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REVIEW ARTICLE

Incorporation of antimicrobial agents into dental materials obtained by additive manufacturing: A literature review

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KEYWORDS

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Abstract *Background:* This review aimed to identify antimicrobial agents incorporated into dental materials obtained through additive manufacturing and their efficacy.

Methods: Protocol registration was performed in Open Science Framework (osf.io/sp3xa/) and an electronic search was carried out in the databases PubMed, Science Direct, Embase, Lilacs, and Scopus, up to February 2022, combining the terms (“additive manufacturing” OR “3D printing”) AND (antimicrobial). Eligibility criteria included: experimental studies that incorporated 3D printing material with an antimicrobial agent for dental application; that evaluated antimicrobial activity; articles published in peer-reviewed journals and in English.

Results: The database search resulted in 1139 references. The manual selection was carried out in 851 studies. Twenty-five articles were selected for full-text reading, of which 8 were included in this review. Polymers were the dental materials most often modified with antimicrobial agents for 3D printing, followed by metal alloy. The antimicrobials used were mainly nanoparticles, metal particles, antifungals, monomers containing quaternary ammonium salt, and antiseptics such as chlorhexidine.

Conclusion: The addition of the antimicrobial agents in polymers and alloy for additive manufacturing showed promising efficacy against *Candida* spp., Gram-positive and Gram-negative bacteria.

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Contents

1. Introduction

Over the last 30 years, there has been expressive growth of additive manufacturing, also known as 3D printing, at a rate of 26.9%/year. This generates billions of dollars for the industry, with great application in the health sector (Turner et al., 2020). This technology is characterized by the development of a physical object through the deposition of layer-by-layer material based on a tridimensional digital model (Vasamsetty et al., 2020; Deng et al., 2018).

In dentistry, 3D printing provided agility in procedures, reproducibility, simplification of processes, reduced office visits, and greater accuracy (Yoon et al, 2018; You et al., 2021; Shim et al., 2020; Tasaka et al., 2019). The technology allows for a wide range of applications, such as models, prostheses, aligners, implants, and surgical guides (Santos et al., 2022; Vasamsetty et al., 2020; Deng et al., 2018). This technique has advantages compared to subtractive manufacturing, since it consumes less material, allows greater reproduction of details, and enables the manufacturing of several products with complex geometry (You et al., 2021; Shim et al., 2020).

Although the use of digital flow is promising in the dental field, a frequent problem is biofilm accumulation, which can lead to the development of diseases, such as caries or mucosal inflammation, or disseminated infections (Santos et al., 2022; Totu et al., 2017). Most materials used in dentistry do not have inherent antimicrobial capacity, which can be achieved by incorporating, coating, or changing the chemical or physical characteristics of the surface (Turner et al., 2020).

The modification of materials using nanomaterials, antifungals, metal oxides and monomers is an alternative consolidated in the literature to prevent the formation of biofilm (Totu et al., 2017; Nagrath et al. 2018; González-Henríquez et al., 2019; Shreshta and Kishen, 2016; AlKahtani, 2018; Castro et al., 2021). In the 3D printing field, this is an innovative subject and there are no reviews that address types of antimicrobials incorporated in printed dental materials and their efficacy. Thus, this review is needed to systematically map the research done on the incorporation of these agents, type of material, 3D technology used, and to identify any existing gaps in knowledge.

2. Materials and methods

2.1. Protocol and structure

This review was structured based on PRISMA and was registered on Open Science Framework (osf.io/sp3xa/). The review question, based on PICO, was: "Which 3D printing materials used in dentistry were modified with antimicrobial agents and showed antimicrobial efficacy?" The PICOS methodology used was:

Population – 3D printing dental materials.
Intervention – modification with antimicrobials.
Comparison – materials that did not receive the intervention.
Outcome – evaluation of antimicrobial efficacy.
Study type - experimental in vitro studies.

2.2. Search and study selection

A custom search was performed in the databases PubMed, Science Direct, Embase, Lilacs, and Scopus checking articles published until February 22, 2022 (Table 1), with the terms (“additive manufacturing” OR “3D printing”) AND (antimicrobial). Manual selection of the articles was conducted by two authors (A.B.V.T. and G.G.C.), duplicate references were identified using EndNote (Clarivate Analytics), and then the results were exported to Rayyan (Qatar Computing Research Institute). The initial selection was performed based on title and abstract, and the second selection, on full-text reading. When there were disagreements between the two researchers, the opinion of a third researcher (A.C.R.) was sought.

2.3. Eligibility criteria

Eligibility criteria included (1) experimental studies that used an antimicrobial agent to modify 3D printing material for application in dentistry; (2) studies that evaluated antimicrobial activity; (3) published in English; (4) published in peer-reviewed journals, considering their impact factor (registered in Journal Citation Reports, Clarivate Analytics).

2.4. Data extraction

The data was extracted to a table with the following information: authors and year; type of study; printing technology, printers, printed material; printing parameters; modification/antimicrobial agent; method of additions; type of restoration/prostheses; antimicrobial evaluation; results and outcome.

2.5. Risk of bias assessment

Risk of bias assessment followed the criteria proposed by the Joanna Briggs Institute (JBI, Tufanaru et al., 2017). This critical appraisal tool is made up of 9 questions, of which 3 were not adequate for this review and were excluded. Representative figures were made using ReviewManager 5.4 (RevMan, Cochrane).

2.6. Data synthesis strategy

A descriptive analysis of the data was performed regarding the antimicrobial agent incorporated, type of printed material, printing technology, and antimicrobial activity evaluation.

3. Results

3.1. Search results and studies included

The search found 326 references on PubMed, 570 in Science Direct, 90 in Embase, 153 in Scopus, and none in Lilacs, for a total of 1139 results. The 176 duplicated references found in EndNote and 87 found in Rayyan were excluded. After the initial selection, 851 references were excluded. Twenty-five studies were selected for full-text reading; of these, 17 were excluded. Eight studies were included in this review (Fig. 1). Information on the included studies is presented in Table 2.

3.2. Risk of bias

Figs. 2 and 3 show the risk of bias assessed according to JBI. Three of the 8 included studies showed a high risk of bias on the criteria related to statistical analysis (Totu et al., 2017; Lu et al., 2018; Li et al., 2019), since they did not report whether a statistical analysis was performed. Turner et al. (2020) were unclear about this, since they did not mention what statistical test was used, and Yue et al. (2015) also were unclear on the statistical test, since only one of the methods was shown.

3.3. Antimicrobial agent incorporated, type of printed material, and printing technology

Only one of the studies evaluated an alloy for dental application (Lu et al., 2018), in which copper (Cu) was incorporated into a CoCrW alloy at a concentration of 3%. The metals were cast, a powder of the CoCrWCu alloy was produced by atomization in argon gas, and the samples were obtained by Selective Laser Melting (SLM) (Lu et al., 2018).

The other studies modified polymers. Polycaprolactone (PCL) microspheres containing the antifungal Amphotericin-B (AmB) were mixed into polymethylmethacrylate (PMMA - at 0.2% w/v), a filament was obtained by extrusion and used to print samples in 1, 5, and 10 layers by Fused Deposition Modeling (FDM) (Nagrath et al., 2018). Selective Laser Sintering (SLS) was used to print the powder of Poly-

Table 1 Custom Search Strategy.

Database	Search	Found
PubMed February 22th, 2022	((“additive manufacturing”) OR (“3D printing”)) AND (antimicrobial) Filters applied: English	326
Science Direct February 22th, 2022	(“additive manufacturing” OR “3D printing”) AND (antimicrobial) Filters applied: research articles	570
EMBASE February 22th, 2022	#1: (’additive manufacturing’ OR ’3d printing’) AND antimicrobial #2: #1 AND (’article’/it OR ’article in press’/it) Filters applied: article, article in press	90
LILACS February 22th, 2022	(“additive manufacturing” OR “3D printing”) AND (antimicrobial)	0
SCOPUS February 22th, 2022	(TITLE-ABS-KEY (“additive manufacturing”) OR TITLE-ABS-KEY (“3D printing”) AND TITLE-ABS-KEY (antimicrobial)) AND (LIMIT-TO (DOCTYPE, “ar”)) AND (LIMIT-TO (LANGUAGE, “English”)) Filters applied: article, English	153

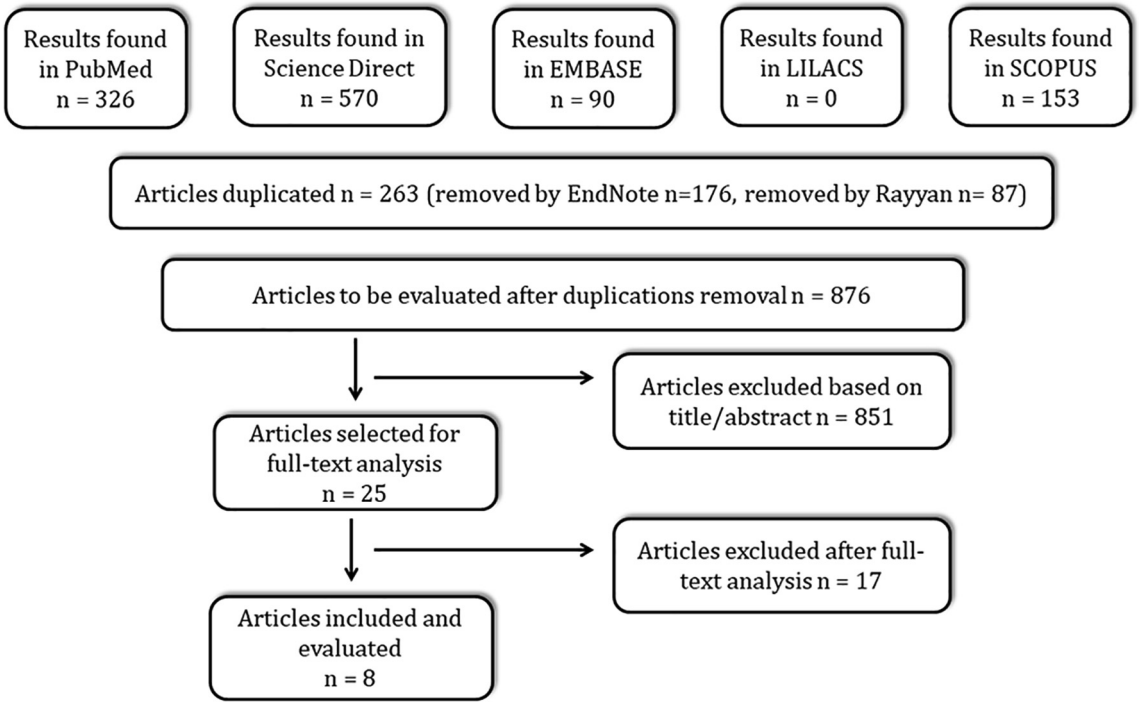

Fig. 1 Diagram containing the steps of the literature research.

amide 12 (PA2200) incorporated with 1.0% of silver phosphate glass powder (B65003) (Turner et al., 2020). Two photocurable composite resins based on UDMA/GDMA were incorporated with 14 wt% of Quaternary Ammonium Methacrylate (QA_C_{12}) and 25 wt% of QA-containing polymer (pQA_C_{12}), respectively, and printed by Stereolithography (SLA) (Yue et al., 2015).

Four studies incorporated antimicrobials into photocurable resins to print by Digital Light Processing (DLP). Totu et al. (2017) incorporated 0.2, 0.4, 0.6, 1, and 2.5 wt% of titanium dioxide (TiO_2) nanoparticles into a commercial PMMA photocurable. A resin was developed and incorporated with 2, 4, 6, and 8 wt% quaternary ammonium salt with methacrylate (QAC), and 10, 20, and 30 wt% quaternary ammonium salt with thiol group (SH-QAC) (Li et al., 2019). A resin for orthodontic applications was incorporated with 0.1 wt% nanodiamonds (ND) and amine-functionalized nanodiamonds (A-ND) (Mangal et al., 2020). Mai et al. (2020) coated a commercial photocurable resin printed by DLP with 0.4 wt% chlorhexidine (CHX) encapsulated in mesoporous silica nanoparticles (MSN) dispersed in polydimethylsiloxane (PDMS).

3.4. Antimicrobial activity evaluation

The CoCrWCu alloy showed less *Escherichia coli* colony formations than the CoCrW alloy after biofilm growth for 24 h (Lu et al., 2018). All antimicrobial polymers demonstrated efficacy. Nagrath et al. (2018) observed that the PMMA-loaded PCL microspheres with single-layer printed AmB reduced the biomass of *Candida albicans* compared to the control group, and the samples printed in 5 and 10 layers did not show an effect. The PA2200 incorporated with 1.0% B65003, on the other hand, showed fewer Colony Forming Units per milliliter (CFU/mL) against *Staphylococcus aureus* and *Pseudomonas aeruginosa* than the normal PA2200 in phosphate-buffered saline (PBS); however, when cultured in brain heart infusion (BHI), no difference was observed (Turner et al., 2020).

As for the UDMA/GDMA incorporated with 14 wt% of QA_C_{12} and 25 wt% of pQA_C_{12}, the modified groups were found to be more efficacy against *Streptococcus mutans* compared to the control group (UDMA/GDMA without Quaternary Ammonium [QA] obtained by polymerization mold and conventional photo illumination). It was observed that the samples with QA had less CFU and live and dead bacteria (Yue et al., 2015).

Regarding antimicrobial resins printed by DLP, great results were observed. Totu et al. (2017) related that TiO_2-PMMA at 0.4%, 1% and 2.5% inhibited the growth of *Candida scotti*, and 0.2%, TiO_2-PMMA did not show efficacy.

The resin with QAC and SH-QAC showed major efficacy against *S. aureus* than *E. coli*. At 4%, 6%, and 8%, QAC was able to inhibit the growth of *E. coli*, with partial effect at 2% QAC. All concentrations of QAC showed activity against *S. aureus*. The lowest concentration of SH-QAC (10%) was able to inhibit *S. aureus*; however, against *E. coli*, only the concentration of 30% showed great effect (Li et al., 2019).

The ND and A-ND resin at 0.1% was found to produce lower biofilm thickness and *S. mutans* biomass than the control group. No differences were observed between the modified groups (Mangal et al., 2020). The CHX-MSN coating printed resin inhibited the growth of *S. mutans* and showed lower values of CFU/mL in relation to the control group (Mai et al., 2020).

4. Discussion

4.1. 3D printing technologies

SLA was the first 3D printing technique, invented by Charles Hull. It uses a high-powered laser to polymerize point-to-point photocurable resin in a vat, converting liquid into solid plastics (Stansbury and Idacavage, 2016; González-Henríquez et al., 2019; Teixeira and Reis, 2021). SLS, a technique introduced after SLA, uses a thermoplastic polymer powder preheated to near the melting point, and a high-power CO_2 laser performs the tracing of the design layer-by-layer. The unsintered powder is a support for the object and can be

Table 2 Printed dental materials incorporated with antimicrobial agents.

Authors/ year	Type of study	Printing technology, printers, printed material	Printing parameters	Modification/ antimicrobial agent	Method of additions	Type of restoration/ prostheses	Antimicrobial evaluation	Results and outcome
Li et al., 2019	Experimental in vitro study.	Digital Light Processing (DLP). Wuxi printer (Jiangsu Minreon Technology Co., Ltd). Experimental photosensitive resin.	Wavelength: 415 nm. Wash in absolute ethanol: 1–2 min. LED light for post-curing: 405 nm, 3–5 min.	Incorporation of 2, 4, 6, and 8 wt% quaternary ammonium salt with methacrylate (QAC), and 10, 20, and 30 wt % of quaternary ammonium salt with thiol group (SH-QAC).	QAC and SH-QAC were synthesized with a mix of monomers, dissolved in absolute ethanol, and added to the experimental resin.	Dental restorations, prosthesis, and molds.	Growth curves of *Escherichia coli* and *Staphylococcus aureus* were evaluated by optical density at different times. The contact-killing after 3 h was evaluated in different dilutions and by colony-forming unit (CFU/mL).	2% QAC showed partial efficacy against *E. coli*, and the major concentrations were able to inhibit 100% of *E. coli* and *S. aureus*. SH-QAC in minor concentrations (from 10%) showed efficacy against *S. aureus*, and only 30% of SH-QAC reduced the survival rate for *E. coli*.
Lu et al., 2018	Experimental in vitro study.	Selective laser melting (SLM). Commercial selective laser melting machine employing a Nd:YAG laser. CoCrW alloy.	Laser power: 50 to 95W. Laser scan speed: 250 to 2000mm/s. Hatch spacing: 0.11 mm. Layer thickness: 0.025 mm. Printing angle: 90°.	Addition of 3% Cu.	Casting and powder production by atomization in argon gas.	Dental alloy for abutments, crowns, and bridges.	CFU/mL of *Escherichia coli* after biofilm formation at sample's surfaces.	The CoCrWCu alloy showed visible less CFU of *E. coli* than CoCrW alloy.
Mai et al., 2020	Experimental in vitro study.	Digital Light Processing (DLP). 3D printer RAM500 (Ray Co., Korea). Photopolymer RAYDent C&B (Ray Co., Korea).	Not reported.	Coating of photopolymer printed with 0.4 wt% chlorhexidine (CHX) encapsulated in mesoporous silica nanoparticles (MSN) dispersed in polydimethylsiloxane (PDMS).	CHX was encapsulated in MSN (CHX@MSN) and mixed in PDMS. Photopolymer was printed, functionalized with oxygen plasma, coated with CHX@MSN.	Dental prostheses.	CFU/mL of *Streptococcus mutans* after biofilm formation at sample's surfaces.	The samples coated inhibited the growth of *S. mutans* more than non-coated samples.
Mangal et al., 2020	Experimental in vitro study.	Digital Light Processing (DLP). 3D printer NextDent 5100 (3D Systems, NextDent B.V.). NextDent Ortho Rigid (3D Systems, NextDent B.V.).	Layer thickness: 100 μm. Printing angle: 0°. Polymerization light: 405 nm. Maximum printing speed: 140 mm/h.	Incorporation of 0.1 wt% nanodiamonds (ND), amine-functionalized (A-ND) and pure non-functionalized (ND).	ND was dissolved in an organic solvent and mixed into resin.	Intra-oral orthodontic applications.	*Streptococcus mutans* biofilm formed in samples was stained and evaluated by CLSM and Image J. Thickness of the biofilm and average biomass was calculated.	The biofilm thickness and biomass of *S. mutans* were less in ND and A-ND groups than in the resin.

(*continued on next page*)

Table 2 (*continued*)

Authors/ year	Type of study	Printing technology, printers, printed material	Printing parameters	Modification/ antimicrobial agent	Method of additions	Type of restoration/ prostheses	Antimicrobial evaluation	Results and outcome
			Post-cured: 15 min.					
Nagrath et al., 2018	Experimental in vitro study.	Fused deposition modeling (FDM). 3D printer BCN3D (Sigma, Spain). Polymethylmethacrylate (PMMA) filaments.	Printing nozzles and temperature: 0.4 mm, 275°C. Printing bed temperature: 65°C. Print speed: 10 mm/s. Printed layers: 1, 5, and 10.	Incorporation of 0.2% w/v polycaprolactone (PCL) microspheres containing amphotericin-B (AmB).	A solution of AmB and gentamycin was incorporated into PCL. PCL-AmB were mixed in PMMA (powder and liquid - 2:1 ratio). The filament was extruded.	Dental prosthesis, splints, orthodontic applications, or interim treatment prosthesis (for drug release).	Biomass of *Candida albicans* was quantified after biofilm formation on samples with 1, 5, and 10 printing layers, and after 3 days in a desiccator.	Sample printed with 1 layer showed a significant reduction of *C. albicans* biomass. Samples printed in 5 and 10 layers did not show effect.
Totu et al., 2017	Experimental in vitro study.	Digital Light Processing (DLP). EnvisonTEC Perfactory 3D printer (Gladbeck, Germany). Polymethyl methacrylate (PMMA, E-Dent 100, Envision Tec GmbH).	Not reported.	Incorporation of 0.2, 0.4, 0.6, 1, and 2.5 wt % TiO_2 nanoparticles.	TiO_2 nanoparticles were synthesized through a modified sol-gel procedure from Ti $(OBu)_4$, and incorporated into the PMMA solution.	3D printed denture	Minimum Inhibitory Concentration (MIC) against *Candida scotti* by reduction of 2,3,5-Triphenyl tetrazolium chloride.	PMMA incorporated with 0.4, 1, and 2.5% showed antifungal action against *Candida scotti*.
Turner et al., 2020	Experimental in vitro study.	Selective Laser Sintering (SLS). Printer Formiga P100 (EOS). Polyamide 12 (PA2200, EOS).	Laser power: 21 W. Scan spacing and speed: 0.25 mm, 2500 mm/ s.	Incorporation of 1% silver phosphate glass (B65003, BioCote).	Powders of PA2200 and B65003 were mixed.	Implants, prostheses, splints, and health devices.	CFU/mL of *Staphylococcus aureus* and *Pseudomonas aeruginosa* after biofilm formation at sample's surfaces in Phosphate Buffered Saline (PBS) and Brain Heart Infusion (BHI).	In PBS, 1% B65003-PA2200 showed a higher effect than normal PA2200, however, in BHI, there was no difference between the groups.
Yue et al., 2015	Experimental in vitro study.	Stereolithography (SLA). Stereolithographic printer (Formlabs Form 1). Photocurable composite resin (UDMA/GDMA).	Layer thickness: 300 μm in XY plane and 25 μm in Z. Washing in isopropanol and photocuring for 5 h.	Incorporation of 14wt % Quaternary Ammonium Methacrylate (QA_C_{12}) and 25wt% QA- containing polymer (pQA_C_{12}).	QA_C12 was synthesized with DMAEMA and HEMA (pQA). 14 wt% UDMA/GDMA/ QA_C12: mix of 50 mol % UDMA, 36 mol% GDMA and 14 mol% QA_C12. 25 wt% pQA_C12: mix of > 40 wt% of UDMA, 55 wt % of GDMA, 25 wt% of pQA.	Molar teeth, crowns, dental splint, and orthodontic retainers.	Contact-killing against *Streptococcus mutans* evaluated with and without salivary film. Bacterial suspensions (30, 300, and 3000 bacteria/cm−2) were dropped at the samples in 3M Petrifilm aerobic count plates. Biofilm formed for 6 days on the sample's surfaces was stained and evaluated by CLSM.	14 wt% UDMA/ GDMA/QA_C12 and 25 wt% UDMA/ GDMA/pQA_C12 showed more efficacy against *S. mutans* than UDMA/GDMA.

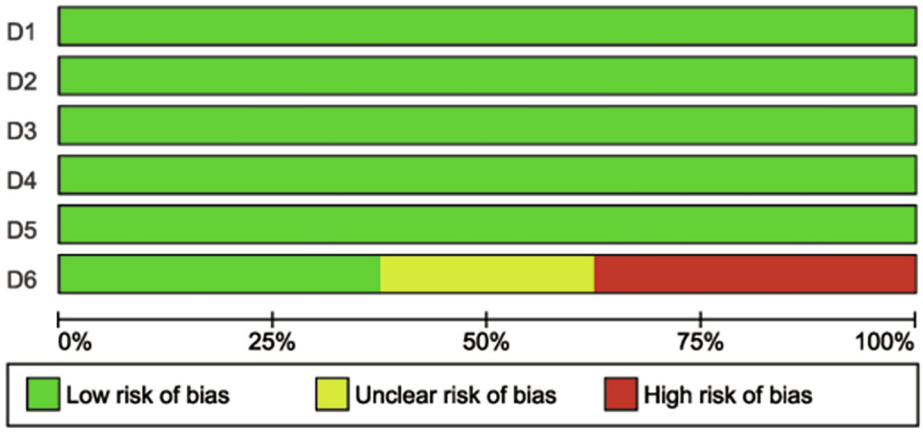

Fig. 2 Risk of bias graph. D1: Is it clear in the study what is the 'cause' and what is the 'effect'? D2: Were the participants included in any comparisons similar? D3: Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest? D4: Was there a control group? D5: Were the outcomes of participants included in any comparisons measured in the same way? D6: Was appropriate statistical analysis used?

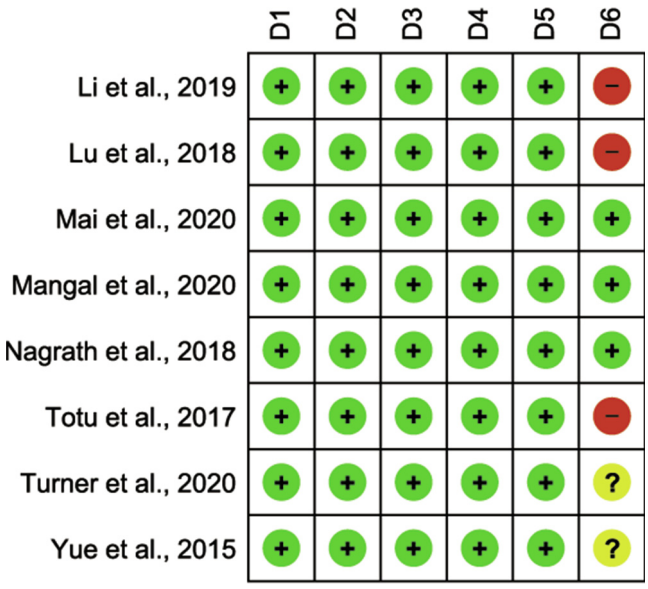

Fig. 3 Risk of bias summary. D1: Is it clear in the study what is the 'cause' and what is the 'effect'? D2: Were the participants included in any comparisons similar? D3: Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest? D4: Was there a control group? D5: Were the outcomes of participants included in any comparisons measured in the same way? D6: Was appropriate statistical analysis used?

reused (Stansbury and Idacavage, 2016). SLM is a technique similar to SLS, providing a total melting of particles, commonly used for sintering metal powder (Oliveira and Reis, 2019). DLP uses photocurable resin and a digital projection screen with micromirrors to display a single image on the entire vat at once. A prepolymerized layer is the basis for the next layer to be cured (Totu et al, 2017; Santos et al., 2022). DLP is an evolution of SLA with the advantage of taking less time to print (Teixeira and Reis, 2021). FDM produces objects by layer-by-layer overlap, using a thermoplastic filament heated to the melting point, obtaining a three-dimensional object by double extrusion (Nagrath et al. 2018; González-Henríquez et al., 2019; Bayraktar et al., 2019).

4.2. Antimicrobial agents and mechanism of actions

Nanoparticles, oxides, and metal particles are known for their antimicrobial activity (Makvandi et al., 2020a). Cu, Ag, and TiO_2 are addressed in this review (Totu et al., 2017; Lu et al., 2018; Turner et al., 2020). Ag is widely used as an antimicrobial agent incorporated into dental materials and medical devices, exhibiting a broad spectrum against Gram-positive and Gram-negative microorganisms (Bapat et al, 2018). The mechanism of action focuses on the direct and indirect interaction of Ag ions and cellular structures, such as the production of reactive oxygen species (ROS) (Bapat et al., 2018; Delfi et al., 2020; Makvandi et al., 2020a; Turner et al., 2020). Ag nanoparticles interact with enzymes of the cell wall containing sulfur functional groups (thiol) and establish bonds that inactivate the bacteria, which happens for methicillin-resistant *S. aureus* (MRSA) (Makvandi et al., 2020a; Turner et al., 2020). ROS interacts with protein and lipids of the membrane and causes damage to DNA, breaking the double bond between nucleotide pairs (Bapat et al., 2018; Makvandi et al., 2020a; Zare et al., 2020).

Cu is reported as a potent antimicrobial against MRSA and used for water purification in filters against *E. coli*. Its mechanism of action is related to ROS production and hydroxyl radicals (OH•) that cause damage to DNA, proteins, and cell death (Delfi et al., 2020; Makvandi et al., 2020a, 2020b). Cu nanoparticles accumulate on the cell surface and rupture the membrane, which facilitates their entry into the cell (Makvandi et al., 2020b).

The bactericidal and fungicidal activity of TiO_2 nanoparticles is related to ROS production and OH• by a photocatalytic reaction in water presence. This reaction damages the polyunsaturated phospholipids of the peptidoglycan cell membrane, invading the cell and damaging the DNA. This prevents the development of microorganisms on the surface of the material

(Totu et al., 2017; Bapat et al., 2019; Makvandi et al., 2020a; Zare et al., 2020).

Two studies included in this review incorporated QA into composite (Yue et al., 2015) and photocurable (Li et al., 2019) resins, and verified their effect against *S. mutans*, *E. coli*, and *S. aureus*. QA acts by contact-killing the bacteria, which, when interacting with the alkyl chain, is invaded through the membrane, causing a rupture of the cytoplasm and autolysis. The long chain of QA is more effective against Gram-positive bacteria, since it penetrates the polyglycane outer layer of this cell type, which is loosely packed. Gram-negative bacteria have a double phospholipidic membrane that functions as additional protection for the cytoplasm (Makvandi et al., 2018; Delfi et al., 2020). Gram-positive and Gram-negative bacteria have negative charges on their surface, and the QA is a cationic salt that shows antimicrobial activity by electrostatic interaction (Nikfarjam et al., 2021). This corroborates the results observed by Li et al. (2019), who found that QAC and SH_QAC were more effective against *S. aureus* (Gram-positive bacteria) than *E. coli* (Gram-negative).

Antifungals perform an important role against microorganisms that cause oral diseases, such as *Candida* spp. AmB binding to ergosterol, the main component of the fungal cell membrane, results in the formation of pores in the membrane. These pores cause fungal acidity with the loss of cytoplasm and cell death (Hamill, 2013).

Nanodiamonds (ND) are nanoparticles that mimic the properties of diamonds and are toxic to Gram-positive and Gram-negative bacteria, depending on concentration and size (Makvandi et al., 2020b; Mangal et al., 2020; Wang et al., 2020). Their hydrophilic properties attribute high reactivity to the surface (Mangal et al., 2020), inhibit biofilm formation, and cluster in bacteria surfaces with a negative charge, causing cellular damages (Makvandi et al., 2020b). The physical mechanism of action can cause damage to the bacterial outer membrane and consequent cell death (Wang et al., 2020).

Chlorhexidine (CHX) is an antibiofilm agent, commonly used in mouthwash, and has a broad spectrum of action. However, it does not have the ability to make monomeric bonds, which makes its incorporation into polymers difficult. Another challenge is controlling its release to be slow and gradual (Makvandi et al., 2020b). For this reason, CHX was encapsulated in carriers such as MSN to be released over time and dispersed in a polymer, to then be incorporated into resin (Mai et al., 2020). CHX causes disruption of the cell membrane and extravasation of intracellular components, such as potassium and nucleic acids. In addition, it inhibits the bacterial glycolytic pathway. It is more effective against Gram-positive bacteria due to the teichoic acids in the cell wall and the absence of the outer membrane that makes up Gram-negative bacteria (Teixeira et al., 2019).

4.3. Antimicrobial effect on 3D printed dental materials

The printing parameters influence the properties (Teixeira and Reis, 2021). Nagrath et al. (2018) observed antifungal effect only in the sample printed as one layer that presented more porous and permittivity in Scanning Electron Microscopy (SEM) analysis. While the multilayer samples formed a solid and impermeable surface, preventing drug release. In addition, during the printing, the heating of AmB reduce drug integrity, which could have occurred in multilayer samples.

Totu et al. (2017) proved that the addition of 0.4% TiO_2 was able to inhibit *Candida* spp., while the literature shows that 5% TiO_2 must be incorporated into conventional resin to obtain any effect (Naji et al., 2018). Thus, 3D printing favored greater efficacy with fewer concentration. The ultraviolet radiation (UV) in 3D printing can be responsible for this effect of TiO_2, since it activates the crystalline form of TiO_2 and generates electrons, ROS, superoxide, and OH• (Naji et al., 2018).

The antimicrobial efficacy of Cu incorporated into metal alloys was also demonstrated in other studies, corroborating the results of Lu et al. (2018): Ti-Cu (Zhang et al., 2013) and Ti_6Al_4V-xCu (Ren et al., 2014) inhibited *E. coli* e *S. aureus*. However, Lu et al. (2018) did a preliminary test and did not mention the number of samples per group or the colony count, which does not guarantee the reproducibility of the results.

The method of incorporation influences the antimicrobial result. QAC incorporated directly into resin showed more efficacy than SH-QAC with the same QA content. The long chain of the SH-QAC may have immobilized the antimicrobial agent in resin (Li et al., 2019). Yue et al. (2015) also incorporated lower concentrations of QA_C_{12} than pQA_C_{12} (a semi-interpenetrating polymer network), ensuring a high molecular weight antimicrobial molecule within the matrix, which minimizes the rapid release of the antimicrobial agent.

The evaluation method also influences the result. 1.0% B65003-PA2200 showed antibacterial effect only when tested in PBS, and in BHI showed no activity. BHI has chemical substances that react with silver ions and make them lose their efficacy, especially the functional thiol groups (Turner et al., 2020).

Some antimicrobial agents, besides reinforcing the structure, change the surface characteristics as hydrophilicity. ND has this ability, influencing bacteria adhesion (Mangal et al., 2020); and CHX coating reduced surface irregularity, influencing the hydrophilicity and lower adhesion of *S. mutans* (Mai et al., 2020).

4.4. Clinical relevance and limitations of antimicrobial agents for clinical implications

The incorporation of antimicrobials into dental materials prevents microorganism adhesion and pathogenic biofilm formation, which cause local and systemic diseases. Antimicrobial metals such as Cu and Ag, when incorporated into alloys and implant materials, avoid *peri*-implantitis, and screw and abutment loss (Lu et al., 2018; Turner et al., 2020). Crowns and bridges made with antimicrobial alloy prevent caries in the surrounding tooth structure and biofilm accumulation (Lu et al., 2018). Antimicrobial composites and resins for dental restorations and orthodontic application also prevent the appearance of caries and periodontal disease-causing biofilm (Yue et al., 2015; Nagrath et al., 2018; Li et al., 2019; Mangal et al., 2020). Antimicrobial prosthetic resin prevents the growth of biofilm that causes chronic mucosal inflammatory responses, such as denture stomatitis, and systemic diseases, such as cardiovascular, joint, pulmonary, and oropharyngeal pathologies, due to the proximity of the pros-

thesis to the respiratory system (O'Donnell et al., 2016; Totu et al., 2017; Mirizadeh et al., 2018; Mai et al., 2020).

The modification of dental materials with antimicrobials is effective; however, it can reduce the resistance of materials or induce cytotoxic effects, which makes their use in clinical practice unfeasible. Research and innovation are important for the production of new materials to overcome clinical disadvantages, such as biofilm formation and diseases caused by this problem, but the industry has a fundamental role in the production of these materials on a large scale and their introduction in the market.

Studies with printed dental materials incorporated with antimicrobials are recent in the literature; therefore, more studies may soon appear on this topic. A future update of this review is recommended, following the evolution of the literature and studies with the incorporation of antimicrobial additives to other printed dental materials such as implants, orthodontic brackets, and aesthetic aligners, for example.

5. Conclusion

Polymers were the class of dental materials for 3D printing that was most often modified with antimicrobial agents, in addition to metal alloys. The antimicrobials used were mainly nanoparticles, metal particles, antifungals, monomers containing quaternary ammonium salt, and antiseptics such as chlorhexidine. The addition of antimicrobial agents in the materials evaluated in the present study showed promising efficacy against *Candida* spp., Gram-positive and Gram-negative bacteria.

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Ethical statement

No ethical issues were during the study presentation.

CRediT authorship contribution statement

Ana Beatriz Vilela Teixeira: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Gabriela Greghi de Carvalho:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Andréa Cândido dos Reis:** Conceptualization, Methodology, Validation, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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