

Antimicrobial effectiveness and Ag⁺ release in antimicrobial-modified PMMA: A systematic review

Eficácia antimicrobiana e liberação de Ag⁺ em PMMA modificado com antimicrobianos: Uma revisão sistemática

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Abstract

Silver, a long-term ionic release antimicrobial, is incorporated into polymethylmethacrylate (PMMA) in order to reduce bacterial adhesion to polymeric materials and increase the longevity of rehabilitation, given the prevalence of pathologies. The aim was to answer: "What is the correlation between ionic release and antimicrobial activity of acrylic resins incorporated with silver?". The search strategy was applied to the PubMed, ScienceDirect, Embase and Scopus databases. The articles were selected in two phases according to eligibility criteria. The risk of bias was assessed using the quasi experimental studies (non-randomized experimental studies) tool from the Joanna Briggs Institute (JBI). A total of 538 articles were found in the databases, of which 28 were duplicated and were excluded. After first reading, 6 studies were selected for full reading, 5 of which made up this review. Silver was evaluated in different incorporations, which had proven antimicrobial efficacy, given its ionic release. No meta-analysis was carried out due to the heterogeneity of the data. The included studies showed a directly proportional correlation between ionic release and antimicrobial activity in PMMA incorporated with silver-containing agents.

Keywords: Acrylic resin; Incorporation; Antimicrobial activity; Ion release; Silver.

Resumo

A prata, um antimicrobiano de liberação iônica de longo prazo, é incorporada ao polimetilmetacrilato (PMMA) para reduzir a adesão bacteriana a materiais poliméricos e aumentar a longevidade da reabilitação, dada a prevalência de patologias. O objetivo era responder: "Qual é a correlação entre a liberação iônica e a atividade antimicrobiana das resinas acrílicas incorporadas com prata?". A estratégia de pesquisa foi aplicada aos bancos de dados PubMed, ScienceDirect, Embase e Scopus. Os artigos foram selecionados em duas fases, de acordo com os critérios de elegibilidade. O risco de viés foi avaliado usando a ferramenta de estudos quase experimentais (estudos experimentais não randomizados) do Joanna Briggs Institute (JBI). Um total de 538 artigos foi encontrado nos bancos de dados, dos quais 28 estavam duplicados e foram excluídos. Após a primeira leitura, 6 estudos foram selecionados para leitura completa, dos quais 5 compuseram esta revisão. A prata foi avaliada em diferentes incorporações, que apresentaram eficácia antimicrobiana comprovada, dada a sua liberação iônica. Não foi realizada meta-análise devido à heterogeneidade dos dados. Os estudos incluídos mostraram uma correlação diretamente proporcional entre a liberação iônica e a atividade antimicrobiana no PMMA incorporado com agentes contendo prata.

Palavras-chave: Resina acrílica; Incorporação; Atividade antimicrobiana; Liberação de íons; Prata.

Resumen

La plata, un antimicrobiano de liberación iónica a largo plazo, se incorpora al polimetilmetacrilato (PMMA) para reducir la adhesión bacteriana a los materiales poliméricos y aumentar la longevidad de la rehabilitación, dada la prevalencia de patologías. El objetivo era responder: «¿Cuál es la correlación entre la liberación iónica y la actividad antimicrobiana de las resinas acrílicas incorporadas con plata?». La estrategia de búsqueda se aplicó en las bases de datos PubMed, ScienceDirect, Embase y Scopus. Los artículos se seleccionaron en dos fases de acuerdo con los criterios de elegibilidad. El riesgo de sesgo se evaluó mediante la herramienta de estudios cuasiexperimentales (estudios experimentales no aleatorizados) del Instituto

Joanna Briggs (JBI). Se encontraron 538 artículos en las bases de datos, de los cuales 28 estaban duplicados y fueron excluidos. Tras una primera lectura, se seleccionaron 6 estudios para una lectura completa, 5 de los cuales conformaron esta revisión. Se evaluó la plata en diferentes incorporaciones, de probada eficacia antimicrobiana, dada su liberación iónica. No se realizó metaanálisis debido a la heterogeneidad de los datos. Los estudios incluidos mostraron una correlación directamente proporcional entre la liberación iónica y la actividad antimicrobiana en el PMMA incorporado con agentes que contienen plata.

Palabras clave: Resina acrílica; Incorporación; Actividad antimicrobiana; Liberación de iones; Plata.

1. Introduction

Incorporating silver into PMMA increases the longevity of the rehabilitation treatment, since ionic release contributes to long-term efficacy, inhibits microbial growth, and reduces the risk of associated infections (Hassan et al., 2019; Phillips et al., 2010).

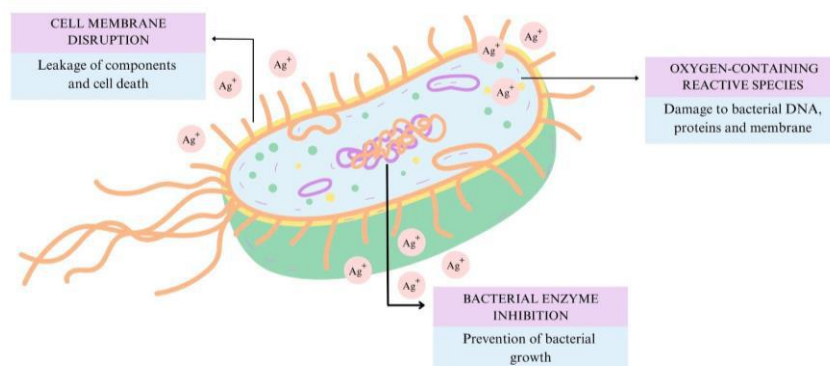
Aesthetics, easy handling and cost-effectiveness characterize polymethylmethacrylate (PMMA) as the gold standard in prosthetic manufacture (Zafar et al., 2020; Al Sunbul et al., 2019; Nikawa et al., 1999), but it has high surface roughness and porosity contributing to bacterial adhesion (Machado et al., 2011; Raszewski et al., 2017; Raszewski et al., 2018). The difficulty in sanitizing these devices leads to pathologies: prosthetic stomatitis, bacterial endocarditis, and aspiration pneumonia (Felton et al., 2011; Kozak et al., 2023; Santacrose et al., 2023; O'Donnell et al., 2016).

Mouthwashes and gels are effective antimicrobials, but they depend on the patient's cooperation, which corroborates the addition of chlorhexidine (CDA) (Wei et al., 2006; Wei et al., 2007; Maluf et al., 2020), sodium fluoride (Yassin et al., 2016), nanographene oxide (nGO) (Lee et al., 2018; Ferreira et al., 2024) and silver (Kassaei et al., 2008; Fan et al., 2011; Monteiro et al., 2012; Oei et al., 2012; Jo et al., 2017; Mukai et al., 2023) to the polymer matrix homogeneously, a process called incorporation, which aims for continuous, and safe ionic release (Souza et al., 2010; An et al., 2021, Cao et al., 2018).

However, there is no consensus on the antimicrobial profile of CDA: in contrast to studies (Salim et al., 2013; Arnold et al., 2008; Salim et al., 2013), Maluf et al. state that it is not quantity-dependent. Furthermore, the F ion does not affect isolated species, only intermicrobial interactions (Yassin et al., 2016) and the dispersion of nGO limits its action, given the formation of agglomerates (Lee et al., 2018; Ferreira et al., 2024; Patil et al., 2006).

Silver, a secular antimicrobial (Wright et al., 1998; Monteiro et al., 2008; Wang et al., 2019; Gligorijević et al., 2022), when incorporated into PMMA, promotes morphological alterations in the bacterial membrane: reduction of molecular oxygen and/or interference in DNA replication (Xiao et al., 2021; Zhang et al., 2018) (Graphical Abstract).

Graphical Abstract - Antimicrobial Mechanism of Action of Ag⁺ Release.



Source: Self-authored.

Silver nanoparticles (AgNPs) have better ionic release due to their large surface area/volume (Oves et al., 2018; Yin et

al., 2020; Silva et al., 2021) and act synergistically with phenazine-1-carboxamide and eugenol, which optimizes antimicrobial performance, that varies with the target microorganism: there is greater sensitivity to *E. coli*, compared to *S. aureus*, given the concentration of peptidoglycan (Durán et al., 2016; Kaur et al., 2016). However, this agent's capacity can be affected by oxidation in aqueous media and the size of the particles (Bruna et al., 2021). In addition, AgNPs affect human cells through cytotoxicity and genotoxicity (de Lima et al., 2012; Park et al., 2010; Park et al., 2011; Tripathi et al., 2022; Noga et al., 2023; Khorrami et al., 2018; Pauksch et al., 2014).

Ion release measures antimicrobial effectiveness and duration, by anodic stripping voltammetry, (Kassae et al., 2008) UV/Vis spectroscopy (Fan et al., 2011), periodic effluent measurement (Yassin et al., 2016) or inductively coupled plasma optical emission spectrometry (Jo et al., 2017). Studies have determined a direct relationship between released ions and antimicrobial effectiveness in resins incorporated with certain concentrations (Kassae et al., 2008; Fan et al., 2011; Monteiro et al., 2012; Oei et al., 2012; Jo et al., 2017).

Maximum antimicrobial efficacy at ideal ionic concentrations is a challenge in dentistry, which seeks to prevent pathological installation. This systematic review aims to analyze studies on the incorporation of Ag⁺ into PMMA and the correlation between antimicrobial action and ionic release, something that has never been reported in the literature.

2. Methodology

This review was registered on the Open Science Framework (osf.io/34t67) and was prepared following the Preferred Reporting Items for Systematic Reviews and Meta Analyses Checklist (PRISMA) (Souza et al., 2021; Mattos et al., 2015; Snyder et al., 2019; Cordeiro et al., 2007; Gomes et al., 2014) with the aim of answering the following question: "What is the correlation between ionic release and antimicrobial activity of acrylic resins incorporated with silver?".

The study design framework (PICOS) applied was P = acrylic resin; I = incorporation of silver as an antimicrobial; C = control group; O = antimicrobial activity and release of silver ions; S = in vitro. The search strategy: 'acrylic resin' OR 'PMMA' OR 'polymethylmethacrylate' AND 'silver incorporation' OR 'Ag incorporation' AND 'antimicrobial activity' AND 'ion release' was applied to the PubMed, ScienceDirect, Embase and Scopus databases on May 19, 2023.

The articles were selected in two stages: firstly, two authors (M.C.F.H and I.F) assessed the articles by reading their titles and abstracts, and secondly, the selected articles were read in full, applying inclusion and exclusion criteria. To resolve disagreement, a consensus meeting was held with a third reviewer (A.C.R.). The data extracted from the included papers was tabulated in Table 1, below:

Table 1 - Extraction of data from the selected studies for full reading.

Author, Year	The aim of the study	Type of antimicrobial and % incorporated	Evaluation of antimicrobial activity	Ion Release	Result
Jo JK, 2017	Induce long-term microbial anti-adhesive effects.	Silver-sulfadiazine (AgSD)-loaded mesoporous silica nanoparticles (Ag-MSNs - 0.5, 1, 2.5 and 5%) were incorporated into PMMA.	<i>Candida albicans</i> and <i>Streptococcus oralis</i> with experimental specimens for up to 28 days.	The specimens were gathered at the determined time points described for the release test.	Anti-adhesive effects were observed in the incorporated samples, except for 0.5% Ag-MSNs against <i>S. oralis</i> . 5% Ag-MSNs exerted a significant, continuous anti-adhesive effect against <i>C. albicans</i> up to 14 days. Anti-adhesive effects for up to 28 days compared to pure PMMA and PMMA incorporating MSNs only.

Fan et al, 2010.	Demonstrate that AgNPs could be synthesized in situ in acrylic dental resins, release Ag ions and provide effective antimicrobial activity.	AgNPs were formed in situ in dental resins using Ag benzoate. Chemically polymerizable acrylic resin: (0, 0.002, 0.02, 0.2 and 0.5%.	<i>S. mutans</i> . In vitro bacterial growth inhibition assay, CFU.	UV/Vis spectroscopy was used to determine the release of Ag ions.	The release study showed that chemically polymerizable specimens showed release with as low as 0.002% AgBz. A zone of inhibition of <i>S. mutans</i> is clearly visible for the 0.5% and barely perceptible for the 0.2% specimens, correlated to 97.5% and 52.4% inhibition of <i>S. mutans</i> growth.
Oei et al, 2011	Investigate the effects of varying the concentrations of the initiator and accelerator in PMMA on Ag loading, mechanical properties, Ag ion release, and antibacterial properties.	AgBz was dissolved in DMAEMA, this was blended with liquid orthodontic monomer and extra BP.	<i>P. aeruginosa</i> , <i>A. baumannii</i> , <i>S. aureus</i> and <i>P. mirabilis</i> . CFUs after 48h.	Ag ion release was measured at 1, 2, and 4 days; 1,2 and 4 weeks using AAS.	The ion release increased until day 7 and kept gradual until day 28. The increase in AgBz concentration doubled Ag ion release, and increased antimicrobial activity 99.9%. Even the smallest concentrations achieved antimicrobial efficacy and the initiator and the accelerator didn't affect the antimicrobial efficacy.
Monteiro et al, 2011	Evaluate a denture base resin containing silver colloidal nanoparticles to check the distribution and dispersion of these particles in the polymer.	Silver nanoparticle suspension was added to the acrylic resin monomer in different concentrations (0.05, 0.5, and 5 vol% silver colloidal).	Zone of growth inhibition and number of <i>C. albicans</i> colonies.	Testing the silver release in deionized water at different time periods.	Nanocomposites had efficacy against <i>C. albicans</i> , especially 5%. Despite the high sensitivity of the technique used, no Ag ⁺ was detected in the deionized water. With lower concentrations, the distribution of silver nanoparticles was reduced, whereas their dispersion was improved in the polymer. After 120 days of storage, nanoparticles were mainly located on the surface of the nanocomposite specimens.

Source: Self-authored.

In vitro experimental articles were included, in which there was: incorporation of Ag⁺ as an antimicrobial in PMMA, antimicrobial evaluation and ionic release and presence of prosthetic purpose, published in English.

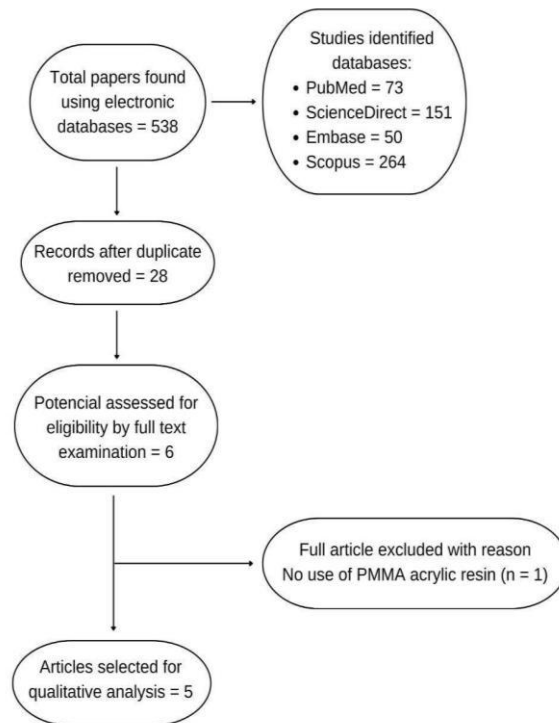
Exclusion criteria: 1) Absence of inclusion criteria; 2) Book chapter; 3) Systematic review; 4) Congress abstract; 5) Letter to the editor; 6) Other type of study, other than in vitro; 7) Antimicrobial agent applied superficially.

Adaptation of the Joanna-Briggs Institute (JBI) quasi-experimental studies (non-randomized experimental studies) to assess the risk of bias. In classifying the methodological quality of the studies, each question was scored with "low", "high" and "unclear" risk of bias. The analysis was carried out using RevMan 5.3 software.

3. Results and Discussion

A total of 538 articles were found in the databases, of which 28 were duplicates and were excluded. After reading the titles and abstracts, 6 studies were selected for full reading, 5 of which made up this review, when applying the eligibility criteria in the second phase (Figure 1).

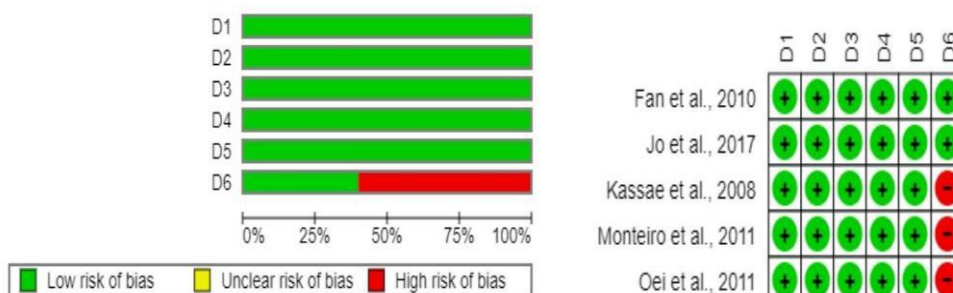
Figure 1 - Systematic review flowchart.



Source: Self-authored.

By analyzing the risk of bias, it can be seen that 3 studies are at high risk for: "Was appropriate statistical analysis used?", as they did not report which statistical test was used to evaluate the data. For the other questions, all the studies had a low risk (Figure 2).

Figure 2 - Risk of bias of the selected studies by JBI tool.



Source: Self-authored.

The AgNPs (Kassae MZ et al., 2008; Fan C et al., 2011; Monteiro DR et al., 2012) were incorporated in different concentrations and methods: 0.5% added to the liquid and sonified for 15 minutes, (Kassae MZ et al., 2008) 0.002%, 0.02%, 0.2% and 0.5% synthesized in situ by silver benzoate (AgBz) (Fan C et al., 2011) and 0.05%, 0.5% and 5% bound to the monomer. (Monteiro DR et al., 2012) Silver benzoate (AgBz) (Oei JD et al., 2012), dissolved in 2% dimethylamino aniline

(DMAEMA), was mixed with polymethylmethacrylate. Mesoporous silica nanoparticles (MSNs) loaded with silver-sulfadiazine (AgSD) were incorporated at 0.5%, 1%, 2.5% and 5% (Jo JK et al., 2017).

The Colony Forming Units (CFUs) evaluated the antimicrobial action: AgNPs at 0.4% for *E. coli* (Kassae MZ et al., 2008), AgBz even at low concentrations against *P. aeruginosa*, *A. baumannii*, *S. aureus* and *P. mirabilis* (initiators or accelerators did not interfere) (Oei et al., 2012), 2.5% of Ag-MSNs for *C. albicans* and 5% for *C. albicans* and *S. oralis*. Zones of inhibition against *C. albicans* (Monteiro et al., 2012) and *S. mutans* (Fan et al., 2011) were analyzed: AgNPs at 5% and 0.5% as the gold standard, respectively.

By anodic stripping voltammetry, the release of Ag⁺ was analyzed for 1, 2, 4, 7, 11 and 15 days: in the first two, it grew and maintained a constant increase until the 15th. (Kassae et al., 2008) By UV/Vis spectroscopy, ionic release was found at all concentrations (Fan et al., 2011).

Atomic Absorption Spectrometry (AAS) was carried out in 2 studies. In the first, for 1, 2 and 4 days and 1, 2 and 4 weeks, an increase in release was observed up to the 7th day, it maintained a constant increase up to the 28th day and a higher concentration of AgBz doubled the release (Oei et al., 2012). In the second, it was investigated for 7, 15, 30, 60, and 120 days and at lower concentrations, the distribution of AgNPs was reduced, but there was better dispersion after 120 days (Monteiro et al., 2012). By Optical Emission Spectrometry with inductively coupled plasma, it was found that 95% of the incorporated was released in the reloaded sample. Due to recharging and ionic release, there were anti-adhesive effects for up to 1 week (Jo et al., 2017).

AgNPs reduce the adhesion of pathogens to prosthesis wearers (Bapat et al., 2018; Singh et al., 2018; Su et al., 2021; Tahmasbi et al., 2020; Wady et al., 2012) by releasing Ag⁺ which: inactivates enzymes, inhibits bacterial DNA replication and alters the plasma membrane (Zafar et al., 2020; Al Sunbul et al., 2019; Nikawa H et al., 1999). The nanometric size, given the large surface area/volume, (Oves M et al., 2018; Tamayo et al., 2014; Zhao et al., 2014) allows for better ionic release which interacts with oxygen, improves dispersion and enhances antimicrobial activity (Furno F et al., 2004; Venkatesan et al., 2014).

Kassae et al. and Oei et al. proved AgNPs to be antimicrobial: after 24 hours of incubation, even with Ringer's solution, no *E. coli* was found (Kassae et al., 2008) and there was widespread inhibition of *A. baumannii*, *P. aeruginosa*, *P. mirabilis* and *S. aureus*, even with the slow release of Ag⁺, which shows the non-interference of the initiator, dimethylamino aniline (DMAEMA) or the amine (Oei et al., 2012).

Monteiro et al. established the influence of the concentration of AgNPs on the dispersion in the polymer, but, in disagreement with studies (Damm et al., 2008; Kong et al., 2008), there was no change in ionic release. Another striking finding was the absence of Ag⁺ in the liquid medium, even with long immersion, which can be explained by the synthesis of AgNPs: the Turkevich method (Turkevich et al., 1951) which reduces silver nitrate with sodium citrate. Furno et al. demonstrated that the diffusion of water can result in the plasticization of the material, which generates the migration of Ag⁺ superficially (Furno et al., 2004).

AgBz (benzoic acid + silver) acts on bacterial ATP production, impedes vital functions and causes death. (Fan et al. established that: the greater the amount of AgBz, the more Ag⁺ is released, which potentiates the antibacterial action. 0.5% was given as the gold standard, as it inhibited *S. mutans*. 0.2% inhibited more than 50% of *S. mutans*, which establishes: efficacy, even at low concentrations (Fan et al., 2011).

Light-curing resins showed Ag⁺ release at 0.1% AgBz, given "visible crack", because at 0.15% there was no release. Chemically polymerizable resins showed a release of up to 0.002% AgBz, due to the homogeneous distribution and smaller size of the AgNPs and/or greater porosity (Fan et al., 2011).

Jo et al. demonstrated significant flexural strength, hardness and anti-adhesiveness of Ag-MSNs for up to 28 days, which were present even after reloading, compared to pure PMMA or PMMA incorporated with MSNs (Jo et al., 2017).

4. Conclusion and Suggestion

We can infer from this systematic review that the majority of studies conclude that there is a correlation between antimicrobial activity and the release of Ag⁺ in modified PMMA, directly proportional to the amount of AgNPs, AgBz or Ag-MSNs incorporated. Further studies correlating ion release with antimicrobial activity are encouraged in order to establish more specific parameters and concentrations that can be used for long-term release with efficacy.

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