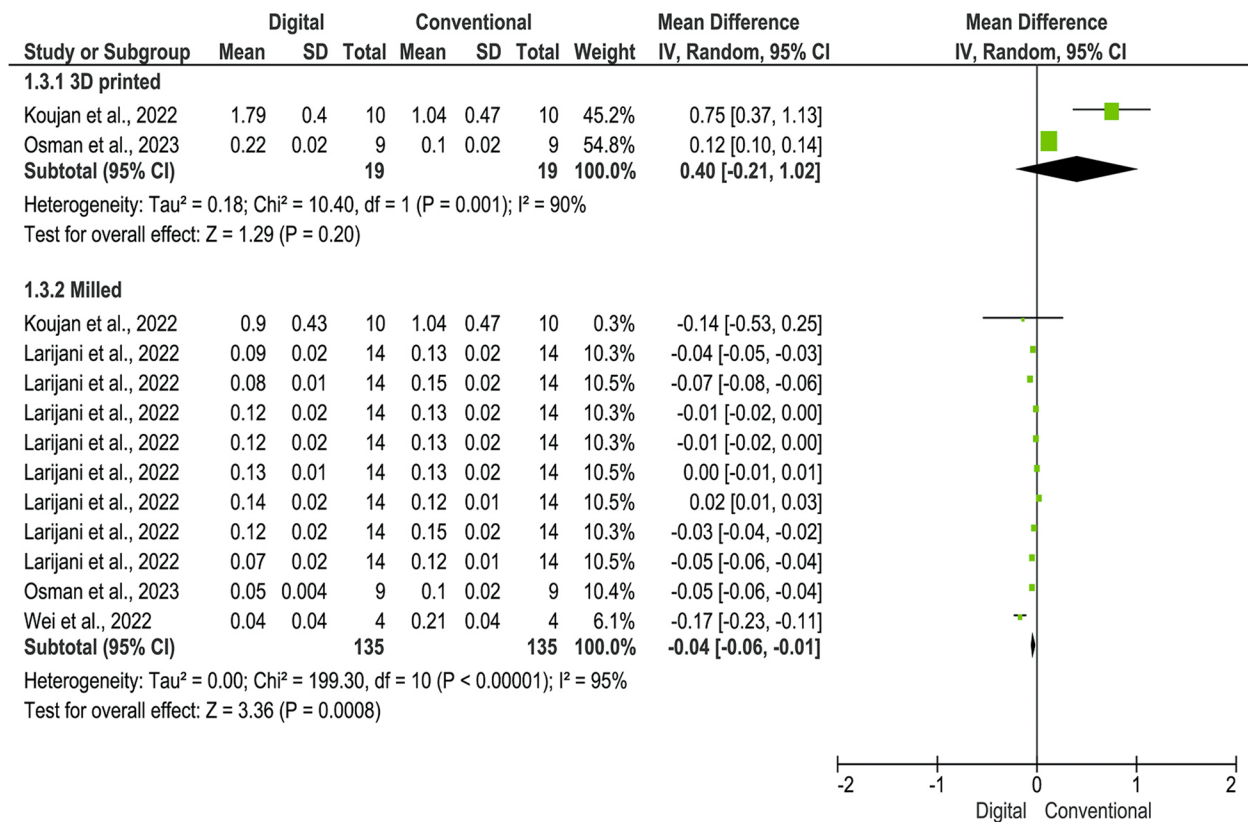


Fig. 3 Forest plot of the pooled data for the included studies that used OD value as measurement unit

test). Similarly, regarding the studies that used OD value, no publication bias was found (Fig. 5A and B) ($P=0.070$, Egger’s test).

Quality of the included studies

The quality results of the included studies are presented in Figs. 6 and 7. All the studies included in this systematic review and meta-analysis were found to be of moderate quality, ranging from 50 to 70%. The majority of the methodological shortcomings were related to sample size calculation, operator details, outcome assessor details, and blinding of the operator(s), outcome assessor(s), and statistician. Details of the statistical part were also missing from some studies.

Discussion

While conventional materials and methods have long been used in denture fabrication, recent advances in dental materials and technologies have led to the development of new denture materials and digital fabrication methods [41–43]. However, these new materials and methods must demonstrate improved biocompatibility, better mechanical properties, and a less

favourable environment for microbial adhesion in order to gain acceptance among dental professionals. In this review, we aimed to systematically summarize the available in-vitro evidence on the potential adhesion of *C. albicans* to digitally-fabricated acrylic resin materials in comparison to conventional ones. The key findings of our meta-analyses indicate that the adhesion of *C. albicans*, as measured by CFU or OD values, is lower on the milled digitally-fabricated resin materials compared to the conventionally-fabricated resin materials. This suggests that these digitally-fabricated materials either provide a less favourable environment for *C. albicans* colonization, or have anti-fungal properties. Regardless of the mechanism, this fact must be emphasized, disseminated among dental professionals, and incorporated into clinical practice.

As we have included studies that compared the same material in both conventional and digital methods, the proposed explanation that the digitally-fabricated resin materials have anti-fungal properties is negated. Yildirim et al. [44] concluded that the adhesion of *C. albicans* to of the resin surface may be influenced by the physicochemical properties more than the surface

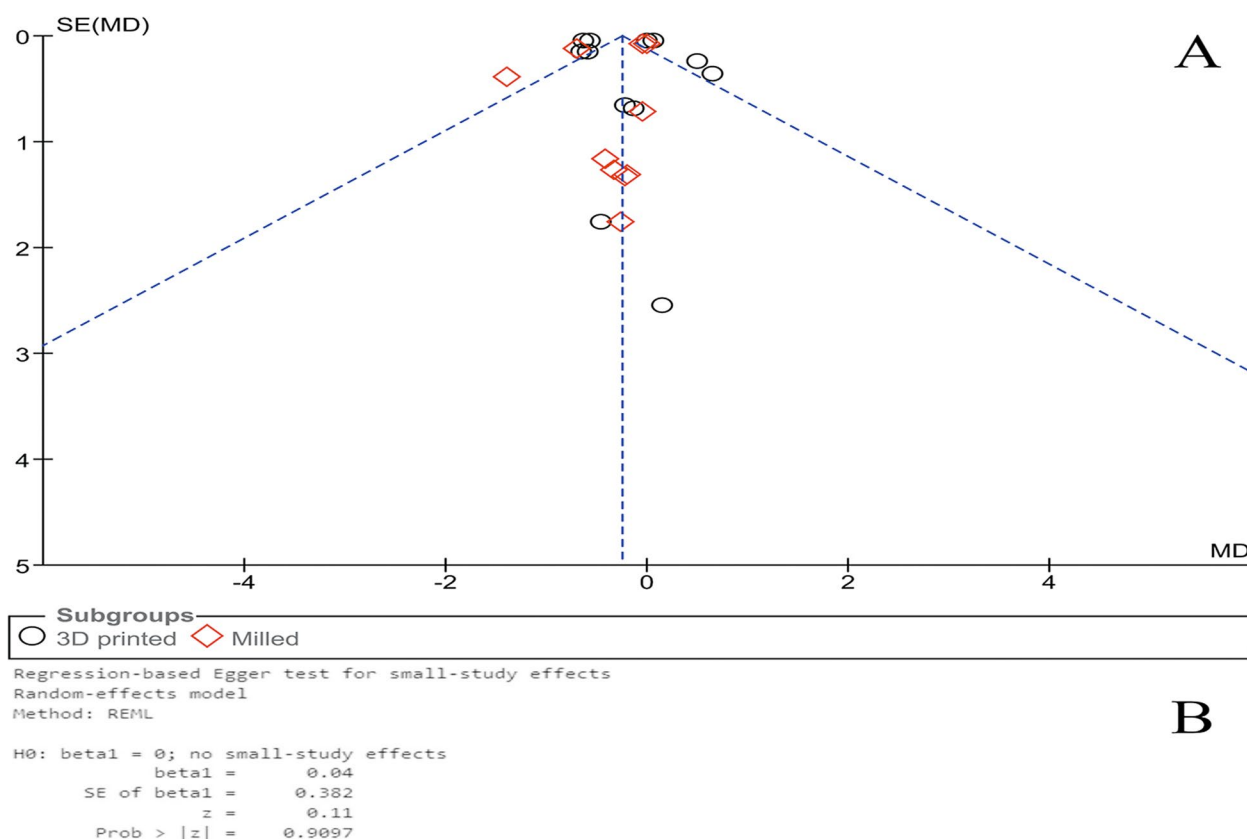


Fig. 4 Publication bias (A) and Egger’s test (B) for the included studies that used log CFU/ml as measurement unit

roughness. Instead, the mechanical properties of the materials are suspected to be the reason for the difference in *C. albicans* adhesion. These mechanical properties vary between the conventionally- and the digitally-fabricated dentures, with the latter providing a less favourable environment for *C. albicans* colonization. Recent systematic reviews have confirmed that many mechanical properties are different between the two methods of denture fabrication, with surface roughness being a key factor that may influence the adhesion of *C. albicans*. These reviews have shown that the conventionally-fabricated resins have higher surface roughness values than the digitally-fabricated resins [45–47]. Moreover, the surface roughness values of the conventionally-fabricated resins were found to be above the threshold value of 0.2 μm [48]. In a recent meta-analysis, three factors were identified as responsible for *C. albicans* adhesion to denture materials, including surface roughness, wettability (measured as contact angle), and surface free energy [49]. Al-Fouzan et al. [22] stated that “the rougher the surface, the greater the Candida colonization will be.” However,

there is a significant debate regarding the effects of these factors [50–53]. Other factors including continued denture wearing, poor cleaning of dentures, non-brushing of tongues, as well as sleeping with dentures are also contributing factors to *C. albicans* adhesion to dentures [14, 54].

Given that the surface porosity and roughness of the acrylic resin are considered responsible factors for *C. albicans* adhesion, Gauch et al. [55] argued that denture surfaces could be considered an infection source. In fact, many studies have reported a strong positive correlation between *C. albicans* adhesion and the surface roughness of denture base polymers [23, 56], although one recent study contradicted such results [40]. Based on many previous scanning electron microscope studies, the conventionally-fabricated resin showed a more porous surface and multiple surface irregularities than CAD/CAM-fabricated resin [23, 36]. The presence of such irregularities, porosity, and/or imperfections on a given surface of a dental device enhances microbial accumulation, even when it is clean [57]. As mentioned earlier, a threshold of

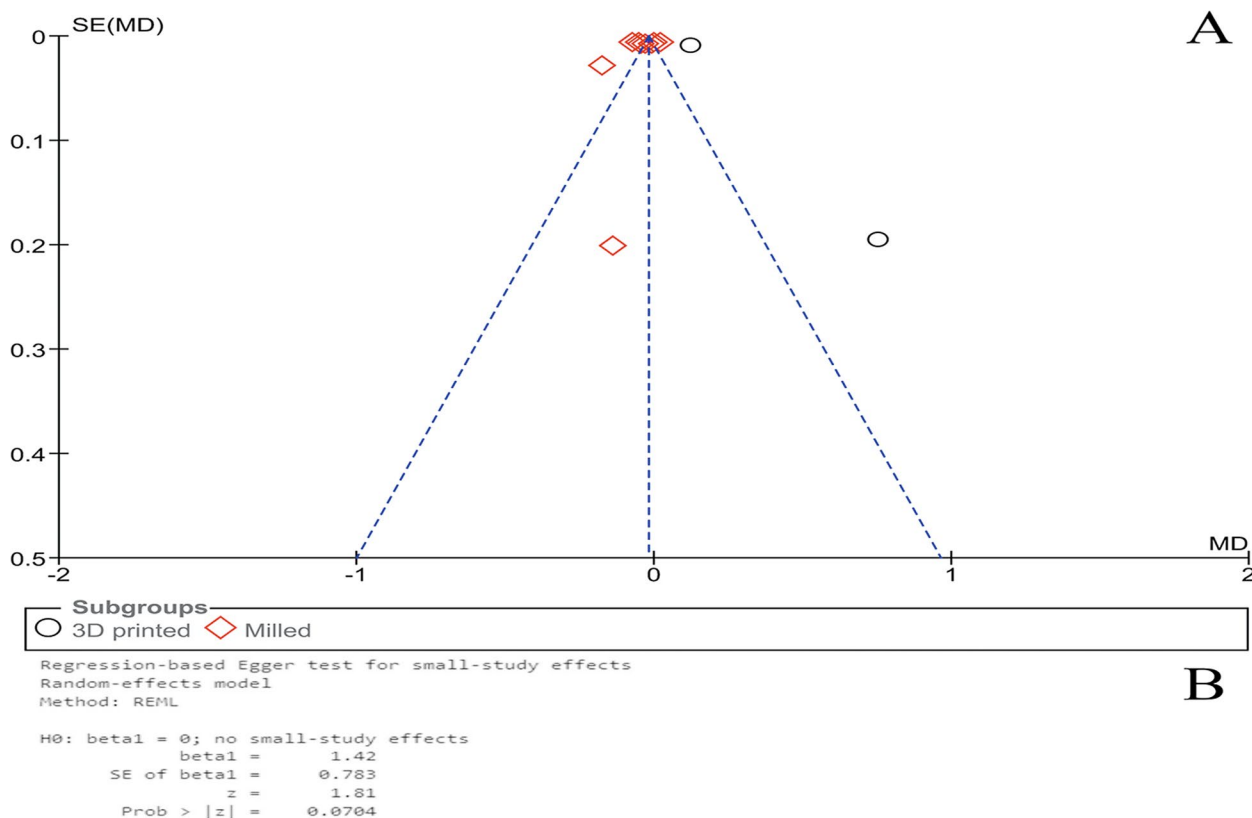


Fig. 5 Publication bias (A) and Egger’s test (B) for the included studies that used OD value as measurement unit

≤0.2 μm is recommended in order to prevent the formation of biofilm on any dental hard and prosthetic surfaces [48].

The findings of the present review are important from a clinical point of view. The milled dentures often have smoother and more polished surfaces than conventionally-fabricated dentures. This, in turn, reduces the potential for Candida adherence. Furthermore, milled dentures are less porous and more resistant to moisture absorption compared to the conventionally-fabricated dentures, potentially reducing the favourable conditions for Candida growth. Moreover, better retention and stability are expected due to the precision and improved fit of the milled dentures. Collectively, this helps to reduce the likelihood of micro-movements that may cause friction and irritation, which in turn may create opportunities for Candida to colonize and adhere.

The current systematic review has both strengths and weaknesses. One of the strengths was focusing on in-vitro studies. Including all studies since inception date was another point of strength. Updating the search and conducting manual search in the references of the

potential studies was a strong point ensuring covering all the potential studies. Also, the review included studies that assessed one type of material using different fabrication technologies and provided information on the effects of fabrication rather than solely on material types or ingredients. The tool used to assess the quality of the included studies is also robust, representing a strength aspect of the study. The quality of all included studies was moderate. Thereby, the overall evidence of the current study might be affected (down-graded). Accordingly, following a thorough, standardized, precise, and detailed methodology is highly recommended in future studies. However, one of the limitations of this review was that it only included studies published in English, which may have missed valuable information in other languages. Although the qualitative and quantitative tests, Funnel plot and Egger’s test, respectively, revealed no publication bias, this cannot be considered conclusive, especially with a few studies included. Therefore, the potential publication bias might be considered to exist, which represents one of the limitations of the current study. The heterogeneity among the included studies was relatively high.

	Clearly stated aims/objectives	Detailed explanation of sample size calculation	Details of comparison group	Detailed explanation of methodology	Operator details	Method of measurement of outcome	Outcome assessor details	Blinding	Statistical analysis	Presentation of results
Al-Fouzan et al., 2017	+	-	+	+	-	+	-	-	?	+
Alfouzan et al., 2023	+	+	+	+	-	+	-	-	+	+
Freitas et al., 2022	+	?	+	+	-	+	-	-	+	+
Jung, 2020	+	+	+	+	-	+	-	-	?	+
Khattar et al., 2023	+	+	+	?	-	+	-	-	+	+
Koujan et al., 2022	+	-	+	+	-	+	-	-	?	+
Larijani et al., 2022	+	-	+	+	-	+	-	-	?	+
Linder, 2022	+	-	+	+	-	+	-	-	?	+
Makke 2014	+	+	+	+	-	+	-	-	+	?
Meirowitz et al., 2021	+	-	?	?	?	+	-	-	?	+
Murat et al., 2019	+	-	+	+	-	+	+	-	+	+
Osman et al., 2023	+	+	+	+	-	+	-	-	+	+
Teixeira et al., 2023	+	-	+	+	-	+	-	-	+	+
Wei et al., 2022	+	-	+	+	-	+	-	-	+	?

Fig. 6 Risk of bias summary of the included studies

This heterogeneity might be due to the different sample sizes, different dimensions and shapes of the specimens, or the different exposure times to the candida

culture among the included studies. Additionally, we tried our best to extract the numerical data from some of the included studies using the relevant software or formula; however, there were three studies that lacked

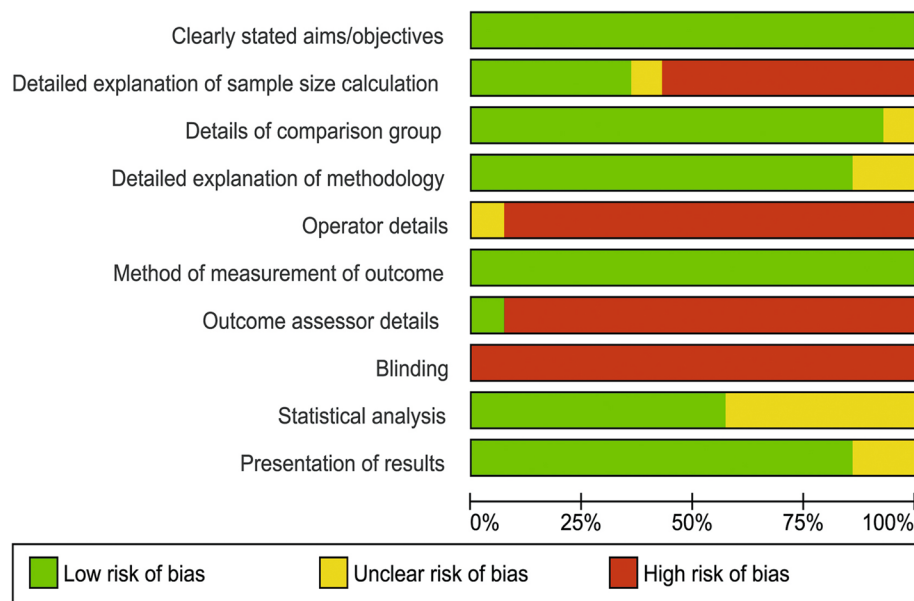


Fig. 7 Risk of bias graph of the included studies

the adopted quantitative data, and thus were excluded from the meta-analysis, which in turn could limit the conclusive evidence of this review. Yet, owing to some methodological limitations of the included studies, more robust in-vitro studies, along with well-designed clinical studies are highly recommended to discern the available evidence.

Conclusion

In conclusion, the limited available evidence suggests that the milled digitally-fabricated acrylic resins provide less adhesion environment to *C. albicans* compared to the conventionally-fabricated materials. Moreover, in cases where a digitally-fabricated denture is the preferred choice, the recommendation leans toward the milled denture over the 3D-printed one.

Abbreviations

PRISMA	Preferred Reporting Items for Systematic Review and Meta-analyses
<i>C. albicans</i>	<i>Candida Albicans</i>
DS	Denture-related Stomatitis
CFU	Colony Forming Unit
OD	Optical Density
CAD/CAM	Computer-Aided Design/Computer-Aided Manufacturing
MD	Mean Difference
CI	Confidence Interval
QUIN	Quality Assessment Tool For In Vitro Studies
ID	Identification
MS	Microsoft
SPSS	The Statistical Package for the Social Sciences

Supplementary Information

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- Supplementary Material 1.
- Supplementary Material 2.
- Supplementary Material 3.

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Authors' contributions

Mohammed Nasser Alhaji: Conceptualization and study design, Data collection, Formal analysis, Writing- Original Draft. Esam Halboub: Data Curation, Formal analysis, Writing- Review & Editing. Norlela Yacob: Data collection, Writing- Original Draft. Sadeq Ali Al-Maweri: Data Curation, Writing- Review & Editing. Asja Celebić: Data Curation, Writing- Review & Editing. Hesham M. Al-Mekhlafi: Data Curation, Writing- Review & Editing. Siti Fauzza Ahmad: Writing- Review & Editing, Supervision. Nosizana Mohd Salleh: Conceptualization and study design, Methodology, Validation, Writing- Review & Editing, Visualization, Supervision, Project administration. All authors read and approved the final version.

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Declarations

Ethics approval and consent to participate

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Competing interests

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