CATÓLICA
FACULDADE DE MEDICINA DENTÁRIA
VISEU

INCORPORATION OF INORGANIC ANTIMICROBIAL SUBSTANCES INTO HEAT-CURED DENTURE BASE RESINS – A SYSTEMATIC REVIEW

Dissertação apresentada à Universidade Católica Portuguesa

para obtenção do grau de Mestre em Medicina Dentária

Por:

Mariana Alexandra Fernandes Lima

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Viseu, 2024

“A journey of a thousand miles begins with a single step.”

Lao Tzu

Aos meus pais:

Pelo apoio incansável e pela crença inabalável nos meus sonhos.

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RESUMO

Introdução: À medida que a esperança de vida e a percentagem de utilizadores de prótese aumentam, uma higiene oral apropriada torna-se crucial. Estomatite Protética, Cárie e Periodontite são as patologias mais observadas na cavidade oral, resultantes de infeções fúngicas e bacterianas. O polimetilmetacrilato (PMMA) é o material de base protética mais utilizado, devido às suas propriedades favoráveis. Vários estudos avaliam a incorporação de agentes anti-infeciosos no PMMA como uma estratégia para prevenir o crescimento de biofilme na superfície protética. Esta revisão sistemática visa avaliar o efeito da incorporação de antimicrobianos inorgânicos em resinas de base protética na prevenção do crescimento microbiano e identificar as partículas mais eficazes para melhorar as propriedades antimicrobianas do PMMA.

Materiais e Métodos: Esta revisão sistemática segue as diretrizes PRISMA, tendo o protocolo de pesquisa sido registado no PROSPERO. A pesquisa foi realizada, usando *Medical Subject Headings* (MeSH) e operadores booleanos no PubMed/Medline®, Cochrane® e Web of Science® Core Collection, a 27 de setembro de 2023 e repetida em 17 de janeiro de 2024. A seleção foi realizada por dois investigadores independentes, começando pelos títulos dos artigos, seguida pelos resumos e, finalmente, pela leitura dos textos integrais. A avaliação da qualidade e a concordância inter-examinador foram avaliadas utilizando a *Checklist for Quasi-Experimental Studies do Joanna Briggs Institute* e o kappa de Cohen.

Resultados: No total, 246 títulos foram lidos, resultando na exclusão de 194 artigos. Dos 52 resumos lidos, 25 foram selecionados para a leitura de texto completo. Assim, 15 estudos foram incluídos nesta revisão, dos quais 14 apresentaram baixo risco de viés, enquanto um apresentou risco moderado. Observou-se uma concordância quase perfeita nas três fases da seleção. A extração de dados foi realizada, focando na preparação das amostras e nos testes antimicrobianos realizados.

Conclusões: A maioria dos antimicrobianos inorgânicos apresentou propriedades favoráveis contra as estirpes microbianas. No entanto, devido à alta heterogeneidade nas amostras, são necessários mais testes e ensaios clínicos.

Palavras-chave: Agentes Anti-Infeciosos, Nanopartículas, Polimetilmetacrilato, Bases Protéticas, Estomatite Protética

ABSTRACT

Introduction: As life expectancy and the percentage of denture wearers increase, appropriate dental hygiene becomes crucial. Denture Stomatitis, Caries, and Periodontitis are the pathologies most observed in the oral cavity, resulting from fungal and bacterial infections. Polymethylmethacrylate (PMMA) is the most widely used denture base material, due to its favourable properties. Various studies have tested the incorporation of anti-infective agents into PMMA as a strategy to prevent biofilm growth on the denture surface. This systematic review aims to evaluate the efficacy of incorporating inorganic antimicrobial particles into the denture base resins in preventing antimicrobial growth, thereby identifying the most effective agents for enhancing PMMA's antimicrobial properties.

Materials and Methods: This systematic review adheres to the PRISMA guidelines and the research protocol was registered in PROSPERO. The search was performed using Medical Subject Headings (MeSH) and Boolean operators in PubMed/Medline®, Cochrane®, and Web of Science® Core Collection, on 27 September 2023 and repeated on 17 January 2024. The screening was conducted by two independent investigators, starting with article titles, followed by abstracts, and finally, full-text readings. Quality assessment and interrater agreement were evaluated using the Joanna Briggs Institute's Checklist for Quasi-Experimental Studies and Cohen’s kappa.

Results: A total of 246 titles were screened, resulting in the exclusion of 194 articles. Out of 52 abstracts, 25 were selected for full-text screening. Finally, 15 studies were included in this review, fourteen of which with low risk of bias, while one presented a moderate risk. Almost perfect agreement was observed in the three phases of the screening. Data extraction was performed, focused on sample preparation and antimicrobial tests performed.

Conclusions: Overall, most of the inorganic antimicrobials presented favourable properties against the microbial strains. However, due to the high heterogeneity in the samples, more tests are necessary, as well as clinical trials.

Keywords: Anti-Infective Agents, Nanoparticles, Polymethyl Methacrylate, Denture Bases, Denture Stomatitis

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List of abbreviations and acronyms

Ag – Silver

AgCl – Silver Chloride

$AgVO_3$ – Silver Vanadate

Au – Gold

CAD-CAM – Computer-aided design / Computer-aided manufacturing

CADS – Candida-associated Denture Stomatitis

CFU – Colony Forming Unit

Cu – Copper

CuO – Copper Oxide

DNA – Desoxyribonucleic Acid

IFCD – Implant-fixed complete dentures

IOVD – Implant-supported overdentures

JBI – Joanna Briggs Institute

MeSH – Medical Subject Headings

ND – Nanodiamond

PICO – Population; Intervention; Comparison; Outcome

PMMA – Polymethylmethacrylate

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analysis

PROSPERO – International Prospective Register of Systematic Reviews

ROS – Reactive Oxygen Species

SEM – Scanning Electron Microscope

SiO_2 – Silicon Dioxide

TeO – Tellurium Oxide

TiO_2 – Titanium Dioxide

1 Introduction

Nowadays, tooth loss continues to be a public health concern. With age, tooth decay and periodontal diseases become more prevalent; consequently, just as systemic health declines, so does oral health[1]. As life expectancy and the percentage of denture wearers increase[2], the proportion of immunocompromised patients in need of oral rehabilitation also rises, making proper dental care and denture hygiene essential to provide a good quality of life[3]. Oral rehabilitation allows patients to recover their ability to eat solid aliments while restoring aesthetics and function, thus improving the psychological well-being of the patient[3,4]. However, just like natural teeth, dentures require proper care and maintenance. Poor literacy on how to properly care for and wear dentures can result in the development of multiple pathologies and subsequent severe decline of the patient's general health[3]. It is important to be aware of the pathologies associated with denture wearing to prevent and treat them, be it by instructing the patient and caregivers on how to clean their denture properly or by taking precautions to prevent the adhesion of the pathogens to the denture in the first place[3].

1.1 Dentures

Edentulism, defined as a lack of teeth, is frequently observed in elderly populations, although it can affect a wider age range[1]. Different patients require different treatment plans and, as such, these procedures entail an individualized approach to provide the best solution to the problem. One of the most important objectives of oral rehabilitation is to restore function and aesthetics without compromising oral health, teeth, and mucosal tissues[4]. Therefore, to provide the best comfort and retention/stability possible, examining the bone quality and quantity is just as important as analysing the smile line, lip support, vertical dimension of occlusion and phonetics[2]. Depending on these factors, dentures can be designed in multiple schemes, ranging from removable to fixed and partial to complete[3].

1.1.1 Types of dentures

As there are various degrees of edentulism, multiple dentures were designed to accommodate all the different necessities observed. Therefore, dentures vary in materials used, extension and type of support. Oral rehabilitation can be achieved through fixed or removable prostheses. Each type of rehabilitation can be partial or complete, varying in the type of support (tooth/implant and/or tissue)[5]. Compared to implant rehabilitations, conventional removable dentures are conservative and minimally invasive, allowing dental practitioners to reinstate the patient's vertical dimension of occlusion, function, and aesthetics without surgery[6].

Edentulism can be differentiated into complete, when no natural teeth remain in the arch, or partial when there are natural teeth surrounding edentulous spaces. Kennedy's classification, one of the most widely accepted systems of classification of partial edentulism, aids in characterizing partial edentulism according to the location of the remaining teeth. According to Kennedy's classification, edentulism can be classified as class I (bilateral and posterior to remaining teeth), II (unilateral and posterior to remaining teeth), III (unilateral with natural teeth mesial and distal to the edentulous space) and IV (single space, bilateral and anterior to remaining teeth)[7–9].

Depending on the location of the edentulous area, partial dentures will be adapted to be solely supported by adjacent teeth (class III) or by both tooth and mucosa (classes I and II), while complete dentures often solely rely on mucous support. Natural roots or implants may be prepared to support overdentures, allowing proprioception and better retention. Fully edentulous arches may be viable for implant-supported dentures, such as implant-fixed complete dentures (IFCD), only removable by dentists, or implant-supported overdentures (IOVD) that can be removed by the patient[2]. Thus, while patients may consider IFCDs may be more comfortable and stable, IOVDs are more affordable and provide better phonetics, with reduced surgical time and morbidity[2].

Partial removable dentures can have metallic frameworks, denominated skeletal dentures, or acrylic frameworks, entitled acrylic dentures, advantageous due to their aesthetics, affordability, and ease of modification/rebasing[10]. However, acrylic dentures have their disadvantages, namely bulkiness and a tendency for plaque

accumulation[10]. To improve aesthetics, nonmetal clasp dentures were developed, using acrylic resin instead of metal clasps; despite their aesthetic advantage, acrylic clasps may result in an increased risk of loss of periodontal support in abutment teeth and bone loss[6].

Generally, a denture will be classified as transitional or definitive; however, another classification, immediate denture, is given to prostheses that are applied immediately after tooth extraction[11]. Immediate dentures are made of acrylic and can be transitional, with plans to change to a new denture once the socket heals, or definitive, with the possibility of rebasing and relining after tissue adaptation to the prosthesis[11].

1.1.2 Denture components

Complete and partial dentures differ in multiple aspects, including in the components. Complete dentures are divided into two main parts: prosthetic base and artificial teeth[9]. On the other hand, the main components of partial removable dentures are retainers, connectors, prosthetic base, and artificial teeth[9]. Retainers provide the denture with stability and retention by attaching to the abutment teeth through a clasp assembly or a precision attachment[9]. Connectors can be differentiated into major, connecting all the edentulous areas in the arch, and minor, connecting the major connector to the other elements of the denture, such as rests, clasps, and indirect retainers[9]. The prosthetic base serves as a base for the artificial teeth, with a saddle covering the edentulous mucosa, and can be made of metal, resin, or metal-resin[9,10]. Artificial teeth can be made of resin, porcelain, or metal, and are the components responsible for restoring the function and aesthetics of the natural teeth they are replacing[9].

1.1.3 Ideal denture

Oral rehabilitations abide by specific parameters on the ideal properties of a denture. Therefore, dentures follow some requirements to provide the best comfort to the patient and to avoid deleterious interaction with the organism: prosthesis should seek

to preserve the remaining teeth, avoid excessive forces, have a good fracture resistance, be biocompatible and favourable to general health[9,12].

An ‘Every’ denture refers to partial removable dentures that follow the six principles for a maxillary removable partial denture described by Every in 1949[10]. Therefore, an ‘Every’ denture complies with the following principles: maintain interdental point of contact between natural and artificial teeth, prevent the distalization of the most distal natural tooth by using distal stabilizers, leave wide embrasures between natural and artificial teeth to lessen plaque accumulation and gingival contact, provide a free-occlusion to avoid occlusal interferences, obtain maximum retention by extending the prosthetic base to cover a wider area, and keep palatal acrylic 3mm away from the gingival margin[10].

1.1.4 Retention

One of the main components of partial dentures, the retainers, provide stability and retention through attachment to abutment teeth, while indirect retainers prevent the displacement and rotation of distal extensions[9,10].

Since complete edentulous patients lack healthy abutment teeth where retentors could attach, these dentures require other mechanisms of retention, such as sub-pressure and tissue adherence[9]. Sub-pressure depends on the formation of a peripheral seal between the denture margin and the mucosa and can be obtained in the vestibulum and the post dam area[9]. Retention is also improved by the formation of a thin film of saliva between the denture base and the mucosa; thus, a wider surface area provides improved retention[9].

Another mechanism of achieving optimal retention is the application of implant or root-supported overdentures[9]. However, root-supported denture requires careful and thorough oral hygiene to preserve the roots and prevent caries and periodontitis[9].

1.1.5 Associated pathologies

Partial denture wearers are at higher risk of developing tooth decay, gingival inflammation, and bone loss, as the abutment teeth are subjected to multiple forces[10,13]. Therefore, even though oral rehabilitation should be carefully planned to achieve the best results and prognosis[13], it is also important to know the main pathologies that affect denture wearers to best prevent and treat them. Denture Stomatitis, tooth decay and periodontal diseases are pathologies caused by microorganisms, specifically fungi and bacteria, and have the potential to severely impact the patient's health.

Interactions between microbes in the oral cavity are common and often essential for their survival and development[14]. In the oral cavity, metabolic communication between bacteria, namely streptococci, and *Candida albicans* (*C. albicans*) is beneficial for both species' subsistence[14]. As such, interactions between *Streptococcus mutans* (*S. mutans*) and *Candida albicans* are frequently observed, which involve *C. albicans* benefiting from carbon sources, adhesion sites and lactic acid and, in return, *S. mutans* profiting from reduced oxygen tension[14,15]. Thus, *C. albicans* is considered a mediator for tooth decay, as *S. mutans* is often found in *C. albicans* biofilms[14]. The interaction between *C. albicans* and periodontitis-related microbes, such as *Fusobacterium nucleatum* is not yet fully understood, despite the certainty that *Fusobacterium nucleatum* can communicate with both Gram-positive and Gram-negative bacteria and fungus, thus contributing to *C. albicans* colonization and impacting the general health of the patient[14,15].

Multiple pathologies can originate from the oral cavity and spread to other organs, a phenomenon that may occur in denture wearers with insufficient denture hygiene and that can result in the onset of diseases such as aspiration pneumonia, septic meningitis, and infective endocarditis[3]. A higher risk of stroke, coronary artery disease, and other cardiovascular diseases may be observed in patients with periodontitis, while caries is mostly associated with Diabetes[16,17]. Thus, it is essential to be aware of such correlation and properly prevent and control biofilm formation with adequate disinfection of the prosthesis[3].

1.1.5.1 Denture Stomatitis

Commonly referred to as “thrush”[14], *Candida spp.* are opportunistic pathogens[18], *Candida albicans* being the species most frequently found in oral mucosa, mainly in the tongue and palate[14]. With a multifactorial aetiology, oral candidiasis is one of the most prevalent diseases caused by this yeast[18]. Although the prevalence of other *Candida* species (*Candida spp.*), such as *Candida krusei*, has been increasing[19], *C. albicans* is still considered the most predominant pathogen responsible for Candida-associated Denture Stomatitis (CADS) in denture wearers[14,15,19,20].

While *Candida spp.* is commonly present in the oral cavity as a commensal species[14,18,19], a shift in the human microbiome can result in a transition to a pathogenic state and, eventually, originate an infection[18,19]. Thus, age and systemic conditions, such as hypertension, or diabetes[18] can result in this imbalance and subsequent onset of infection. Since *C. albicans* is prevalent on the dorsal surface of the tongue, this is the most frequent location of infection in most manifestations of oral candidiasis[14]. In denture wearers, however, the focus of infection is the palate[21], as the low oxygen and pH favour *Candida spp.* proliferation, commonly resulting in the development of erythema and hyperplasia[14].

In dentures, *C. albicans* can normally be found on the palatal surface[18]. This can lead to the development of CADS, as the friction between the denture and the palate traumatizes the mucosa and allows *Candida spp.* to infiltrate the mucosal tissues, thus resulting in Denture-Induced Stomatitis (DIE)[14,19]. This inflammatory pathology is known to have a multifactorial aetiology, generally attributed to infections and trauma[21]. As a consequence, denture wearers have a higher risk of developing CADS due to poor oral hygiene, prolonged denture wear, decreased salivary flow and complications during denture fabrication[15,18,21].

1.1.5.2 Tooth decay

Tooth decay is a multifactorial pathology of the oral cavity that evolves from surface roughness to cavitation, pulp involvement, abscess and systemic symptoms, if left untreated[22]. Cavities are generally associated with bacteria, namely streptococci and

lactobacilli, and their metabolization of carbohydrates, such as sucrose, into acids, resulting in demineralization of the enamel[22]. Since caries is one of the most observed pathologies of the oral cavity, proper hygiene is required to avoid tooth decay and consequent tooth loss[1].

The oral microbiota generally present on the tooth surface includes two main cariogenic bacteria, *Streptococcus mutans* and *Lactobacillus acidophilus*, that ferment carbohydrates and produce acid[17,22]. While the salivary flow removes food debris[14] and contributes to the remineralization of surface enamel eroded by the acid, a prolonged exposure to acidic substances results in a deeper demineralization and, afterwards, a discontinuity in the enamel colour and surface[22]. Following the evolution of a white spot into a cavity, the patient often reports higher sensitivity to temperature shifts and sweet or sour ingredients, associated with food impaction in the enamel discontinuity and interdental spaces[22]. Other than plaque and food debris, there are multiple factors responsible for tooth demineralization, such as tooth morphology and medication[22].

Denture hygiene is essential both in complete and partial dentures, as well as in overdentures[9]. Partial dentures are more predisposed to food accumulation in the clasps and adjacent natural teeth, thus increasing the risk of tooth decay and root caries[1]. Roots in teeth-supported overdentures are also susceptible to tooth decay, thus making good oral hygiene essential[9]. Since streptococci find acidic environments favourable to their colonization, dentures require careful and thorough disinfection to remove any food buildup and prevent biofilm adherence[1]. Floss and brushes are essential for proper hygiene in abutment teeth and interdental spaces, aiding in removing accumulated food debris and preventing biofilm formation in those sites[4].

1.1.5.3 Gingivitis and Periodontitis

The tooth surface is generally covered with plaque, composed of microbes and food particles[22], that is constantly removed by salivary flow and oral hygiene[14,22]. When this hygiene isn't thorough and buildup remains on the tooth surface for a longer time, it hardens into calculus, consisting of calcified plaque[23].

The surface of supragingival calculus is, generally, rough[24] and, in association with microbial lipopolysaccharides and toxins, may result in gingival inflammation and gingivitis[23]. Subgingival calculus formation occurs after bacteria colonize the root surface, following which bacterial matrix-metalloproteinases initiate the dissolution of collagen and establishment of periodontal pockets, leading to periodontal support tissue loss (periodontitis)[23].

While the oral microbiota is so vast, there are two main groups of microbes associated with periodontitis, the red and orange complexes[25]. The major pathogens responsible for periodontitis belong to the red complex and include *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, while other bacteria possibly connected to periodontitis, such as *Fusobacterium nucleatum*, are sorted into the orange complex[25,26]. However, other species have been reported to be probable pathogens responsible for periodontitis[25].

Gingivitis and periodontitis are often observed in elderly patients, culminating in tooth loss as a result of bone resorption and gingival inflammation. In partial denture wearers, periodontitis is commonly found in abutment teeth, with a lower incidence in non-abutment teeth[4,10]; thus, to maintain tooth support, partial dentures should be carefully cleaned[1,10]. Roots in teeth-supported overdentures are at higher risk of periodontitis and, consequently, require careful oral hygiene[9]. Therefore, appropriate hygiene is fundamental to eliminate food and biofilm that may have remained in interdental spaces and abutment teeth[4].

1.2 Denture resins

Denture base materials have progressed greatly through the centuries, from ivory to metal and finally resins. Nowadays, denture bases are composed mostly of metal alloys or resin, with each material having its advantages and disadvantages. Although PMMA is the most used denture base material, multiple types of resins have been studied and used as base materials for dentures, varying in properties, preparation methods and treatment prognostic.

1.2.1 Chronological evolution of denture bases

While dentures are reported to have been developed in 3000BC[15], denture materials have evolved across the centuries, with historical mentions of dentures made of different materials, such as bone, wood, ivory, and gold[27]. Although gold, used centuries ago, had excellent biocompatibility and corrosion resistance, it lacked aesthetics and was expensive[27,28]. In the 17th century, Purmann (1648-1721) described for the first time the process of fabricating a denture out of wax that lacked retention, a problem resolved by Fauchard (1678-1761) with the introduction of springs[27].

Material such as ivory are reported to have been used to carve dentures in the 18th century, as observed with US President George Washington; however, ivory easily stained and deteriorated in the mouth, proving not to be an ideal material[27]. Porcelain, also fabricated in the 18th century, had an unpredictable contraction during firing, which made it difficult to replicate and produce porcelain dentures[27,28].

In the 19th century, being cheap and easily adaptable, vulcanite became the main material used in denture bases[27]. Vulcanite had many advantages, such as comfort, low density and weight, ease of fabrication and dimensional stability; however, it was unesthetic, lacked a chemical bond to porcelain[28] and required a payment of royalties for dentists to be allowed to use it[27]. An attempt at replacing vulcanite was made with aluminium, which provided an accurate fit but failed due to its expensive and demanding technique[27,28]. Celluloids could be coloured to simulate mucosa but also presented disadvantages, such as a tendency to distort (due to the lack of dimensional stability), being prone to food stains and resulting in modified taste and odour as a result of the presence of camphor[27,28].

In the 20th century, resins such as bakelite were discovered and applied in dentistry, offering good aesthetics (although easily stainable), but were brittle and required a sensitive and careful manipulation[27,28]. A copolymer of acetate and vinyl chloride, Polyvinyl Chloride (PVC), presented many disadvantages, namely being susceptible to discolouration and having weak mechanical properties[28].

Metal alloys were first introduced as denture-base materials in the 20th century, with an evident increase in their application in the manufacturing of fixed and removable dentures[28–30]. A good compatibility with the oral tissues is desirable, thus an ideal metal alloy exhibits resistance to chemical degradation and corrosion[30]. Niquel-chromium and cobalt-chromium are the two metal alloys that are most frequently used, although the addition of other metals, such as molybdenum, tungsten, and titanium, has presented advantageous properties[29,30]. Molybdenum is reported to present a lower metal ion leaching, compared to nickel and chromium[30].

Despite their multiple advantages, such as good mechanical properties, affordability, and low density, nickel-chromium dentures require difficult manipulation and their hard and metallic frame result in an unesthetic appearance, with a risk of allergic reactions due to the presence of nickel[28]. On the other hand, cobalt-chrome alloys are compatible with oral tissues and present high resistance and strength[29]. With a high retention load, cobalt-chrome-molybdenum is the most frequent alloy used in the framework of removable partial prosthesis[31,32].

In 1843, polymethylmethacrylate (PMMA) was reported by Redtenbacher for the first time; nevertheless, its introduction as a denture base material only occurred in 1937[12,28,33]. By 1946, PMMA had replaced other materials and had become the most used denture base material, with its uses increasing and expanding in the following two decades[28]. Bypassing disadvantages presented by other materials, PMMA was able to chemically bond to acrylic teeth, was easy to repair, inert, translucent and had a good colour that could mimic tissues and teeth[28,33]. However, PMMA's polymerization contraction led to the development of a new injection technique, thermoplastic resins (nylon in the 1950s) and a new system of rapid injection[12,34]. Despite its weaknesses, PMMA remains the most used acrylic resin in dentures[27], with modifications, such as the addition of fibres and other materials, being made to overcome some of its disadvantages[28].

1.2.2 Denture base resins

Removable partial dentures are generally composed of acrylic saddles covering a metal framework, resulting in a denture that is stronger and fracture-resistant, while

presenting several disadvantages regarding aesthetics, allergies, and corrosion[35]. The American Dental Association (ADA) groups denture resins according to their activation mechanism into type I (heat-polymerized), type II (auto-polymerized), and type III (thermoplastic resins), while the ISO 20795-1 2013 added type IV (light-polymerized) and type V (microwave-polymerized) into the classification[28].

Although polymethylmethacrylate is still the most used resin in conventional dentures, other resins, namely thermoplastic resins, have been developed and incorporated into flexible dentures[34–37]. Nonmetal clasp dentures provide better aesthetics and comfort than conventional skeletal partial dentures, as there are no metal clasps to be visible whilst smiling or in function, while their translucency allows a better colour match with mucosa and gingiva tissues[6,12,34,37]. A light-curing resin, based on urethanedimethacrylate (UDMA), has been proposed as an alternative to PMMA, presenting a lack of methyl methacrylate residues, as well as easy and time-efficient preparation and manipulation[38]. However, this resin was reported to have a higher degree of deformation when compared to PMMA, thus having a low dimension accuracy[38].

1.2.2.1 Thermoplastic resins

Thermoplastic resins have been developed to overcome some of the main disadvantages of polymethylmethacrylate, as well as provide an aesthetic, metal-free alternative to partial dentures[12,34]. Possessing advantages such as transparency, high dimensional and colour stability, and decreased odour, thermoplastic resins are used as clasps in nonmetal clasps dentures, replacing the metallic clasps and, therefore, increasing the patient's satisfaction[6,12]. Flexible dentures are softer and have a lower module of elasticity, comparatively to the hard and dense acrylic resins, and pose no risk of metal allergy, thus being more comfortable for the patient[6,34,37,39]. Meanwhile, they also possess good mechanical properties, such as flexibility, bonding and impact strength, fatigue endurance, and lower quantities of residual monomer[12,39,40]. However, nonmetal clasp dentures require complex preparation and polish, being at higher risk of clasp fracture, abrasion roughness, discolouration and causing periodontal disease[6,34,37,39].

There are multiple thermoplastic resins, such as polyamide, polyester, polycarbonate, and polypropylene[12,34,37,39,40]. Despite their well-known high fracture resistance, polyamides, namely nylon, have variable physical characteristics, depending on the fabricant[35,40]. Providing generally good aesthetics and lower water sorption and solubility compared to PMMA[36], as well as higher flexibility due to modifications with fibres, polyamides present a risk of discolouration and staining, have a poor fit, and are difficult to repair due to being chemical-resistant[35,39,40]. Even though the risk of fracture is higher as a result of its reduced impact resistance, polyester resins have a great fit, good flexural strength and modulus, are soft, and are easy to repair, due to good bonding to self-polymerizable resins[40]. Polycarbonates have high flexural strength and modulus as well as a good fit and bonding strength to self-polymerizing resins, thus making repairs easier[40]. Being a recently developed resin, polypropylenes' properties are still under investigation[37,40], with results stating this resin presents a lower flexural strength and modulus comparatively to other thermoplastic resins[37].

1.2.2.2 Polymethylmethacrylate

Polymethylmethacrylate (PMMA) is currently the most used denture base material[41], generally used in the fabrication of prosthodontic and orthodontic appliances[28]. Having replaced previously used dental base materials, such as gold and porcelain, this resin is not devoid of disadvantages[28]. Although PMMA's properties are majorly favourable, there is ongoing research with the intent of developing novel resins or modifying PMMA to achieve ideal mechanical and chemical properties without loss of biocompatibility[28,35,42].

1.2.2.2.1 Types of polymerizations

PMMA is generally presented in a powder-liquid formulation, with powder containing the polymer PMMA and the liquid encompassing the monomer methyl methacrylate, the accelerator, and the inhibitor[28]. PMMA polymerization consists of four steps: initiation, activation, propagation, and termination[28]. The creation of a free radical

following the initiation and activation phases occurs chemically or through exposure to microwaves, heat, or light[28]. Microwave-cured PMMA polymerization requires a short curing time through exposure to microwave energy in a non-metallic flask, resulting in a similar dimensional accuracy to heat-polymerized PMMA[28].

Heat-cured PMMA requires heat energy to activate the initiator, namely benzoyl peroxide, after combining the powder and liquid[15,28]. The heat cycle can be achieved through cycles of varying lengths, ranging from a 9-hour water bath to a shortened polymerization time of 20 minutes for rapid-heat PMMA, and results in the generation of free radicals[28]. The heating cycle allows a high degree of polymerization, thus resulting in good physical properties, and reducing residual monomers[28]. Nevertheless, heat polymerized PMMA has an unsatisfactory poor fit and polymerization[28].

Cold polymethylmethacrylate, also known as chemically or self-cured PMMA, does not require heat energy, since polymerization occurs from the activation of benzyl peroxide by a tertiary amine initiator, resulting in a lower polymerization degree and, therefore, compromised mechanical properties, poor colour stability, and residual monomers[15,28]. Although it has advantages such as good dimensional stability and adaptation, its disadvantages result in a reduced use in oral applications[28].

Light-cured PMMA contains a photo-sensitive compound, camphorquinone, that activates when exposed to visible light for a specified time[15,28]. Therefore, light-cured PMMA allows easy and controlled processing and manipulation, with higher dimensional stability and reduced residual monomers and microbial adhesion[28]. However, light-cured PMMA is expensive and restricted to denture repair and relining, due to its sensitive technique, difficulties curing thicker layers, and lower mechanical properties[28].

1.2.2.2.2 Denture fabrication

When PMMA powder and liquid components[28] are mixed, an exothermic reaction occurs, resulting in a progressive change of consistency, and ensuing multiple phases, specifically sandy, stringy, doughy, rubbery, and stiff[28,43]. The time required for the

mixture to reach the doughy stage after mixing depends on multiple factors, such as the powder-liquid ratio, the temperature, and the surface area[28]. Firstly, the sandy stage is characterized by a grainy texture, originating from the PMMA powder becoming soaked by the methyl-methacrylate liquid[28]. Next, in the stringy stage, PMMA dissolves and the mixture gains a stringy and sticky texture[28]. Thirdly, the viscosity is lost in the doughy phase, making it the ideal stage to place the mixture in a dental flask[28]. Afterwards, PMMA reaches the rubbery stage as the residual monomers evaporate, turning into a rubber-like texture that can no longer be moulded[28]. Finally, the resin hardens and dries, as residual monomers continue to evaporate, reaching the stiff phase[28].

Heat-polymerized PMMA dentures are generally fabricated through a flask-pack-press technique, in which PMMA is placed in a flask, pressed, and positioned in a water bath[28]. In the compression moulding method, while PMMA is in the doughy stage, it is placed into a polymerization flask with the gypsum mould inside, exposed to high pressure to fill the entire free space, and then polymerized at a high-temperature[28,43]. On the other hand, the injection moulding method requires the presence of a sprue in the flask, through which the material is injected, and a vent hole, to allow gases to escape[28]. The constant injection of material counters polymerization shrinkage, allowing a better fit[28,41]. After removing the flask from the water bath and allowing it to cool, the denture should be thoroughly polished and prepared before it is placed in the oral cavity[28].

Computer-aided design / Computer-aided manufacturing (CAD-CAM) polymethylmethacrylate can be obtained through subtractive or additive processes[44]. While the subtractive technique requires a pre-polymerized block of PMMA that is milled until the desired denture base shape and size, the additive technique, also known as rapid prototyping, consists of using a light-polymerizable liquid to 3D print the denture[44]. Since the data used to manufacture CAD-CAM is stored, new identical dentures can be produced without a new clinical study, thus reducing chair time[44]. While milled PMMA has no risk of polymerization shrinkage, due to requiring a fully polymerized block of resin, it also results in increased waste of PMMA[44]. On the other hand, being an additive technique, 3D-printed PMMA only uses the exact quantity needed to build the denture as designed[44]. Fabrication of frameworks for removable

partial dentures can also be achieved through CAD-CAM technology, using either metallic or non-metallic materials, and resulting in reduced material waste[45].

1.2.2.2.3 PMMA properties

An ideal denture material should provide acceptable biological, physical, mechanical, and chemical properties, to increase patient satisfaction and comfort. While PMMA has great properties, it also has disadvantageous properties, such as polymerization shrinkage[12,34]. PMMA has good translucency and colour, thus providing good aesthetics[28,33], while being durable, easy to repair and affordable[28]. However, it is prone to staining and discolouration[28].

Regarding biological properties, PMMA is mostly biocompatible, although residual monomers may result in cytotoxicity, mucosa irritation, and tissue inflammation[28]. Thus, a thorough polymerization, a proper liquid-powder ratio and immersion of the denture in water are essential to improve biocompatibility[28]. Therefore, heat and microwave-cured PMMA present the best biological properties[28].

Heat-polymerized PMMA has generally good physical properties, namely dimensional stability and lower water sorption and solubility when compared to self-polymerized PMMA[28]. On the other hand, polymethylmethacrylate has a low thermal conductivity, resulting in surface crazing and lack of temperature perception by the patient[28]. Other disadvantages include lack of opacity, as well as polymerization shrinkage and residual monomers, more frequently observed in heat and cold-polymerized PMMA, when compared to light-polymerized PMMA[28].

While the flexural strength of heat, cold and microwave-polymerized PMMA is considered good, heat-cured PMMA has a higher fracture toughness than cold-cured PMMA[28]. However, polymethylmethacrylate has a low wear resistance and surface hardness, as well as impact and fatigue strength[28,41]. Chemically, even though PMMA is not water-soluble, storing the resin in water may lead to dimension changes and cracking[28]. On the other hand, due to its organic resin composition, PMMA is highly soluble in organic solvents and plasticizes when in contact with alcoholic solutions[28].

Denture bases fabricated through CAD-CAM additive or subtractive techniques provide a better fit, good mechanical, and physical properties, having advantageous characteristics when compared to conventional PMMA, such as flexural modulus and strength, antibacterial capacity, impact strength, less residual monomer, colour stability, as well as surface roughness, wettability, and hardness[28,33,45]. Removable partial dentures metallic frameworks can also be fabricated through CAD-CAM techniques, resulting in superior mechanical strength[45]. While subtractive CAD-CAM PMMA, made from milling a pre-polymerized block of resin[33,44], has an increased hydrophobicity, it is less porous and, thus, might reduce microbial adhesion[33].

1.2.2.2.4 PMMA modifications

Although PMMA's properties are majorly favourable, various modifications, such as the addition of fibres, have been made to PMMA to achieve ideal mechanical and chemical properties and overcome limitations presented by this acrylic resin, such as surface roughness and porosity[28,35]. Polymerization shrinkage, residual monomers, low fatigue, bonding and impact strengths, porosity, low colour stability, and low heat conductivity are some of the major disadvantages of PMMA that have been improved through modifications[15,28,46].

Fibers, such as carbon, aramid, nylon, polyethylene, polypropylene, and glass[28,47], have been added to PMMA, improving fatigue resistance and impact and flexural strengths[47], with their effect depending on the length, diameter, and orientation of the fibres[28]. The fibres used to modify PMMA result in various effects: carbon, although unesthetic, improves tensile strength, fracture resistance, elasticity, and flexibility; polyamides, namely nylon and aramid (unesthetic, potential irritant, and hard to polish), are biocompatible and present increased flexural strength and modulus; requiring surface treatment, polyethylene and polypropylene improve impact strength, toughness and ductility; glass fibres enhance flexural and impact strength, Vickers hardness and toughness while decreasing deformation[28,47].

Filler particles, such as metal oxides, strengthen denture resin and improve its mechanical and physical properties; being thermal conductors, fillers allow the patient to experience temperature perception while wearing the denture[28,47]. Therefore, the

addition of fillers to PMMA results in reduced water sorption and solubility, as well as improved dimensional stability and antimicrobial and mechanical properties[28,47]. These particles can consist of alumina, silicon dioxide, titanium dioxide, platinum, zirconia, nanodiamond, silver, and hydroxyapatite[28,47]. The effect obtained by adding fillers to the resin varies greatly. While nanosilica particles can improve material properties such as abrasion resistance, mechanical properties, and refractive index[42], silver has antimicrobial properties, thermal conductivity, and improved mechanical properties[28,47].

Although chemical modifications to PMMA still require research, a clear example of such modification is rubber-PMMA[28]. By enhancing impact resistance and reducing crack propagation, this modification increases fatigue resistance and flexibility, allowing the prosthesis to endure larger impacts[28].

To prevent biofilm growth, antimicrobial agents may be incorporated in denture base resins, either through inorganic particles, antimicrobial polymers, surface functionalization, or medicaments[28]. Examples of such antimicrobials include fluoride, fluorapatite, titanium dioxide coated with apatite, silver, nanodiamonds, amphotericin B, thymoquinone, and quaternary ammonium compounds[28]. On the other hand, surface functionalization consists of modifying the surface of the denture to avoid altering other properties[28]. Through oxygen plasma and thermal treatment, chlorhexidine, graphene oxide, sodium metabisulfite, and potassium sorbate can be incorporated into PMMA surface with minor alteration to the mechanical properties and cytotoxicity[28].

1.3 Antimicrobials

The oral microbiota is composed of varied microorganisms that communicate and interact among species and even with microbes belonging to other domains[17]. However, a previously commensal microbial has the potential to develop into a pathogen, as a result of dysbiosis, and cause pathologies[18]. As a result, antimicrobials were developed to treat or prevent infections, either by inhibiting microbial colonization or by killing existing pathogens[48]. Therefore, for different pathogens, multiple

antimicrobials have been produced to act by targeting different microbial components[48].

1.3.1 Types of antimicrobials

While antimicrobials are commonly associated with chemical substances, different types of organic and inorganic substances and particles have been researched for their antimicrobial properties[49,50]. Antimicrobial resistance is one of the major factors behind the increasing interest in studying the antimicrobial properties of alternative substances and particles[51].

1.3.1.1 Chemical antimicrobials

Antimicrobials can be classified as antifungals, antibiotics, antiseptics, antivirals, and antiparasitic[48].The mechanisms of action of antimicrobials include the inhibition of cell wall synthesis, as well as nucleic acid metabolism and protein synthesis blocking[52,53].

Antiseptics, such as chlorhexidine (damages the inner cytoplasm membrane), antimicrobial peptides (cell permeabilization and membrane disruption), fluoride (inhibits enolase, a glycolic pathway enzyme), or quaternary ammonium salts (suspected that it causes bacterial lysis by binding to its membrane), are examples of antimicrobials presenting multiple mechanisms for inhibiting the development of microbes[17].

Antibiotics can target cell wall (β-lactams and glycopeptides), protein synthesis (inhibitors of 30S subunit, inhibitors of 50S subunit, tetracyclines, macrolides, and oxazolidinones), DNA replication (quinolones), or folic acid metabolism (sulphonamides and trimethoprim)[53]. There are multiple types of antibiotics, such as penicillin (blocks the synthesis of the peptidoglycan layer of the bacterial cell walls), macrolides (inhibit ribosomal translation), tetracyclines (block protein synthesis), clindamycin (disrupts ribosomal translocation), and metronidazole (block the synthesis of nucleic acid)[17].

Antifungals act by direct interaction or by inhibiting the synthesis of ergosterol, the main component of the fungal cell membrane[54]. Antifungals can be classified as azoles (block ergosterol synthesis), polyenes (bind to ergosterol, altering permeability and causing cell death), or thiocarbamates (inhibit ergosterol biosynthesis)[54]. Examples of such antifungals include miconazole, amphotericin B, or nystatin[15,20].

Antivirals target the virus (inhibiting virus attachment, virus entry, uncoating, polymerase, protease, integrase, or nucleoside and nucleotide reverse transcriptase) or the host cell[55]. Some examples of antivirals are ritonavir, atazanavir, acyclovir, valacyclovir, and raltegravir[55].

Antiparasitic drugs can be characterized as anthelmintic or antiprotozoal drugs, depending on which microbes they target[56]. Anthelmintic drugs, such as albendazole, ivermectin, or praziquantel, target parasitic helminths by binding to tubulin, opening chloride-sensitive channels, or disrupting the surface membrane[56]. On the other hand, antiprotozoal drugs, such as albendazole, fumagillin, trimethoprim-sulfamethoxazole, or paromomycin, target protozoan parasites[56].

1.3.1.1.1 Applications in oral health

Since *C. albicans* is still considered the most predominant pathogen responsible for Candida-associated Denture Stomatitis (CADS) in denture wearers[14,15,19,20], symptoms, namely palatal irritation, and inflammation, are generally treated through the application of topical antifungals, such as miconazole, amphotericin B, or nystatin[15,20]. However, CADS has an increased chance of recurrence, either due to non-compliance or to yeast infiltration through cracks in the denture, thus increasing the risks of developing antimicrobial resistance[20,57].

The application of antibiotics, such as penicillin, macrolides, tetracyclines, clindamycin, and metronidazole, as well as antimicrobial agents, namely fluoride, quaternary ammonium salts, chlorhexidine, remineralizing agents, and antimicrobial peptides, may be used to eliminate pathogens responsible for tooth decay or prevent it altogether[17].

For periodontal disease, an anti-infective strategy is required to eliminate or reduce plaque and achieve a successful treatment, either through mechanical (debridement) or chemical methods[16,23]. A chemical approach includes both systemic approaches (antibiotics and host modulation drugs) and topical application (through antiseptics and sustained-release drugs) while maintaining periodic dentist visits[16]. Although mechanical therapy is generally enough to control periodontitis, chemical therapy may be beneficial to remove tissue-invasive microbes such as *Actinobacillus actinomycetemcomitans*[16]. However, topical chemical therapy requires higher doses or longer exposures to the antimicrobial[16].

While antiseptics, such as chlorhexidine, triclosan, fluoride, or essential oils, allow an antimicrobial effect on the whole mouth, sustained-release drugs, such as chlorhexidine chips or 10% doxycycline gel, only act on teeth close to their place of application, thus losing usefulness on generalized loss of attachment[16]. When topical antimicrobial therapy fails, systemic antimicrobials may be required in conjunction with mechanical therapy[16]. Host-modulating drugs, such as doxycycline in sub-antimicrobial doses, prevent attachment loss by blocking enzymes, both host and bacterial-derived[16].

1.3.1.2 Organic antimicrobials

The worrisome increase in antimicrobial resistance has been the instigator of research for alternative substances with bactericidal properties, namely natural extracts[58]. Honey, grapes, cranberries, and black and green tea are some of the multiple biological products that have been proven to possess antimicrobial properties, either through bacteriostatic, anti-biofilm or anti-adhesive mechanisms, while cacao, pomegranates and garlic are some natural extracts with promising antimicrobial properties[58]. These herbal extracts' properties originate from molecules, named phytoalexins, which are formed when plants are subjected to stress[58].

1.3.1.3 Inorganic antimicrobials

Often found as nanoparticles, most inorganic antimicrobials originate from metal-based elements, such as silver (Ag), copper (Cu), gold (Au) and zinc (Zn), metal oxides, namely titanium oxide (TiO_2), zinc oxide (ZnO) and magnesium oxide (MgO), and magnetic oxides, specifically iron oxide (Fe^3O^4)[49,51]. However, the mechanisms behind these nanoparticles' antimicrobial effects are yet to be fully understood[51]. While the size and concentration are decisive factors in the antimicrobial effect's efficacy, a cautious approach when incorporating these nanoparticles is required to achieve a balance between toxicity and biocompatibility[51].

In an aqueous environment, positively charged silver particles and negatively charged cell membranes interact, leading to the ionization of silver nanoparticles and subsequent release of Ag^+ and Ag^{2+} ions[49,51]. Therefore, silver nanoparticle's antimicrobial properties can be related to the ability of these ions to penetrate peptidoglycan membranes and cell walls or fully destroy bacterial membranes, eventually leading to cell death[49,51]. Consequently, silver has been proven to provide an antimicrobial effect against both Gram-positive and Gram-negative bacteria, viruses, and fungi[51]. On the other hand, silver nanoparticles can cause a host reaction and result in toxic effects, thus increasing the need to cautiously evaluate the concentrations of silver used[51].

Identically to silver, copper presents antibacterial properties related to the particles' ability to penetrate bacterial membranes, after which the particles bind to DNA molecules, disrupting the helical structure[49]. Even though copper is effective against bacteria, including silver-resistant species, and fungi, there are risks of cytotoxicity that must be taken into consideration[51]. Despite being more affordable than silver and gold, copper has an increased risk of oxidization[51]. This can be avoided with capping agents, which may be ineffective at completely preventing the formation of the oxide layer, or through the formation of copper oxide (CuO), which decreases the particle's antimicrobial effect[51].

Gold, on the other hand, achieves its antimicrobial effect either by inhibiting the ribosome subunit or by interacting with the cell wall[49,51], being effective against bacteria (especially Gram-negative) and viruses[51]. Besides its antimicrobial

properties, gold also provides the highest stability among metal nanoparticles, being known for its biocompatibility[49,51]; however, the concentration of nanoparticles must be carefully assessed to avoid toxic effects[51].

Zinc's antimicrobial properties, effective against bacteria, viruses, and fungi, are attributed to zinc oxide's (ZnO) tendency to release Zn^{2+} ions in acidic environments[51]. Resembling silver, positively charged zinc oxide interacts with negatively charged cell membranes, resulting in the internalization of the particle into the cell and exerting a bactericide effect after entering the intercellular matrix[49,51]. Although the mechanisms through which the particles affect the cell are yet to be fully understood, these involve direct contact between the particles and the cell walls, releasing Zn^{2+} ions, disrupting cell integrity and producing reactive oxygen species (ROS)[49].

Effective against multiple species of bacteria, titanium oxide's antimicrobial properties result from various mechanisms, namely ROS production, internalization of particles and buildup of free radicals in the surface[51].

Besides the antimicrobial action against bacteria, mainly Gram-positive, viruses and fungi, following the release of Mg^{2+} ions in higher pH environments, magnesium oxide also exerts a biofilm-inhibiting effect[51]. However, other causes, such as ROS formation and quorum sensing, may be responsible for these antimicrobial properties[51].

Iron oxide nanoparticles provide an antibacterial action especially effective against Gram-negative bacteria[51]. These antimicrobial properties are attributed to various mechanisms, namely ROS production, particles' adherence to cell walls with consequent depolarization and loss of integrity, and release of Fe^{2+} and Fe^{3+} ions[51].

1.3.2 Microbial adhesion

While there are still studies trying to determine the mechanism of microbial adhesion to denture base resin, it is known that surface roughness is one of the multiple risk factors[18,46]. In denture wearers, *Candida spp.* is most frequently found in denture surface rather than mucosal tissues[14,20], which can be explained by the physical

properties of the acrylic resin being more favourable for *Candida spp.* adhesion[57]. A rough base surface is beneficial to the formation of biofilms of *C. albicans*[15,18,20,33], thus increasing the risk of developing CADS and other oral pathologies, due to the complex interactions in the oral microbiota[14].

Candida albicans is present in about 80% of the human population, with a high percentage being carriers of the fungi[14]. While carriers are, generally, asymptomatic, they are at higher risk of developing CADS when subjected to oral rehabilitation with removable dentures[57]. Therefore, if the oral microbiota is devoid of *C. albicans* when a denture is worn for the first time, the adhesion of the yeast is hampered, while if there is a developed biofilm before denture wear, the adhesion is facilitated[57]. Nevertheless, good oral hygiene is essential to prevent adhesion to new and old dentures, considering that microcracks and air bubbles allow *C. albicans* to infiltrate the surface, thus making any future attempts at cleaning the denture futile[57]. However, abrasive denture pastes and toothbrushes should be avoided, as they can result in surface roughness and, ultimately, denture surface infiltration[15,57]. Rough tooth and root surfaces are favourable to microbial adhesion and, consequently, plaque formation[24]. Therefore, periodic dentist appointments and mechanical tooth and root debridement may be required to remove supra and subgingival calculus that may have formed, thus preventing and controlling gingivitis and periodontitis[16,23].

Acrylic dentures are more prone to plaque accumulation when compared to metallic dentures, with buildup formation being more evident in interdental spaces, thus making these sites susceptible to inflammation and periodontitis[4,10]. Salivary flow is an important mechanism of oral hygiene, as saliva removes food particles and microorganisms from the teeth and mucosa, reducing biofilm formation and, thus, preventing infections and cavities[14,22], while the salivary pellicle that forms between the denture and mucosal tissue not only contributes to retention but also has a protective role at inhibiting colonization[9,20,57]. However, oral hygiene is necessary, as this protective effect disappears after more than two days have passed since the last time cleaning the denture and mucosa surfaces[57]. Consequently, in patients with xerostomia, *Candida spp.* and other pathogens are allowed to colonize, as the salivary flow is reduced, allowing biofilm formation, while pH and the concentration of antifungal molecules and mucins generally found in saliva decreases[57]. CAD-CAM

PMMA, being less porous, might reduce microbial adhesion to the denture base, thus reducing *C. albicans* colonization[28,33].

1.4 Justification and objectives

Without removing the surface acrylic and rebasing the prosthesis, attempts at denture sanitizing with antifungal disinfectants have been proven to fail at removing yeast biofilm that infiltrated the denture base surface, thus permitting *Candida spp.* to continue colonizing the denture and provoking an inflammatory response to the pathogen[21]. Consequently, the adhesion of *Candida spp.* and other pathogens to the acrylic surface should be prevented, either through thorough regular denture hygiene or the application/incorporation of antimicrobials to the acrylic resins used in dentures.

By focusing solely on the incorporation of inorganic particles into conventional heat-cured denture base resins, this systematic review aims to provide targeted and concise research. This results in a comprehensive and well-defined analysis of the effectiveness of incorporating a specific type of antimicrobial substance in polymethylmethacrylate. In turn, the influence of other factors, such as type of polymerization, in the results is reduced. Therefore, a higher level of homogeneity among the studies is achieved, which increases the validity of comparisons made in the review.

The main objective of this systematic review is to attest to the efficacy of incorporating inorganic antimicrobial agents to denture base heat-cured resin in preventing associated pathogenesis when compared to conventional resins, through a thorough assessment of modified resin's antimicrobial properties. So, we intend to be able to determine which inorganic agent with the best antimicrobial properties can be incorporated into PMMA.

2 Materials and Methods

Systematic reviews can be described as a type of literature review that, through organized and unbiased research and selection, aims at gathering and synthesizing evidence on a specific subject[59,60]. The clear and organized approach allows an easier replication of the research, increasing the review's relevance and reliability[59,60]. Following a search in electronic databases, the articles are screened according to previously defined inclusion and exclusion criteria, so that only relevant studies are selected[59,60]. A systematic review requires a methodical data extraction from the selected articles, after which are analysed and evaluated to answer the question that prompted the review[59,60]. The present review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines and the PRISMA flow diagram template depicted in Annex I, page 87[61,62].

2.1 Research question

To initiate a systematic review, a research question should be formulated[59]. The PICO acronym is commonly used to formulate this question, following the four main components of this template: Population (P), Intervention (I), Comparison (C), and Outcome (O)[63].

Taking into consideration the PICO elements outlined in Table 1, the resulting question was formulated: Does the incorporation of inorganic antimicrobial particles (I) into heat-cured denture base resins (P) result in enhanced antimicrobial properties (O) compared to conventional heat-cured resins (C)?

Table 1: PICO question

Population	Heat-cured denture base resins
Intervention	Incorporation of inorganic antimicrobial particles
Comparison	Conventional heat-cured resins
Outcome	Antimicrobial properties

2.2 Information sources and search strategy

The research, which took place on 27 September 2023, and was repeated on 17 January 2024, was conducted on three online databases: PubMed/Medline®, Cochrane® and Web of Science® Core Collection. The search was performed using search terms and MeSH terms (Medical Subject Headings) connected through Boolean operators such as “AND” and “OR”. The research strategies employed in each of the databases are comprehensively explained in Tables 2 through 4.

Table 2: Research equation used on PubMed/Medline®

(("Dentures"[MeSH]) OR ("Denture Bases"[MeSH]) OR ("Dental Prosthesis"[Mesh]) OR (dental prostheses) OR (denture) OR (denture base) OR (Prostheses, Dental) OR (Dental Prostheses) OR (Prosthesis, Dental))	AND	(("Acrylic Resins"[MeSH]) OR (resin) OR (pmma) OR (polymethylmethacrylate) OR (denture base material))	AND	(("Anti-Infective Agents"[MeSH]) OR ("Cariostatic Agents"[MeSH]) OR (anti-cariogenic) OR (cariostatic) OR (antibiotic) OR (antimicrobial) OR (antibacterial) OR (antifungal) OR (anti-infective))
((("Dentures"[MeSH]) OR ("Denture Bases"[MeSH]) OR ("Dental Prosthesis"[Mesh]) OR (dental prostheses) OR (denture) OR (denture base) OR (Prostheses, Dental) OR (Dental Prostheses) OR (Prosthesis, Dental)) AND (("Acrylic Resins"[MeSH]) OR (resin) OR (pmma) OR (polymethylmethacrylate) OR (denture base material)) AND (("Anti-Infective Agents"[MeSH]) OR ("Cariostatic Agents"[MeSH]) OR (anti-cariogenic) OR (cariostatic) OR (antibiotic) OR (antimicrobial) OR (antibacterial) OR (antifungal) OR (anti-infective)))				

Table 3: Research equation used on Web of Science®

ALL=	(("Dentures"[MeSH]) OR ("Denture Bases"[MeSH]) OR ("Dental Prosthesis"[Mesh]) OR (dental prostheses) OR (denture) OR (denture base) OR (Prostheses, Dental) OR (Dental Prostheses) OR (Prosthesis, Dental))	AND	(("Acrylic Resins"[MeSH]) OR (resin) OR (pmma) OR (polymethylmethacrylate) OR (denture base material))	AND	(("Anti-Infective Agents"[MeSH]) OR ("Cariostatic Agents"[MeSH]) OR (anti-cariogenic) OR (cariostatic) OR (antibiotic) OR (antimicrobial) OR (antibacterial) OR (antifungal) OR (anti-infective))
ALL= ((("Dentures"[MeSH]) OR ("Denture Bases"[MeSH]) OR ("Dental Prosthesis"[Mesh]) OR (dental prostheses) OR (denture) OR (denture base) OR (Prostheses, Dental) OR (Dental Prostheses) OR (Prosthesis, Dental)) AND (("Acrylic Resins"[MeSH]) OR (resin) OR (pmma) OR (polymethylmethacrylate) OR (denture base material)) AND (("Anti-Infective Agents"[MeSH]) OR ("Cariostatic Agents"[MeSH]) OR (anti-cariogenic) OR (cariostatic) OR (antibiotic) OR (antimicrobial) OR (antibacterial) OR (antifungal) OR (anti-infective)))					

Table 4: Research equation used on Cochrane®

ID	Search
#1	MeSH descriptor: [Dentures] explode all trees
#2	MeSH descriptor: [Denture Bases] explode all trees
#3	MeSH descriptor: [Dental Prosthesis] explode all trees
#4	MeSH descriptor: [Acrylic Resins] explode all trees
#5	MeSH descriptor: [Anti-Infective Agents] explode all trees
#6	MeSH descriptor: [Cariostatic Agents] explode all trees
#7	#1 OR #2 OR #3 OR (dental prostheses) OR (denture) OR (denture base) OR (Prostheses, Dental) OR (Dental Prostheses) OR (Prosthesis, Dental)
#8	#4 OR (resin) OR (pmma) OR (polymethylmethacrylate) OR (denture base material)
#9	#5 OR #6 OR (anti-cariogenic) OR (cariostatic) OR (antibiotic) OR (antimicrobial) OR (antibacterial) OR (antifungal) OR (anti-infective)
#10	#7 AND #8 AND #9

The research protocol was registered in PROSPERO (International Prospective Register of Systematic Reviews) on 3 January 2024 and was recorded with ID CRD42024496013, as seen in Annex II, page 88.

2.3 Studies selection

The inclusion and exclusion criteria employed in the review are delineated in Table 5, requiring precise and transparent reporting for a clear, reproducible screening with minimized bias[59].

Table 5: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
In vitro studies, published since 2019	Clinical studies and trials
Denture materials	Reviews, protocols, and pilot studies
Antimicrobial incorporation	Hygiene and pharmacological antimicrobials
Inorganic antimicrobials	Liners and tissue conditioners
Studies with more than 5 samples	Coating and surface treatments
Antimicrobial properties and tests	3D, autoclave, cold, and light polymerization resins
Conventional heat polymerization resins	

The database filters presented in Table 6, obtained from the inclusion and exclusion criteria, were applied before articles were exported.

Table 6: Database filters

Database	Included	Excluded
PubMed		Books and documents Meta-analysis Review Systematic review
Web of Science Core Collection	Article Early Access	Review Article Proceeding Paper
Cochrane	Trials	

After exporting the filtered articles from the three databases, duplicates and triplicates were removed as well as studies published before 2019. Acknowledging the inclusion and exclusion criteria, screening was conducted by two independent investigators, starting with a selection based on the article title, followed by a selection based on the abstract. Afterwards, a final screening, based on full-text reading, was performed, in which the reasons for the exclusions were clearly stated. Cohen's kappa statistic, as shown in Table 7, was adopted to evaluate inter-rater reliability during screening[64].

Table 7: Cohen's kappa statistic interpretation

Value of Kappa	% of Reliable Data	Level of Agreement
0- .20	None	0-4%
.21- .39	Minimal	4-15%
.40- .59	Weak	15-35%
.60- .79	Moderate	35-63%
.80- .90	Strong	64-81%
Above .90	Almost Perfect	82-100%

2.4 Data extraction

Data from defined variables was extracted from included articles into a Microsoft© Excel spreadsheet. These variables include information such as title, author, year, country, journal, resin brand, resin processing, sample number, shape, and size, inorganic antimicrobial incorporated, concentrations of antimicrobial, control group, microorganisms tested, results, and conclusions. All the selected articles were stored in the Mendeley Reference Management Software.

2.5 Quality assessment

Quality assessment for the included studies was conducted using a critical appraisal tool specially designed by the Joanna Briggs Institute for this purpose, the Checklist for Quasi-Experimental Studies (Non-Randomized Experimental Studies), depicted in Annex III, page 88[65]. This tool is designed to evaluate the methodological quality of the study and ascertain the extent to which biases could be introduced, encompassing study design, conduct, and analysis. The checklist comprises nine questions, each offering four response options: "yes", "no", "unclear", and "not applicable".

To evaluate the risk of bias, the total number of “yes” was counted and divided by the total number of questions. Afterwards, the fraction was converted to percentage and rated as seen in the article by Paes *et al.*[66] with the scores presented in Table 8.

Table 8: Risk of bias scoring

% of “Yes”	Risk of bias
0% to 49%	High
50% to 69%	Moderate
70% to 100%	Low

3 Results

3.1 Search results

As shown in the PRISMA flow diagram depicted in Figure 1, the initial database search resulted in a total of 2645 articles: 2008 on PubMed®, 501 on Web of Science® and 136 on Cochrane®. Before the screening, 1821 articles were excluded based on database filters. The remaining articles were exported and listed on a Microsoft© Excel spreadsheet and, afterwards, 117 duplicates and triplicates were identified and removed. Lastly, a time filter was applied, leading to the removal of 461 articles published before 2019. Therefore, the final number of studies to be screened was 246.

The screening began with title reading, leading to the exclusion of 194 articles following the established inclusion and exclusion criteria. The abstracts of the remaining fifty-two articles were read, resulting in the rejection of twenty-seven studies. Finally, the twenty-five articles selected underwent full-text reading, culminating in the exclusion of ten articles: two due to the type of study, six due to the type of acrylic resin, and two due to the methodology. Fifteen studies, identified in Table 9, were selected for inclusion in the current review.

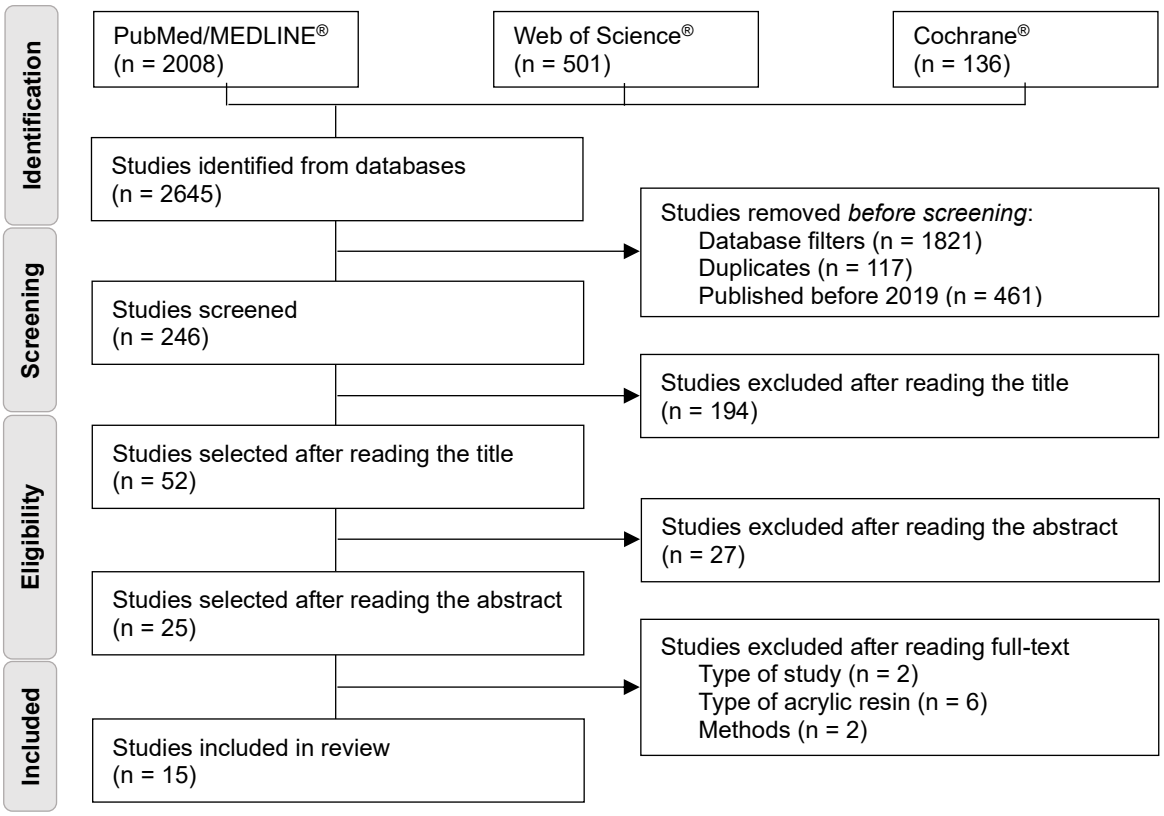

Figure 1: PRISMA flow diagram

Table 9: Included studies

No.	Citation
1 [67]	De Matteis V, Cascione M, Toma CC, *et al*. Silver Nanoparticles Addition in Poly(Methyl Methacrylate) Dental Matrix: Topographic and Antimycotic Studies. Int J Mol Sci. 2019 Sep 21;20(19):4691.
2 [68]	Souza Neto FN, Sala RL, Fernandes RA, *et al*. Effect of synthetic colloidal nanoparticles in acrylic resin of dental use. Eur Polym J. 2019 Mar 1;112:531–8.
3 [69]	Gopalakrishnan S, Mathew TA, Mozetič M, Jose J, Thomas S, Kalarikkal N. Development of biocompatible and biofilm resistant silver-poly(methylmethacrylate) nanocomposites for stomatognathic rehabilitation. Int J Polym Mater Polym Biomater. 2019;69:186–99.
4 [70]	Giti R, Zomorodian K, Firouzmandi M, Zareshahrabadi Z, Rahmannasab S. Antimicrobial Activity of Thermocycled Polymethyl Methacrylate Resin Reinforced with Titanium Dioxide and Copper Oxide Nanoparticles. Int J Dent. 2021 Jan 30;2021:6690806.
5 [71]	Pinheiro MCR, Carneiro JAO, Pithon MM, Martinez EF. Thermopolymerized Acrylic Resin Immersed or Incorporated with Silver Nanoparticle: Microbiological, Cytotoxic and Mechanical Effect. Mat Res. 2021;24(2):e20200115.
6 [72]	Alzayyat ST, Almutiri GA, Aljandan JK, *et al*. Antifungal Efficacy and Physical Properties of Poly(methylmethacrylate) Denture Base Material Reinforced with SiO2 Nanoparticles. J Prosthodont. 2021 Jul;30(6):500-8.
7 [73]	Takamiya AS, Monteiro DR, Gorup LF, *et al*. Biocompatible silver nanoparticles incorporated in acrylic resin for dental application inhibit Candida albicans biofilm. Mater Sci Eng C Mater Biol Appl. 2021 Jan;118:111341.
8 [74]	Fouda SM, Gad MM, Ellakany P, *et al*. Effect of Low Nanodiamond Concentrations and Polymerization Techniques on Physical Properties and Antifungal Activities of Denture Base Resin. Polymers (Basel). 2021 Dec 10;13(24):4331.
9 [75]	Hazim RH, Fatalla AA. The Effect of Tellurium Oxide Micro Particles Incorporation into PMMA on Candida Albicans Adherence. J Res Med Dent Sci. 2021;9(9):129-35.
10 [76]	Ivanovic V, Popovic D, Petrovic S, *et al*. Unraveling the Antibiofilm Activity of a New Nanogold Resin for Dentures and Epithesis. Pharmaceutics. 2022 Jul 21;14(7):1513.
11 [77]	Gligorijević N, Mihajlov-Krstev T, Kostić M, *et al*. Antimicrobial Properties of Silver-Modified Denture Base Resins. Nanomaterials (Basel). 2022 Jul 18;12(14):2453.
12 [78]	Ismaeil MA, Ebrahim M. Antifungal effect of acrylic resin denture base containing different types of nanomaterials: A comparative study. J Int Oral Health. 2023 Jan 1;15(1):78-83.
13 [79]	Teixeira ABV, Valente MLDC, Sessa JPN, Gubitoso B, Schiavon MA, Dos Reis AC. Adhesion of biofilm, surface characteristics, and mechanical properties of antimicrobial denture base resin. J Adv Prosthodont. 2023 Apr;15(2):80-92.
14 [80]	Marić I, Zore A, Rojko F, *et al*. Antifungal Effect of Polymethyl Methacrylate Resin Base with Embedded Au Nanoparticles. Nanomaterials (Basel). 2023 Jul 22;13(14):2128.
15 [81]	Correa S, Matamala L, González JP, *et al*. Development of novel antimicrobial acrylic denture modified with copper nanoparticles. J Prosthodont Res. 2024 Jan 16;68(1):156-65.

3.2 Inter-rater reliability

To assess inter-rater reliability, Cohen's kappa was evaluated after each screening phase, and the results are presented in Table 10.

The value of k, representative of inter-rater agreement, was 0.927859238 after title screening, indicating almost perfect agreement between both raters. After abstract selection, an almost perfect coefficient was calculated, with k reaching 0.961538462. Lastly, following full-text reading, a final measurement of Cohen's kappa yielded a value of 0.918032787, indicative of almost perfect agreement.

Table 10: Inter-rater reliability

Screening	Value of kappa	Meaning
Title	0.93	Almost perfect
Abstract	0.96	
Full text	0.92	

3.3 Quality evaluation

The methodological quality of the fifteen studies selected and included in this systematic review was assessed using a tool specially designed for the effect: the Joanna Briggs Institute checklist for quasi-experimental studies (non-randomized experimental studies). Question 6 was assessed as "not applicable" due to the *in vitro* nature of all the included studies.

The results of this appraisal are explicitly detailed in Table 11 and demonstrate that fourteen of the fifteen included studies present a low risk of bias, while one presented a moderate risk of bias.

Table 11: Quality evaluation using JBI Checklist for quasi-experimental studies (non-randomized Experimental Studies)

No.	Question									% "Yes"	Risk of Bias
	1	2	3	4	5	6	7	8	9		
1	Yes	Yes	Yes	Yes	Yes	Not applicable	Yes	Yes	Yes	88.89%	Low
2	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
3	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
4	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
5	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
6	Yes	Yes	Yes	Yes	Unclear		Yes	Yes	Yes	77.78%	Low
7	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
8	Yes	Yes	Yes	Yes	Unclear		Yes	Yes	Yes	77.78%	Low
9	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
10	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
11	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
12	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
13	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low
14	Yes	Yes	Yes	Yes	Unclear		Yes	Yes	Unclear	66.67%	Moderate
15	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	88.89%	Low

1. Is it clear in the study what is the "cause" and what is the 'effect' (i.e. there is no confusion about which variable comes first)?
2. Were the participants included in any comparisons similar?
3. Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?
4. Was there a control group?
5. Were there multiple measurements of the outcome both pre and post the intervention/exposure?
6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analysed?
7. Were the outcomes of participants included in any comparisons measured in the same way?
8. Were outcomes measured in a reliable way?
9. Was appropriate statistical analysis used?

3.4 Study characteristics

The details of each analysed study, including publishing year and journal, are provided in Table 12. All the included articles were *in vitro* studies, as determined by the inclusion criteria. While two of the selected studies were published in the Nanomaterials journal, thirteen were published in various journals in Dentistry, Biomaterials, and Pharmacology.

In Figure 2, the distribution of studies across countries and respective continents is illustrated. A total of six studies originated from Asia, five from South America, and four from Europe.

As shown in Figure 3, even though 2020 showed the lowest number of studies disseminated, with only one publication[69], six out of the fifteen studies were issued in 2021[70–75]. Additionally, a total of three articles were released in 2023[78–80], whereas 2022[76,77] and 2019[67,68] recorded the publication of two articles each. One study[81] has been published in 2024.

Figure 2: Distribution by continent and country

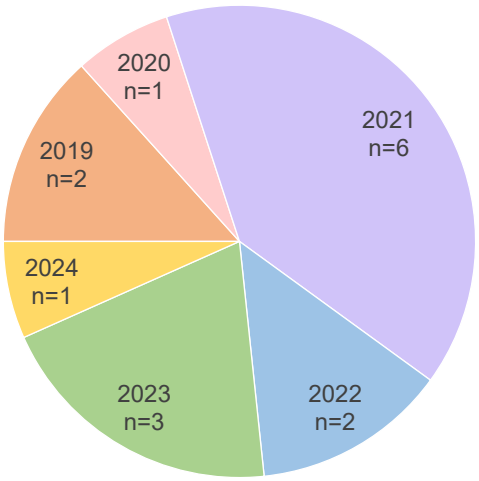

Figure 3: Distribution by publication year

Table 12: General information about the included studies

No.	Year	Country	1st Author	Title	Journal	Type of study
1	2019	Italy	De Matteis V	Silver Nanoparticles Addition in Poly(Methyl Methacrylate) Dental Matrix: Topographic and Antimycotic Studies	Int J Mol Sci	*in vitro*
2		Brazil	Souza Neto FN	Effect of synthetic colloidal nanoparticles in acrylic resin of dental use	Eur Polym J	
3	2020	India	Gopalakrishnan S	Development of biocompatible and biofilm-resistant silver-poly(methylmethacrylate) nanocomposites for stomatognathic rehabilitation	Int J Polym Mater Polym Biomater	
4	2021	Iran	Giti R	Antimicrobial Activity of Thermocycled Polymethyl Methacrylate Resin Reinforced with Titanium Dioxide and Copper Oxide Nanoparticles	Int J Dent	
5		Brazil	Pinheiro MCR	Thermopolymerized Acrylic Resin Immersed or Incorporated with Silver Nanoparticle: Microbiological, Cytotoxic and Mechanical Effect	Mat Res	
6		Saudi Arabia	Alzayyat ST	Antifungal Efficacy and Physical Properties of Poly(methylmethacrylate) Denture Base Material Reinforced with SiO(2)Nanoparticles	J Prosthodont	
7		Brazil	Takamiya AS	Biocompatible silver nanoparticles incorporated in acrylic resin for dental application inhibit Candida albicans biofilm	Mater Sci Eng C Mater Biol Appl	
8		Saudi Arabia	Fouda SM	Effect of Low Nanodiamond Concentrations and Polymerization Techniques on Physical Properties and Antifungal Activities of Denture Base Resin	Polymers (Basel)	
9		Iraq	Hazim RH	The Effect of Tellurium Oxide Micro Particles Incorporation into PMMA on Candida Albicans Adherence	J Res Med Dent Sci	
10	2022	Serbia	Ivanovic V	Unraveling the Antibiofilm Activity of a New Nanogold Resin for Dentures and Epithesis	Pharmaceutics	
11			Gligorijevic N	Antimicrobial Properties of Silver-Modified Denture Base Resins	Nanomaterials (Basel)	
12	2023	Saudi Arabia	Ismaeil MA	Antifungal Effect of Acrylic Resin Denture Base Containing Different Types of Nanomaterials: A Comparative Study	J Int Oral Health	
13		Brazil	Teixeira ABV	Adhesion of biofilm, surface characteristics, and mechanical properties of antimicrobial denture base resin	J Adv Prosthodont	
14		Slovenia	Marić I	Antifungal Effect of Polymethyl Methacrylate Resin Base with Embedded Au Nanoparticles	Nanomaterials (Basel)	
15	2024	Chile	Correa S	Development of novel antimicrobial acrylic denture modified with copper nanoparticles	J Prosthodont Res	

Information about the acrylic resins used in the different studies, consisting of brand and country of manufacture, as well as the details of the preparation of PMMA samples, namely shape, size, and number, are stated in Table 13.

Table 13: Heat-cured PMMA used in antimicrobial tests

No.	PMMA Brand	Country of manufacture	Shape	Size (mm)	Quantity
1	Paladon® 65 (Kulzer)	Germany	disk	Ø≈20	-
2	Lucitone® 550 (Dentsply® Ind. e Com. Ltda.)	Brazil	coupon	60x10x3	-
3	Alfa Aesar.	USA	-	-	-
4	SR Triplex Hot (Ivoclar Vivadent®)	Liechtenstein	disk	10×2	total n=150 5 groups per modification (n=30)
5	Vipicril (Vipi® Ind. e Com. Ltda.)	Brazil	disk	15x2	total n=108 4 groups per modification (n=27)
6	Major Base 20 (Major Prodotti Dentari SPA®)	Italy	disk	15x2	total n=50 5 groups per modification (n=10)
7	Lucitone® 550 (Dentsply® Ind. e Com. Ltda.)	Brazil	disk	10x3	total n=63 7 groups per modification (n=9)
8	Major base 20 (Major Prodotti Dentari SPA®)	Italy	disk	15x2	total n=80 4 groups per modification (n=20)
9	-	-	disk	10x2	total n=25 5 groups per modification (n=5)
10	PMMA Biogal® (Galenika)	Serbia	disk	5x2	total n=48 control n=24 4 groups per species (n=6)
11	Triplex Hot (Ivoclar Vivadent®)	Liechtenstein	disk	10x2	total n=375 5 groups per modification (n=75)
12	Major base, Trevalon/Universal Clear (Dentsply® Ind. e Com. Ltda.)	Germany	disk	10x2	total n=100 5 groups per modification (n=20)
13	Classic Dental Articles Ltda.	Brazil	disk	9x1	total n=9 Unspecified groups
14	Ivoclar Vivadent®	Liechtenstein	coupon	10x10x3	-
15	Acryl BH (GDF)	Germany	disk	10x4	-

Figure 4 shows the prevalence of different brands among the tested PMMA samples. There is an equally high prevalence of product lines from Dentsply® Ind. e Com. Ltda.[68,73,78] and Ivoclar Vivadent®[70,77,80], followed by Major Prodotti Dentari SPA®[72,74]. The remaining brands are only cited in one study each, while there is a study[75] that does not list the brand used.

The distribution of PMMA manufacturing across various countries is described in Figure 5, depicting Brazil[68,71,73,79] as the most prevalent country, identified in four articles, followed by Liechtenstein[70,77,80] and Germany[67,78,81], both with an identical incidence of three studies each. Appearing in two articles, Italy[72,74] displays a comparatively lower prevalence, while the remaining countries have a single

occurrence each. The same study that does not specify the brand name in Figure 4 also omits the manufacturing country[75].

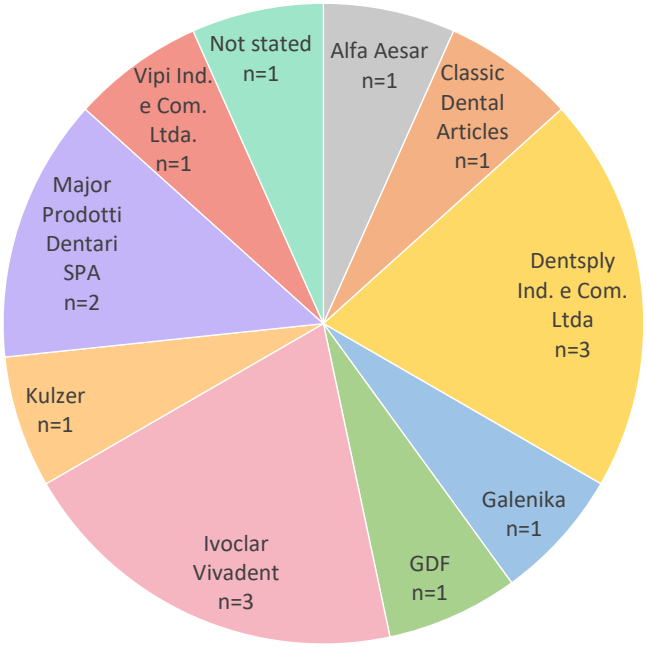

Figure 4: Prevalence of PMMA brands

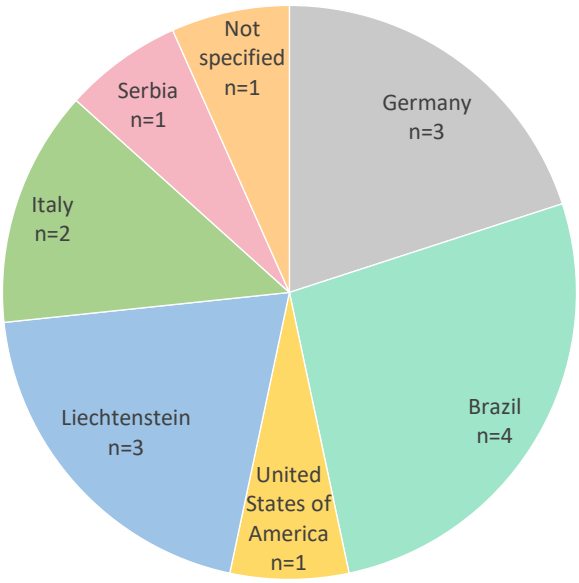

Figure 5: Distribution per manufacturer country

The various shapes and dimensions of the PMMA samples used in the antimicrobial tests performed in the different studies are displayed in Figure 6. Regarding the shape, out of the fifteen selected studies, one did not specify the dimensions or shape of the mould used[69], twelve used disk moulds to cut the PMMA samples for the antimicrobial tests, and two prepared coupon-shaped acrylic resin.

Both coupon-shaped samples displayed different dimensions: one measuring 60mmx10mmx3mm[68] and the other 10mmx10mmx3mm[80]. Concerning the disk-shaped specimens, four studies used moulds with dimensions of 10mmx2mm[70,75,77,78], while three articles cut samples with a diameter of 15mm and thickness of 2mm[71,72,74]. The remaining articles used disk moulds of varying diameter and thickness. Even though one of the studies did not provide the exact measurements of the disks, a picture of the samples with a scale on the side was provided, leading to the conclusion that the specimens were cut with an approximate diameter of 20mm[67].

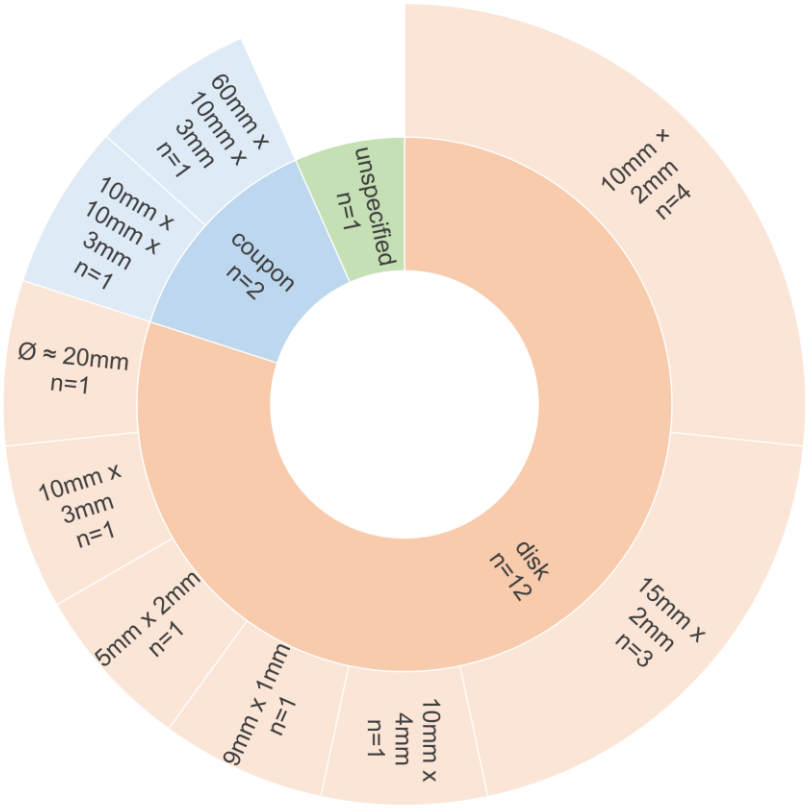

Figure 6: Shapes and sizes of PMMA samples used in antimicrobial tests

Out of the fifteen included studies, five[67–69,80,81] did not specify the number of samples prepared, while a total of 1008 samples were prepared for antimicrobial tests. The distribution of samples, as illustrated in Figure 7, displays a wide span across the studies, ranging from a minimum of 9[79] to a maximum of 375[77].

The number of groups prepared varies, spanning from two to seven, with one study not providing any group-related information[79]. Excluding the nine samples that were not grouped in the study by Teixeira *et al.*[79], the remaining 999 specimens are distributed across a total of forty-two groups. Five studies[70,72,75,77,78] organize the specimens in a total of five groups, while two studies[71,74] prepare four groups for antimicrobial tests. One study[76] mentions the organization of the samples into two groups, further dividing each group into four subgroups, leading to a total of eight subgroups. The number of samples in each group varies from five[75] to seventy-five[77], with two studies[74,78] mentioning the distribution of twenty samples per group.

Figure 7: Distribution of groups and subgroups across the total of samples in antimicrobial tests

The samples tested in each study were prepared in different ways depending, not only on the strain they aimed to neutralize but also on the antimicrobial tested and incorporated into the acrylic resin. Table 14 describes the characteristics of the inorganic antimicrobials incorporated into heat-cured PMMA, how the modification of the acrylic resin was performed, and the microorganisms used in the antimicrobial tests.

While the selected antimicrobials vary from study to study, some particles are more prevalent than others. Over half of the studies[67–69,71,73,77–79] test the incorporation into heat-cured PMMA of a compound containing silver, such as silver vanadate[79] or silver chloride[77]. Gold[76,80], titanium[70,78], and copper[70,81] are inorganic antimicrobials that are tested in two articles each, while tellurium[75], diamond[74], and silica[72] are mentioned in one study each. Overall, half of the total tested nanoparticles are composed of silver, as depicted in Figure 8. For all included studies, the control group consisted of unmodified heat-cured PMMA.

Table 14: PMMA modifications tested for antimicrobial properties

No.	Particle	Size (nm)	Concentration (%)	Control	Incorporation	Microorganism
1	Ag	20 ± 3	3; 3.5	Pure PMMA	Added to the powder and manually mixed	*C. albicans*
2		7.6 ± 2.3	0.05; 0.5; 5		Dispersion added to the monomer	*C. glabrata*
3		<100	1; 2; 5;10		Added to monomer and sonicated for 15min	*S. mutans; C. albicans*
4	CuO	40	2.5; 7.5		Particles and powder were mixed with monomer and stirred	*C. albicans; C. dubliniensis; S. mutans; S. sobrinus; S. salivarius; S. sanguis*
	TiO_2	17				
5	Ag	50	1; 2.5; 5		Aqueous solution added during polymerization	*C. albicans*
6	SiO_2	15	0.05; 0.25; 0.5; 1		Silanized particles independently blended with powder	
7	Ag	5	0.05; 0.5; 5		Added to the liquid component	
		10				
8	ND	-	0.1; 0.25; 0.5		Added to powder, blended manually and in an electric mixer	
9	TeO	-	1; 3; 5; 7		Added to monomer, sonicated, and mixed with powder	
10	Au	69.4 ± 12.42	2		Liquid particles added to the monomer	*S. aureus; E. coli; C. albicans; S. mitis*
11	Ag	<100	2; 5; 10		Particle powder added to PMMA powder	*S. aureus; C. albicans*
	AgCl	1 µm	10			
12	Ag	40	0.5; 1		Particles added to PMMA powder and mixed in an amalgamator	*C. albicans*
	TiO_2	50				
13	$AgVO_3$	Wires: Ø=150 Particles: 25	2.5; 5; 10		Added to powder and manually mixed with liquid	*C. albicans; C. glabrata; S. mutans*
14	Au	11	20		Suspension added to the monomer	*C. albicans*
15	Cu	30 to 150	0.015; 0.045; 0.055; 0.06; 0.068		Aqueous solution was added to monomer and ethanol, stirred, and then mixed with powder	*C. albicans; S. mutans; A. actinomycetemcomitans; S. aureus*

Ag – Silver
AgCl – Silver Chloride
$AgVO_3$ – Silver Vanadate
Au – Gold
Cu – Copper
CuO – Copper Oxide
ND – Nanodiamond
SiO_2 – Silicon Dioxide
TeO – Tellurium Oxide
TiO_2 – Titanium Dioxide

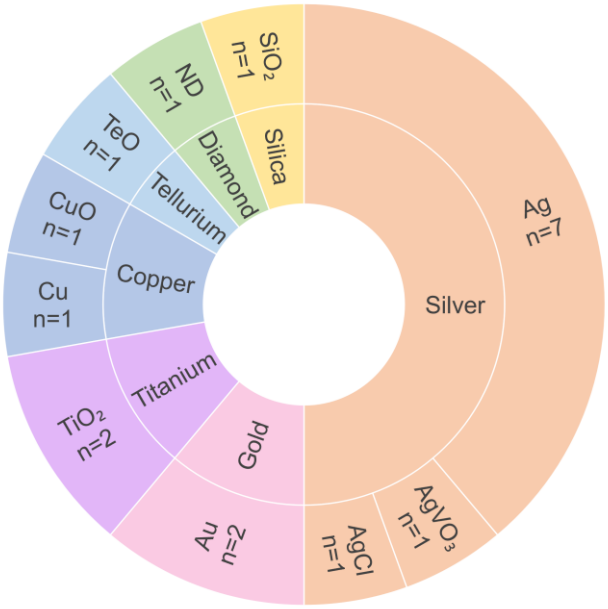

Figure 8: Inorganic antimicrobials tested and respective incidence

Figure 9 illustrates nanoparticle sizes incorporated into PMMA for each study and antimicrobial. In this chart, bars were used to indicate the ranges and lines represent the mean and exact values specified in the articles. To improve clarity and readability, the dimensions of silver chloride in the study by Gligorijevic *et al.*[77] were not represented in the chart, due to a significative deviation from the average range, while the two studies that did not specify the sizes[74,75] were also excluded from the graph. Therefore, a total of thirteen studies are illustrated in the chart, representing a total of fifteen inorganic compounds.

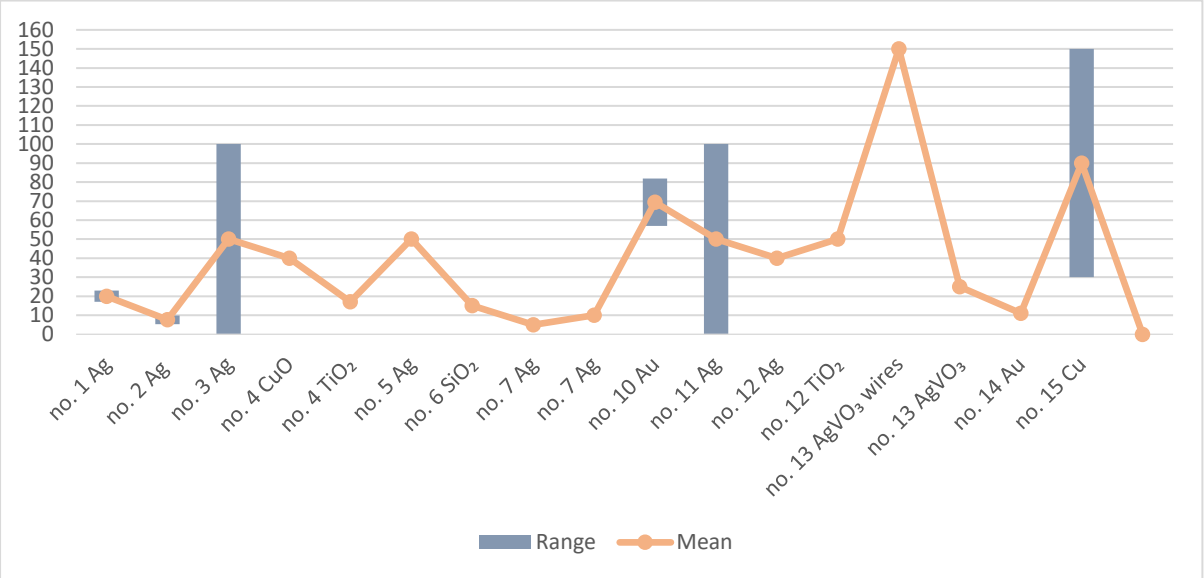

Figure 9: Range of tested particle sizes per study and antimicrobial (nm)

While the average size of nanoparticles incorporated in PMMA varied, most values fell within the range of 5nm[73] to 150nm[81]. On the other hand, silver chloride presented dimensions of 1µm, obtained through SEM images[77]. Two studies[69,77] mention acquiring nanoparticles with sizes smaller than 100nm, without specifying the exact values, while four[67,68,76,81] papers present the sizes within a range. The study by Takamiya *et al.*[73] tested two different sizes of nanoparticles, with 5nm and 10nm, whereas the study by Teixeira *et al.*[79] measured different dimensions for wires and particles observed through SEM images.

The methodology employed to modify the acrylic resin differed greatly, depending on the nanoparticles' properties. Some antimicrobials were incorporated as solids, while others were in a liquid state or suspension. Consequently, since the particles in different states were mixed either with monomer or with PMMA powder, the mixing method varied from manual techniques to the use of electric mixers and amalgamators.

The concentrations incorporated into the samples varied greatly within the same antimicrobial particle, as evident in Table 15. However, some concentrations were tested in multiple studies, such as 5% Ag, which appeared in five out of the fifteen studies[68,69,71,73,77]. Additionally, 0.5% Ag[68,73,78] and 1% Ag[69,71,78] were each tested in three articles, while 0.05% Ag[68,73], 2% Ag[69,77], and 10% Ag[69,77] were analysed in two studies each. Even though most articles tested multiple concentrations to assess which modification provided the best antimicrobial results, two studies[76,80] compare a single concentration of gold against unmodified PMMA.

Multiple antimicrobial species were targeted in the antimicrobial tests performed in the included studies, involving various bacterial and fungal strains, as shown in Figure 10. *Candida albicans* was the most frequently cultured, being present in fourteen out of the fifteen studies, only absent in the study by Souza Neto *et al.*[68]. *Streptococcus mutans* was observed in four studies[69,70,79,81], followed by *Staphylococcus aureus* in three[76,77,81], and *Candida glabrata* in two[68,79]. The remaining seven strains were analysed in one study each.

Table 15: Antimicrobials and respective concentrations tested in each study

No.	Silver												
	Ag									AgCl	$AgVO_3$		
	0.05%	0.5%	1%	2%	2.5%	3%	3.5%	5%	10%	10%	2.5%	5%	10%
1						x	x						
2	x	x						x					
3			x	x				x	x				
5			x		x			x					
7	x	x						x					
11				x				x	x	x			
12		x	x										
13											x	x	x

	Gold		Diamond			Copper						
	Au		ND			Cu					CuO	
	2%	20%	0.1%	0.25%	0.5%	0.015%	0.045%	0.055%	0.06%	0.068%	2.5%	7.5%
4											x	x
8			x	x	x							
10	x											
14		x										
15						x	x	x	x	x		

	Silica				Tellurium				Titanium			
	SiO_2				TeO				TiO_2			
	0.05%	0.25%	0.5%	1%	1%	3%	5%	7%	0.5%	1%	2.5%	7.5%
4											x	x
6	x	x	x	x								
9					x	x	x	x				
12									x	x		

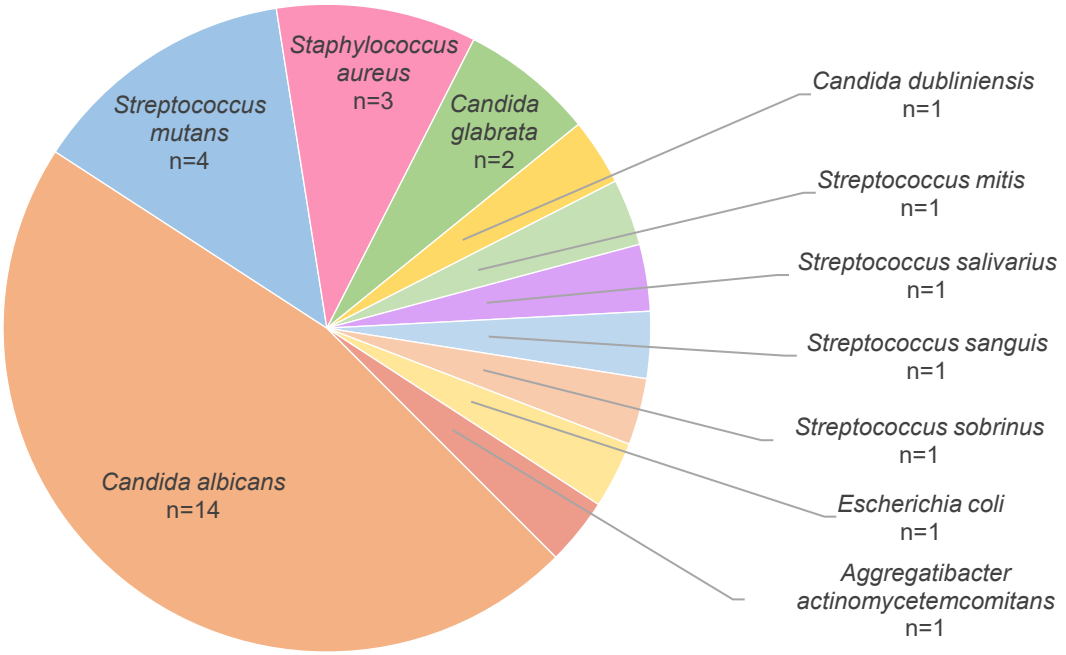

Figure 10: Number of studies with each microorganism strain tested

3.5 Parameters evaluated

The selected studies aim to test the antimicrobial properties of incorporating different inorganic particles in PMMA. Therefore, each study employed various tests, attempting to achieve the most credible results. Table 16 displays the assays and time intervals used to attest the effectiveness of the different concentrations tested and the results obtained. Some studies performed tests involving solely one concentration and the control group, either due to only preparing samples with a single concentration of particles[76,80] or due to performing a specific test with an individual sample group[67,69,81]. However, most of the tests performed in the studies involved multiple concentrations, obtaining results that assessed the most and least effective concentrations of the specific antimicrobial being tested against each of the strains.

Candida albicans was cultured in fourteen out of the fifteen included studies, excluding the study by Souza Neto *et al.*[68]. Silver vanadate, proposed by Teixeira *et al.*[79], was discovered to favour *C. albicans* growth. Two studies tested the incorporation of gold into PMMA and its effectiveness against *C. albicans*, with a concentration of 2% by Ivanovic *et al.*[76], and Marić *et al.*[80] at 20%. These two articles concluded that the incorporation of gold is effective at reducing *C. albicans* viability, although no statistically significant effect was observed in surrounding medium CFU for any of the strains in the tests performed by Ivanovic *et al.*[76].

Giti *et al.*[70] tested the incorporation of copper oxide (CuO) and titanium dioxide (TiO_2), both at 2.5% and 7.5%, concluding that, while CuO presented intermediate effectiveness, 7.5% TiO_2 showed the best properties against *C. albicans*, while the lowest concentration of the same particle presented the worst effectiveness. Correa *et al.*[81] incorporated copper (Cu) at smaller concentrations for CFU tests, while performing SEM assay and surface inhibitory tests between 0.045% Cu and the control group, observing an almost total absence of hyphal growth in modified resin. Even though all groups significantly decreased cell counts, the intermediate concentration, 0.045% Cu, presented the best antimicrobial properties, while the highest concentration, 0.068% Cu, performed the worst[81]. Titanium dioxide (TiO_2) was compared with silver (Ag) in the study by Ismaeil and Ebrahim[78], concluding that 0.5% TiO_2 had the lowest efficacy in both tests, while 1% Ag was the most efficient. The

incorporation of nanodiamond (ND), proposed by Fouda *et al.*[74], silicon dioxide (SiO_2), by Alzayyat *et al.*[72], and tellurium oxide (TeO), by Hazim and Fatalla[75], led to improved antimicrobial properties at higher concentrations of inorganic antimicrobial. Overall, four studies[67,69,77,78] reported higher antimicrobial activity of Ag nanoparticles against *C. albicans* when incorporated at higher concentrations, while two studies[71,73] reported the inverse, with lower concentrations presenting the best antifungal effects. However, the incorporation of silver vanadate ($AgVO_3$) led to increased metabolic activity in a multispecies biofilm, most evident at 10% concentration, as reported by Teixeira *et al.*[79].

For *Candida glabrata*, Souza Neto *et al.*[68] described a higher antifungal effect at 0.05% Ag, with most tests reporting 5% Ag as the least effective. However, in the CV assay, 0.5% Ag presented the worst antimicrobial properties. On the other hand, Teixeira *et al.*[79] reported an increase in antifungal properties for 10% $AgVO_3$, while the lowest concentration favoured growth. For *Candida dubliniensis*, however, copper was proven to be more effective than TiO_2, in a concentration-dependent efficacy, according to Giti *et al.*[70].

Streptococcus mutans was cultured in a total of four articles, obtaining a resulting increase in antibacterial activity when higher concentrations of nanoparticles are incorporated, namely Ag, such as in the study by Gopalakrishnan *et al.*[69], or $AgVO_3$, proposed by Teixeira *et al.*[79], as opposed to lower concentrations that, in the case of $AgVO_3$, tend to favour growth. While the incorporation of 2% gold (Au) resulted in the observation of small bacterial conglomerates[76], the surface inhibitory capacity tests revealed that 0.045% Cu presented favourable antimicrobial properties against *S. mutans Aggregatibacter actinomycetemcomitans*[81].

Equally to *S. mutans*, the modification of PMMA with 2% Au reduced *Staphylococcus aureus* colonies to small conglomerates[76], with promising results in the surface inhibitory capacity[81]. A higher antibacterial effect was observed at higher concentrations of Ag, with the study by Gligorijevic *et al.*[77] concluding that both Ag and AgCl at 10% presented the best antimicrobial properties while 2% Ag was the least effective. Nevertheless, microdilution method tests report a higher antimicrobial efficacy of AgCl over Ag particles against both *C. albicans* and *S. aureus*[77]. The incorporation of 2% Au, as proposed by Ivanovic *et al.*[76], resulted in dispersed cells

and chains of *Streptococcus mitis* and *Escherichia coli* in the modified PMMA. Giti *et al.*[70] reported that 7.5% CuO was the most effective modification against both *Streptococcus sobrinus* and *Streptococcus sanguis*, while 7.5% TiO_2 provided the best antibacterial properties against *Streptococcus salivarius*.

Table 16: Effectiveness of different PMMA modifications

No.	Particle	Tests	Microorganism	Best effect	Worst effect
1	Silver (Ag)	Viability CFU assay	*C. albicans*	3.5% Ag	3% Ag
		Circularity SEM assay		3.5% Ag	- control
		Area covered Colonization assay		3.5% Ag	- control
2	Silver (Ag)	CFU assay	*C. glabrata*	No statistically relevant difference	
		Biomass reduction CV assay		0.05% Ag	0.5% Ag
		Metabolic activity reduction XTT assay		0.05% Ag	5% Ag
		Micrographs of biofilms		0.05% Ag 0.5% Ag	5% Ag
3	Silver (Ag)	Cell count	*S. mutans*	10% Ag	1% Ag
		CFU assay	*C. albicans*	10% Ag	1% Ag
		Fluorescent microscopy	*S. mutans*	PMMA / Ag	- control
4	Copper oxide (CuO) Titanium dioxide (TiO_2)	Optical density	*C. albicans*	7.5% TiO_2	2.5% TiO_2
			C. dubliniensis	7.5% CuO	2.5% TiO_2
			S. mutans		
			S. sobrinus	7.5% CuO	2.5% CuO
			S. salivarius	7.5% TiO_2	2.5% TiO_2
			S. sanguis	7.5% CuO	2.5% TiO_2 2.5% CuO
		Biofilm inhibition	*C. albicans*	7.5% TiO_2	2.5% TiO_2
			C. dubliniensis	7.5% CuO	2.5% TiO_2
			S. mutans	7.5% CuO	7.5% TiO_2
			S. sobrinus	7.5% CuO	2.5% CuO
			S. salivarius	7.5% TiO_2	2.5% TiO_2
			S. sanguis	7.5% CuO	2.5% CuO
5	Silver (Ag)	Viability assay Absorbance	*C. albicans*	1% Ag	2.5% Ag 5% Ag
6	Silicon dioxide (SiO_2)	Direct culture	*C. albicans*	1% SiO_2	0.05% SiO_2 0.25% SiO_2
		Slide count (CFU/mL)		1% SiO_2	0.05% SiO_2
7	Silver (Ag)	CFU assay	*C. albicans*	0.5% Ag 0.05% Ag	5% Ag
8	Diamond (ND)	CFU assay	*C. albicans*	0.5% ND	0.1% ND
9	Tellurium oxide (TeO)	Adherence test	*C. albicans*	5% TeO 7% TeO	1% TeO

Table 17: Effectiveness of different PMMA modifications, Continued

No.	Particle	Tests	Microorganism	Best effect	Worst effect
10	Gold (Au)	CFU assay on discs	*S. aureus*	2% Au	- control
			E. coli		
			C. albicans		
			S. mitis		
		MTT assay	*S. aureus*		
			E. coli		
			C. albicans		
			S. mitis		
		SEM assay	*S. aureus*		
			E. col		
			C. albicans		
			S. mitis		
		CFU assay in the surrounding medium	*S. aureus*	No statistically relevant difference	
			E. coli		
			C. albicans		
			S. mitis		
11	Silver (Ag)	Inhibition zone	*S. aureus*	10% Ag 10% AgCl	2% Ag
			C. albicans	10% AgCl 10% Ag	2% Ag 5% Ag
		CFU assay	*S. aureus*	10% Ag 10% AgCl 5% Ag	2% Ag
			C. albicans		
		Microdilution method Minimum inhibitory concentrations	*S. aureus*)	10% AgCl	10% Ag
			C. albicans		
		Microdilution method Minimum microbicidal concentrations	*S. aureus*		
			C. albicans		
12	Silver (Ag) Titanium dioxide (TiO_2)	Disc diffusion Antifungal activity	*C. albicans*	1% Ag 1% TiO_2 0.5% Ag	0.5% TiO_2
		Elution test Colony counts		1% Ag 0.5% Ag 1% TiO_2	0.5% TiO_2
13	Silver vanadate ($AgVO_3$)	CFU assay	*C. albicans* In multispecies biofilm	- control	10% $AgVO_3$ 5% $AgVO_3$ 2.5% $AgVO_3$
			C. glabrata In multispecies biofilm	10% $AgVO_3$	2.5% $AgVO_3$
			S. mutans In multispecies biofilm		
		Metabolic activity	Multispecies biofilm*:* *C. albicans* *C. glabrata* *S. mutans*	- control	10% $AgVO_3$
14	Gold (Au)	Yeast adhesion	*C. albicans*	20% Au	- control
15	Copper (Cu)	CFU assay	*C. albicans*	0.045% Cu	0.068% Cu
		SEM assay		0.045% Cu	- control
		Surface inhibitory capacity	*A. actinomycetemcomitans*	0.045% Cu	-
			S. aureus		
			C. albicans		
			S. mutans		

Table 17 sums up the main conclusions regarding the outcome of the performed antimicrobial tests, with two studies[71,74] failing to mention alterations in the antimicrobial properties of the heat-cured acrylic resin resulting from the incorporation of the inorganic particles. Seven of the included articles simply state that this modification induces a general improvement of the antimicrobial properties of

PMMA[67,69,76–78,80,81]. Even though Teixeira *et al.*[79] only reported the effectiveness of 10% $AgVO_3$ against *S. mutans* in the conclusions, the remaining studies describe a relationship between the concentration of particles and the effect produced.

The studies by Souza Neto *et al.*[68] and Takamiya *et al.*[73] concluded that a decrease in antimicrobial effect occurs at higher concentrations of nanoparticles, an outcome that Souza Neto *et al.*[68] justified with the formation of agglomerates of silver in the test specimens with 5% Ag, stating that this organization of clusters led to a decreased antifungal effect against *C. glabrata* in the PMMA with the highest concentration of silver.

Giti *et al.*[70] concluded that TiO_2 and CuO presented varying effectiveness against different species, CuO proving to be effective against *S. mutans* while 7.5% TiO_2 was effective against *C. albicans*. Consequently, this study stated that a combination of any of the two tested concentrations of CuO with 7.5% TiO_2 could provide the best antimicrobial properties. Alzayyat *et al.*[72] reported opposing findings for PMMA modified with silica, concluding that the antimicrobial effect improved at higher concentrations of SiO_2. However, this study also concluded that doses of 0.05% and 0.25% are overall preferable, being able to provide enhanced antimicrobial effects while preserving the mechanical properties of the acrylic resin. Hazim and Fatalla[75] observed that incorporating TeO in PMMA resulted in an improvement in the antifungal properties of the acrylic resin, even though concentrations in the middle of the tested range, namely 3% and 5%, proved to be the most effective.

Table 18: Conclusions on antimicrobial tests performed in heat-cured PMMA with incorporated antimicrobials

No.	Main conclusions
1	Ag reduces the viability of *C. albicans* due to the reduction of its ability to adhere to and colonize PMMA.
2	5% Ag led to the formation of agglomerates and reduced efficiency of *C. glabrata* biofilm inhibition. 0.05% Ag showed the most promising properties, effectively reducing the metabolic activity of *C. glabrata* biofilms, as well as the biofilm inhibition and adhesion.
3	Ag showed a significant reduction in the adherence of *S. mutans* and *C. albicans*.
4	All modifications enhanced the antimicrobial activity against *S. salivarius*, *S. sanguis*, and *C. dubliniensis*. CuO provided substantial properties against *S. mutans*, while TiO_2 didn't show major effectiveness. 7.5% TiO_2 was the only modification presenting an antimicrobial effect against *C. albicans*. Incorporating 2.5% or 7.5% CuO with 7.5% TiO_2 into PMMA can inhibit the growth of different species of Streptococcus and Candida.
5	-
6	SiO_2 significantly contributed to a concentration-dependent reduction of *C. albicans* adhesion. 0.05% and 0.25% SiO_2 concentrations reduced *C. albicans* adhesion (retaining mechanical properties).
7	0.05% and 0.5% Ag exhibited antimicrobial effects against *C. albicans*.
8	-
9	TeO incorporation might result in an antifungal activity against Candida strains. 3% and 5% TeO showed better activity against *C. albicans* compared to control, 1% and 7% TeO
10	Au showed a significant antibiofilm effect against *C. albicans*, *S. mitis*, *S. aureus* and *E. coli*. This effect is stronger against Gram- bacteria and *C. albicans* than against Gram+ bacteria.
11	Ag can significantly reduce the risk of infection with *C. albicans* and *S. aureus* at the support mucosa.
12	Ag and TiO_2 can control *C. albicans* proliferation, thus presenting considerable antifungal activity against *C. albicans*.
13	10% $AgVO_3$ showed antimicrobial action against *S. mutans*.
14	Au improves the resistance to the adhesion of *C.* albicans, without severely impacting the mechanical properties.
15	Cu presented potent antimicrobial activity while retaining the mechanical properties, biocompatibility, and aesthetic qualities of PMMA.

4 Discussion

Systematic reviews are a methodical type of literature review that, following a careful study selection, quality evaluation and data extraction, aims at summarising the information available on a specific subject[59,60]. This systematic review aimed to attest the antimicrobial effect of incorporating different inorganic antimicrobial agents in various concentrations to heat-cured denture base resin and their efficacy at preventing associated pathogenesis when compared to conventional resins. For this review, a total of 15 studies were selected with the purpose of determining the inorganic particle with the best antimicrobial effect.

Regional distribution of the included studies revealed varying degrees of incidence of each continent, with Asia leading with six studies, followed by South America with five, and Europe with four. This distribution reveals, as observed in the study by Adam and Khan[82], a higher frequency of studies originated from developing and developed countries. There's a noticeable absence of studies published in North America, Africa, and Oceania, potentially resulting from lack of research within these regions or concerns regarding cytotoxicity. Regarding the temporal distribution, 2021 was the year with the highest volume of publications followed by 2023, with six and three studies respectively. Both 2022 and 2019 convey lower engagement, each presenting a total of two publications, succeeded by 2020 and 2024, the two years with the lowest publication activity, individually contributing only one study each.

4.1 Sample preparation

A diverse distribution of sample number and size was observed in the selected studies, with different concentrations and particle sizes. The included studies prepared the samples using one of two different shapes, moulding the acrylic resin either as disks or coupons. Even though the diameters varied between different articles, a total of 12 studies opted for disk-shaped specimens, eight of which presented a thickness of 2mm, while two used coupon-shaped mould, with a thickness of 3mm. The antimicrobial particles were synthesised and obtained in various dimensions, ranging from 5nm to 150nm, a wider margin than observed in the study by Garcia *et al*.[83] where the particles ranged from 10nm to 100nm. Out of all the tested inorganic antimicrobials,

silver was the inorganic particle with the highest prevalence in the antimicrobial tests, a tendency also observed by Garcia *et al*. 2021[83] and An *et al*. 2023[84].

Garcia *et al*.[83] concluded that the concentration of nanoparticles evaluated in the antimicrobial tests performed ranged from 0% to 30%. Similarly, in the studies included in this systematic review, the control groups consist of unmodified PMMA, while the modified samples define a concentration interval spanning from 0.015% to 20%. As observed in the study by Garcia *et al*.[83], various methods were used to incorporate the particles into the denture resin, through mixing or agitation. Seven authors mixed the particles with the monomer, while the particles were mixed with the polymer in seven of the remaining studies. Consequently, the comparison between the results obtained in the different studies should be performed prudently.

4.2 Microbial strains and antimicrobial tests

Various antimicrobial tests, namely CFU assays and SEM analysis, were conducted in the studies and the results were examined to assess the antimicrobial properties of different concentrations of particles and their effectiveness against each of the eleven bacteria and fungi strains. CFU assay, as observed by Garcia *et al*.[83], was the antimicrobial test performed with the highest frequency. However, as stated by the author, this test presents limitations, such as the fact that cells with low metabolic activity are counted, thus increasing the need for tests that evaluate metabolic activity. As noticed in the study by An *et al*.[84], *C. albicans* was the most frequent pathogen, being tested in 14 of the studies included in this systematic review, followed by *S. mutans* and *S. aureus*. This can be explained by *Candida albicans* prevailing as the species of fungi most prevalent in the oral cavity, with a higher incidence in the palatal mucosa of denture wearers[18], often leading to the development of CADS due to the constant friction against the denture base[14,19]. On the other hand, tooth decay is generally associated with streptococci and lactobacilli, such as *Streptococcus mutans* and *Lactobacillus acidophilus*, and the resultant bacteria-induced enamel demineralization[22].

4.2.1 Fungi

Four studies[67,69,71,73] compared the effectiveness of different concentrations of pure silver nanoparticles against *C. albicans*, having reached different results. De Matteis *et al.*[67] and Gopalakrishnan *et al*.[69] concluded that higher concentrations of Ag presented enhanced antimicrobial properties. Adam and Khan[82] reached a similar conclusion on the effectiveness of silver particles against *C. albicans*, having found a correlation between higher concentrations of Ag nanoparticles and the lower values obtained in the CFU assays performed. On the other hand, Pinheiro *et al*.[71] and Takamiya *et al*.[73] reached an opposing conclusion with better antimicrobial results at lower silver concentrations, while the highest concentrations proved ineffective in some tests[73].

Overall, it can be concluded that there is a tendency for a concentration-dependent increase in effectiveness for silver particles sized between 20nm and 100nm, with one exception. Pinheiro *et al*.[71] tested particles measured at 50nm, having concluded that the results obtained in antimicrobial tests for modified PMMA were not statistically different from the control group. The reduced antimicrobial activity is justified by Pinheiro *et al*.[71] as possibly resulting from the bigger size of the particles and the incorporation into PMMA preventing the release of silver to the environment.

All concentrations of silver vanadate tested in the study by Teixeira *et al*.[79] resulted in higher metabolic activity compared to the control group, favouring *C. albicans* growth. As proved in the study by Gligorijevic *et al*.[77], even though higher concentrations enhanced the antimicrobial effect, silver chloride still provided better results when compared to pure silver.

Titanium dioxide was tested in two studies, authored by Ismaeil and Ebrahim[78] and Giti *et al*.[70], being compared with other particles in both articles. A study by Yudaev *et al*.[85] concluded that PMMA with TiO_2 provided a favourable effectiveness against *C. albicans* when compared to unmodified PMMA. Titanium dioxide was tested against silver in the study by Ismaeil and Ebrahim[78], with both tests attesting to the increase in the effectiveness of higher concentrations of either modification. Ismaeil and Ebrahim[78] determined that 1% Ag presented the best properties, followed by 1% TiO_2 in the disc diffusion assay and 0.5% Ag in the elution test, while 0.5% TiO_2 presented

the lowest effectiveness in both tests. Therefore, it can be concluded that silver particles with 40nm present better properties against *C. albicans* than titanium dioxide measured at 50nm.

Giti *et al*.[70] compared titanium dioxide with copper oxide, having concluded that TiO_2 provided the best results at 7.5%, the sole significantly relevant effect, followed by CuO at 7.5% and 2.5%, while 2.5% TiO_2 showed the worst properties. Copper nanoparticles, as observed in the study by Correa *et al*.[81], provided favourable properties against *C. albicans*, resulting in a significant decrease in cell count, proving the most effective at 0.045%.

Both nanodiamond and tellurium oxide presented better antimicrobial properties at higher concentrations, as observed in the studies by Fouda *et al*.[74] and Hazim and Fatalla[75], respectively. Similarly, Alzayyat *et al*.[72] concluded that silicon dioxide, at a concentration of 1%, provided better properties against *C. albicans* compared to the lower concentrations of 0.5%, 0.25% and 0.05%. A singular concentration of gold was tested in each of the studies by Ivanovic *et al*.[76] and Marić *et al*.[80], with positive results, proving the effectiveness of concentrations of 2% and 20%, respectively. However, Ivanovic *et al*.[76] tested the cell count in the surrounding medium of the modified discs and obtained no statistically relevant difference, indicating a possible lack of antimicrobial release.

Candida glabrata strains were analysed in two articles, authored by Souza Neto *et al*.[68] and Teixeira *et al*.[79], where the effectiveness of silver, with a size of 7.6nm, and silver vanadate was tested. The study by Souza Neto *et al*.[68] concluded that higher concentrations of silver provided worse antimicrobial properties against the yeast, while the CFU assay resulted in no statistically significant difference for any of the concentrations. The author justified these findings with the formation of agglomerates when higher concentrations of silver were mixed with PMMA, a tendency observed by An *et al*.[84] and Yudaev *et al*.[85], instead of forming a homogeneous dispersion.

Giti *et al*.[70] compared the effectiveness of copper oxide and titanium dioxide against *Candida dubliniensis*, having concluded that CuO, at 7.5% and 2.5%, surpassed the effectiveness of both concentrations of TiO_2. Therefore, it can be concluded that

copper oxide presents better antimicrobial properties against *C. dubliniensis*, most evident at the highest concentration.

4.2.2 Gram-positive bacteria

Contrary to what was observed with *Candida spp.*, silver vanadate proved to reduce *Streptococcus mutans* cell count at higher concentrations of $AgVO_3$, even though Teixeira *et al*.[79] observed a tendency to favour cell growth at the lowest concentration of 2.5%. This concentration-dependent increase in effectiveness was also stated in the study by Gopalakrishnan *et al*.[69], which concluded that PMMA modified with silver nanoparticles provided higher antibacterial activity. The incorporation of copper particles, as evident in the studies by Giti *et al*.[70] and Correa *et al*.[81], led to favourable antimicrobial properties. Giti *et al*.[70] concluded that 7.5% copper oxide provided the best antibacterial effect, closely followed by 2.5% CuO, while PMMA modified with titanium dioxide was less effective. Similarly, 7.5% copper oxide proved effective against *Streptococcus sobrinus* and *Streptococcus sanguis*, albeit the least favourable concentration was 2.5% copper oxide, while the optical density assay proved 2.5% titanium dioxide to be similarly ineffective against *S. sanguis*. *Streptococcus salivarius* presented a different tendency, showing higher susceptibility to 7.5% TiO_2, followed closely by 7.5% CuO, while 2.5% TiO_2 demonstrated a lower antimicrobial effect.

Ivanovic *et al*.[76] concluded that the incorporation of 2% gold in PMMA samples resulted in good antimicrobial properties against both *Streptococcus mitis* and *Staphylococcus aureus*, although no relevant effect was observed in the medium surrounding the modified samples. Copper, at a concentration of 0.045%, proved effective against *S. aureus* in the study by Correa *et al*.[81]. Both silver and silver chloride presented similarly favourable antibacterial properties in the CFU and inhibition zone assays performed by Gligorijevic *et al*.[77], while AgCl revealed higher effectiveness in the microdilution methods tested.

4.2.3 Gram-negative bacteria

Two Gram-negative strains were used in the antimicrobial tests in the included studies. Similar to what has been observed with other bacterial strains, the study by Ivanovic *et al*.[76] obtained different CFU results on the discs modified with 2% gold, which proved to be effective at reducing *Escherichia coli* cell count and on the surrounding medium, where the difference was not statistically relevant. Likewise, the SEM and MTT assays obtained favourable results, revealing lower *E. coli* viability and higher cell dispersion in the modified resin compared to the larger conglomerates observed in the control group. Correa *et al*.[81] examined the difference in surface inhibitory capacity between PMMA modified with copper at a concentration of 0.045% and pure PMMA against a strain of *Aggregatibacter actinomycetemcomitans*, obtaining a favourable result in the inhibition tests with the modified resin. While the incorporation of particles in PMMA proved to be effective against these Gram-negative strains, more tests are required to assess the difference in the effectiveness of different concentrations of these inorganic antimicrobials, as a singular concentration was tested against each pathogen.

4.3 Particles and antimicrobial properties

Heat-cured PMMA needs heat energy to activate the initiator[15,28] and is often moulded into denture bases through a flask-pack-press technique, requiring the resin to be placed in a flask, pressed, and positioned in a water bath[28]. Despite its generally advantageous properties, the use of PMMA in denture bases may result in cytotoxicity and mucosa irritation, which increases the importance of testing the incorporation of fibres in the acrylic resin to achieve ideal biomechanical properties[28,35]. The incorporation of antimicrobial particles in acrylic resins could prove effective at preventing the adhesion of *Candida albicans* and other pathogens to the acrylic surface. Nanoparticles, such as silver (Ag), copper (Cu), gold (Au), titanium oxide (TiO_2) and nanodiamond (ND), present antimicrobial properties that make them ideal inorganic antimicrobials to incorporate in denture base resins[49,51].

The combination of silver and vanadium, as explained by An *et al*.[84], aims to overcome changes in the properties of modified PMMA due to the tendency of Ag particles to agglomerate.

The study by Teixeira *et al.*[79] demonstrated that incorporating $AgVO_3$ in PMMA resulted in an increase of microbial metabolic activity compared to pure PMMA, with a tendency to generally favour *C. albicans* cell growth, a phenomenon also observed in strains of *C. glabrata*. Overall, the incorporation of silver vanadate in PMMA resulted in decreased antimicrobial properties compared to pure PMMA. However, unlike what was observed in this study, Garcia *et al*.[83] concluded that, at a concentration of 5%, $AgVO_3$ presented favourable properties against *C. albicans*, while De Campos *et al*.[86] attested to the efficacy of $AgVO_3$ against bacteria and fungi. Teixeira *et al.*[79] stated that a percentage of silver vanadate higher than the minimum inhibitory concentration previously determined was used. Therefore, the justification that Teixeira *et al.*[79] presents to support the difference between the results and the literature lies in the use of a multispecies biofilm that included *S. mutans*. The study by Vila *et al*.[14] confirmed the existence of communication and interaction between bacteria, such as *S. mutans*, and *C. albicans*, thus validating the results found by Teixeira *et al.*[79].

As observed in the study by De Campos *et al*.[86], $AgVO_3$ presented a higher antimicrobial activity against *S. mutans* in concentrations of 10%, proving to be effective against the bacterial strain, and a reduction in cell counts when incorporated with a concentration of 5%. However, resembling what was observed with *Candida* spp., PMMA modified with a concentration of 2.5% favoured *S. mutans* growth. Gopalakrishnan *et al*.[69] concluded that PMMA modified with higher concentrations of silver nanoparticles proved effective against *S. mutans*, while Gligorijevic *et al*.[77] obtained promising results with both silver and silver chloride against *S. aureus* and *C. albicans*.

Diverse results were observed in the studies that tested the incorporation of silver particles. Overall, it was noticeable that smaller silver nanoparticles provided higher antimicrobial effectiveness against *Candida spp.* at lower concentrations, as evident in the studies by Souza Neto *et al*.[68], Pinheiro *et al*.[71] and Takamiya *et al*.[73]. On the other hand, larger nanoparticles seemed to require higher concentrations to obtain better results against *C. albicans*, a tendency observed by De Matteis *et al.*[67],

Gopalakrishnan *et al*.[69], Gligorijevic *et al*.[77] and Ismaeil and Ebrahim[78]. The studies by Parameswari *et al*.[87] and Adam and Khan[82] concluded that higher concentrations of silver particles resulted in better results in the CFU assays regardless of the methodology and the measurements of samples and particles. In contrast, and as suggested by Pinheiro *et al*.[71], Garcia *et al*.[83] asserted the existence of a relationship between the size of the nanoparticles and the antimicrobial efficiency, associating smaller nanoparticles with better antimicrobial properties as it is easier for these particles to penetrate cell membranes.

A justification for unsatisfactory results in the antimicrobial tests was proposed by Souza Neto *et al*.[68] and Pinheiro *et al*.[71], residing in the theory that the antimicrobial properties of silver result from direct contact with the microorganisms and ion release. However, as stated by both authors, incorporating silver in PMMA prevents the Ag^{+} ions from being released, thus reducing the effectiveness of the modified resin. Souza Neto *et al*.[68] and Takamiya *et al*.[73] presented another justification for the reduction in antimicrobial properties, an explanation also found in studies by An *et al*.[84] and Yudaev *et al*.[85], which correlated the formation of silver agglomerates to the incorporation of higher concentrations of the nanoparticle. A third reason for the decrease in the antimicrobial properties at higher concentrations was presented by Takamiya *et al*.[73], who denoted an increase in the irregularities in the surface of the samples with the highest concentration, which would facilitate the adherence of the pathogens.

Higher concentrations of copper oxide, as observed by Giti *et al*.[70], resulted in an overall favourable effect against *Candida spp*. and *streptococci*. In contrast, *C. albicans* and *S. salivarius* proved to be more susceptible to 7.5% TiO_2. Similarly, Yudaev *et al*.[85] concluded that the antimicrobial properties of PMMA incorporated with copper or titanium dioxide particles increased at higher concentrations. Gad and Abualsaud[88] observed that lower concentrations of TiO_2, ranging from 1% to 2%, presented favourable properties against *C. albicans*, while particle size appeared to not affect the evaluated properties. A link between the method of incorporating the particle and the properties of the modified resin was proposed by Gad and Abualsaud[88], who concluded that a better homogeneity was obtained when the particle was added to the monomer.

Albeit titanium dioxide presented promising properties, silver and copper oxide provided the best results in most of the tests performed in the studies by Giti *et al*.[70] and Ismaeil and Ebrahim[78]. The efficacy of copper against fungal and bacterial strains was verified by Correa *et al*.[81], who stated that *S. mutans* was the most susceptible species in the surface inhibitory capacity assay. Correa *et al*.[81] detected that *C. albicans* biofilms on the modified resin did not present the hyphal forms commonly observed in more aggressive phenotypes of the fungi.

Silicon dioxide, nanodiamond and tellurium oxide presented a concentration-dependent effectiveness against C. albicans, providing the best antimicrobial properties at higher concentrations, as evident in the studies by Alzayyat *et al*.[72], Fouda *et al*.[74] and Hazim and Fatalla[75], respectively. Yudaev *et al*.[85] and Bajunaid[89] concluded in their studies that the incorporation of ND increased the antimicrobial properties of PMMA, with better results at higher concentrations. Likewise, gold at 2% and 20%, as individually tested by Ivanovic *et al*.[76] and Marić *et al*.[80], obtained favourable results in the antimicrobial tests against *C. albicans* and three different bacterial strains. Ivanovic *et al*.[76] observed that gold presented better antimicrobial properties against Gram-negative bacteria and *Candida albicans*, with worse results in the antimicrobial assays against Gram-positive bacteria, justified by the author as resulting from the cell wall of Gram-positive bacteria presenting higher thickness.

Similar to the observations by Souza Neto *et al*.[68] and Pinheiro *et al*.[71] on the incorporation of silver, Alzayyat *et al*.[72] linked a higher antimicrobial effectiveness with the presence of silicon dioxide nanoparticles in the surface of the resin, as direct contact with the pathogens results in enhanced antimicrobial effect. Giti *et al*.[70] concluded that an acrylic resin modified with both 7.5% TiO_2 and CuO at either 2.5% or 7.5% would provide promising antimicrobial properties against *Candida* spp. and *Streptococcus* spp.

Even though most studies included in this review concluded that higher concentrations of nanoparticles resulted in better antimicrobial properties, there were exceptions. Souza Neto *et al*.[68], Pinheiro *et al*.[71] and Takamiya *et al*.[73] observed that PMMA at lower concentrations provided a better efficacy against fungi strains, while Teixeira *et al*.[79] concluded in their study that $AgVO_3$ favoured *Candida* spp. growth. Curiously, besides the fact that these four studies investigated the incorporation of silver-based

particles, all studies that observed a decrease in effectiveness at higher concentrations originated from Brazil, thus having tested PMMA manufactured in that country. However, since the brands used varied in these studies, there is no certainty that there is a relationship between the findings and the origin of the resin.

Overall, most of the particles tested in the included studies provided favourable antimicrobial properties against fungi and bacteria. However, it is important to consider the biocompatibility and physical properties of modified resins. As observed by Takamiya *et al*.[73], the incorporation of nanoparticles could result in surface roughness, thus increasing the adhesion of biofilms in denture bases, which may justify some unfavourable results in the antimicrobial assays. Even though Fouda *et al*.[74] observed that the effectiveness of the incorporation of nanodiamonds resided in the reduction of surface roughness, the author noted that higher concentrations could result in the opposite effect due to the formation of agglomerates.

4.4 Limitations

Inclusion and exclusion criteria and temporal filters were applied to increase the homogeneity of the included studies. However, it was observed that there was a great difference in the methodology and the size and shape of the samples tested in the various studies. This lack of homogeneity was also observed in the studies by Garcia *et al*.[83] and Adam and Khan[82] and complicates the organization of the findings and elaboration of an effective comparison, as the differences observed may be a result of the different methodologies applied during sample preparation instead of the concentrations tested.

A remarkably low number of clinical studies on this subject was observed, which may be a result of an increased cost of producing and obtaining modified dentures. However, *in vitro* studies are incapable of truthfully replicating oral conditions, which increases the necessity of *in vivo* and clinical studies.

This systematic review focuses solely on the antimicrobial properties of the modified resin. Nonetheless, as stated by Garcia *et al*.[83] and Bangera *et al*.[90], other properties need to be assessed to guarantee functionality and safety when wearing dentures with

modified acrylic bases. Bajunaid[89] also observed the necessity of considering the effect of modifying PMMA in the general properties and aesthetics of the resin.

4.5 Clinical significance

Despite the large number of *in vitro* studies focused on the antimicrobial properties of modified PMMA and the fact that most of the included studies obtained favourable results, there is a low number of clinical trials, as stated by Adam and Khan[82]. Since nanoparticles, namely silver, can be toxic to humans, as acknowledged by Garcia *et al*.[83], biocompatibility tests must be performed to assess the safety of incorporating these particles. Therefore, considering the difference between the environment used in the *in vitro* studies and actual oral conditions, more clinical trials must be performed to obtain evidence on the cytotoxicity and performance of modified PMMA in the oral cavity. Besides, as stated by Adam and Khan[82], analysing the effectiveness of nanoparticles against CADS requires clinical studies.

Overall, the incorporation of nanoparticles in PMMA provided positive results, demonstrating a promising antimicrobial effect against common oral pathogens. Nevertheless, to assess the interactions between the particles and the organism, *in vivo* and clinical trials are necessary.

5 Conclusion

Multiple studies have been conducted to assess the effectiveness of modifying denture base resins to prevent the growth of fungi and bacteria biofilms responsible for Denture Stomatitis, tooth decay and periodontitis. Inorganic antimicrobials, such as silver and gold, have been subjected to various *in vitro* tests to investigate their antimicrobial properties when incorporated into PMMA. Prompted by this increasing interest in the development of an antimicrobial resin, this systematic review aimed to answer the research question: “Does the incorporation of inorganic antimicrobial particles into heat-cured denture base resins result in enhanced antimicrobial properties compared to conventional heat-cured resins?”

To increase the homogeneity of the included studies, inclusion and exclusion criteria were applied. Nonetheless, while analysing the articles, a great variation in methodology and sample preparation was observed. Therefore, an exact comparison of the results obtained in the included studies is not possible, due to the wide range of variables that could have prompted such differences in outcomes. Other limitations encountered consisted of a low number of studies that compare different particles and a reduced number of clinical trials.

A tendency was observed in the included articles, as most studies tested the antimicrobial properties of nanoparticles against *Candida albicans*. Silver was the most prevalent particle, being used to modify PMMA in multiple studies, either as pure silver or as silver chloride and silver vanadate. However, despite the promising results observed in the samples modified with silver, the antimicrobial efficacy varied significantly based on the concentration and size of the particles, a result that may be attributed to particle agglomeration or the physical properties of the modified resin. Gold, titanium dioxide and copper proved to be effective against a larger variety of fungi and bacteria, with most studies presenting a concentration-dependent increase in effectiveness. An acrylic resin modified with particles of titanium dioxide and copper could provide a favourable antimicrobial effect against *Candida* spp. and *Streptococcus* spp.

Overall, almost all the inorganic antimicrobials presented promising properties against tested strains. Therefore, to answer the main question that prompted this review, modified PMMA did exhibit better antimicrobial properties than pure PMMA. However, it is not possible to declare a particle as the most efficient, due to the high

heterogeneity in the samples and antimicrobial tests performed in the studies. Besides, few authors compare different particles in the same test, which would have facilitated a comparison of the effect. More tests are necessary, especially *in vivo* studies and clinical trials, as the oral environment and microbiota are vastly different from *in vitro* strains. It is important to analyse the effect of PMMA modifications in multispecies biofilms, to gauge the interactions between different pathogenic strains in the presence of inorganic antimicrobials and its effect on the effectiveness of the modified resin.

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7 Annexes

Annex I: PRISMA

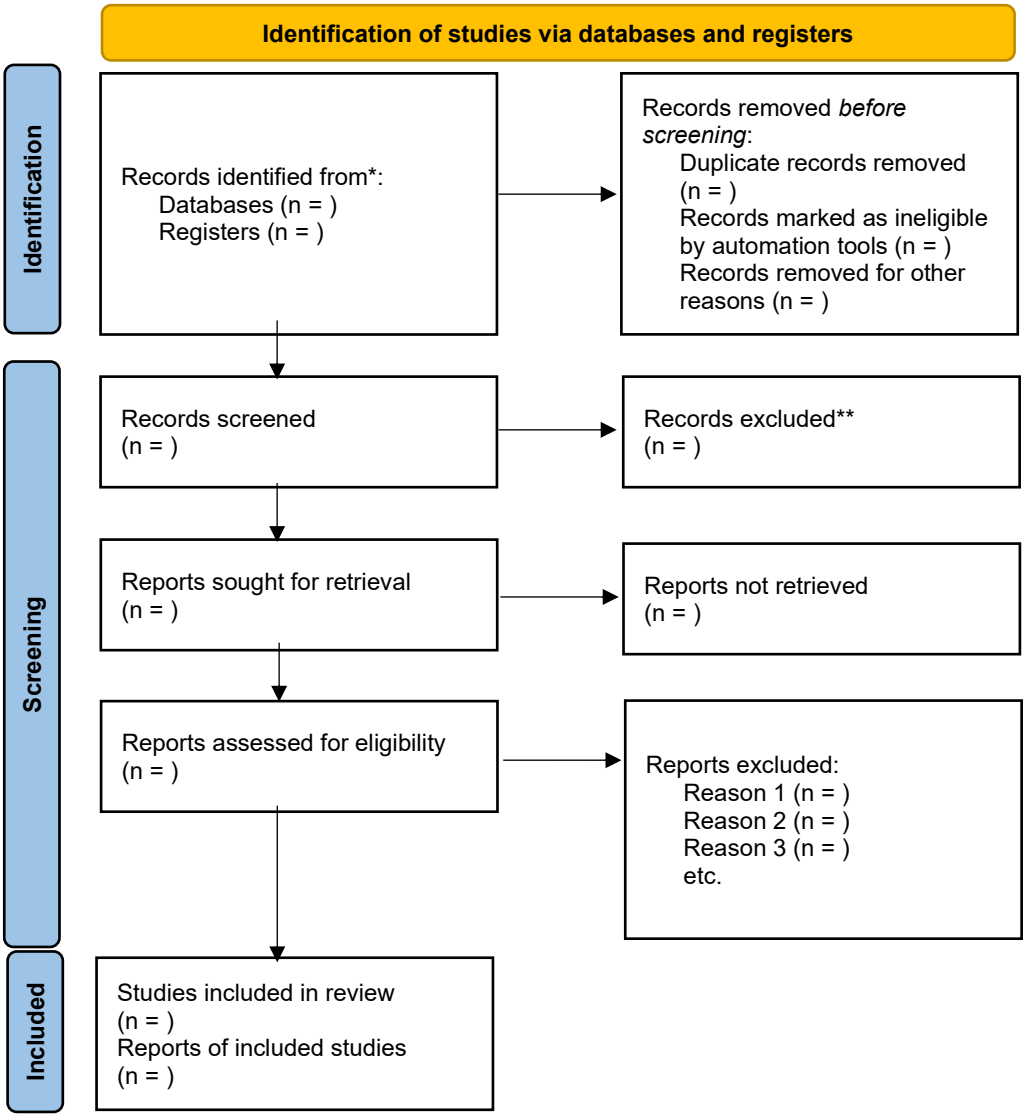

Figure 11: PRISMA flow diagram 2020

Annex II: PROSPERO registration

You have 1 records

My other records

These are records that have either been published or rejected and are not currently being worked on.

ID	Title	Status	Last edited
CRD42024496013	Incorporation of Inorganic Antimicrobial Substances into Heat-cured Denture Base Resins – A Systematic Review	Registered	15/01/2024

Figure 12: PROSPERO registration

Annex III: Joanna Briggs Institute Critical Appraisal Checklist for Quasi-Experimental Studies

	Yes	No	Unclear	Not applicable
1. Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?	☐	☐	☐	☐
2. Were the participants included in any comparisons similar?	☐	☐	☐	☐
3. Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?	☐	☐	☐	☐
4. Was there a control group?	☐	☐	☐	☐
5. Were there multiple measurements of the outcome both pre and post the intervention/exposure?	☐	☐	☐	☐
6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	☐	☐	☐	☐
7. Were the outcomes of participants included in any comparisons measured in the same way?	☐	☐	☐	☐
8. Were outcomes measured in a reliable way?	☐	☐	☐	☐
9. Was appropriate statistical analysis used?	☐	☐	☐	☐

Figure 13: JBI Critical Appraisal Checklist for Quasi-Experimental Studies

Membros do Júri das Provas Públicas

Presidente: _Professor Doutor Nuno Rosa_

(Professor Associado, Universidade Católica Portuguesa)

Arguente: _Professor Doutor André Correia_

(Professor Associado, Universidade Católica Portuguesa)

Orientador: _Professora Doutora Patrícia Fonseca_

(Professor Auxiliar, Universidade Católica Portuguesa)

Data das provas públicas: _24_ / _07_ / _2024_

Validação e confirmação pelos serviços escolares:

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