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Review

Antimicrobial Poly (methyl methacrylate) with Silver Nanoparticles for Dentistry: A Systematic Review

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Abstract: Poly(methyl methacrylate) (PMMA) is a widely used polymer for dental applications, and it is mainly used in the fabrication of dental prostheses. In an increasing number of these applications, the risk of suffering bacterial or fungal infection is higher than 60% among oral-prosthesis users. Some authors have reported the failure of other implants in the human body due to biofilm formation on the surface (mainly for total hip implants). In the dental field, the formation of bacterial and fungal biofilms on prosthesis's surface is the etiologic factor for stomatitis, mainly caused by *Candida albicans* and bacteria such as *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis*, as well as many others. The antibacterial and antifungal properties of silver nanoparticles (AgNPs) have been widely reported, and their use in dental materials can prevent oral infections, such as candidiasis and stomatitis, and promote better oral health in dental-prosthesis users. They can even be used in other biomedical applications that require controlling biofilm formation on surfaces. In this review, the reported studies that use composites of PMMA and AgNPs (PMMA-AgNPs) for dental applications are listed and checked, with the aim of gaining a wider perspective of the use and application of this approach in the dental field.

Keywords: dental prostheses; oral infection; stomatitis; *Candida albicans*; denture base; nanotechnology

1. Introduction

Poly(methyl methacrylate) (PMMA) is a polymer used as a denture base acrylic resin in dentistry; due to its properties, it has been used in the biomedical field as a bone cement in implant surgery (mainly in total hip replacements) [1,2]. For biomedical applications, the evaluation of biological properties such as cytotoxicity, in vitro and in vivo biocompatibility, and antimicrobial effects provides essential information about the interaction between material and biological systems, which can be used for the development of new materials or new applications. In addition, the use of additives to improve properties requires the modification of synthesis methods and the evaluation of new properties relating to the resulting material [3].

Silver nanoparticles (AgNPs) have been used in the biomedical field as an antimicrobial agent to prevent infections or colonization of biomedical devices by pathogenic microorganisms [4]. In dentistry, AgNPs have been used to improve the mechanical properties of restorative materials and to promote colonization of dental prostheses' surfaces. The antifungal effect has been proved and has attracted great interest from researchers seeking to develop materials that can inhibit the growth of microorganisms that cause oral infections [5].

This review focuses on the use of AgNPs in dentistry. Synthesis methods, biocompatibility, antimicrobial effects, properties and other uses are some of the fields that we considered when performing the search through the electronic databases. Our aim was to summarize the methods that use PMMA and silver nanoparticles to promote an antifungal effect and that are intended to be used in the dental field. This review provides information that gives an insight into materials that have properties that fulfil the needs of researchers who want to develop materials for dental prosthetics.

2. Methodology

To perform this systematic review, the data that were available from electronic databases were collected according to PRISMA recommendations for systematic reviews [6]. The electronic search was conducted in a ScienceDirect database with the following keywords: "poly(methyl methacrylate)", "silver nanoparticles" and "dentistry". To refine the search, additional keywords were chosen for the following topics: biocompatibility, antimicrobial effect, antimycotic effect and dental applications. The following inclusion criteria were used: full-text articles focused on the aim of this review, i.e., dental applications of silver nanoparticles and poly(methyl methacrylate), and research articles published between 2010 and 2019 in peer-reviewed journals that include the keywords in their abstracts. Papers that focused on other research topics were excluded. An additional search of the PubMed database was performed following the above-mentioned inclusion criteria. The bibliographies of the consulted articles were also searched for pertinent references that were not included in the electronic search and to adequately follow up promising and relevant research lines for the present review. The electronic search was conducted from August 2018 to January 2019. The data and information extraction was focused on dental applications. The data-collection management and analysis consisted of the description of relevant evidence, which is presented in diagram flow format according to PRISMA. Data about relevant biomedical applications were included, excluding reports about silver nanoparticles or poly(methyl methacrylate) with other uses, such as photovoltaic cells, fibers and filling materials. The PRISMA flow chart can be seen in Figure 1.

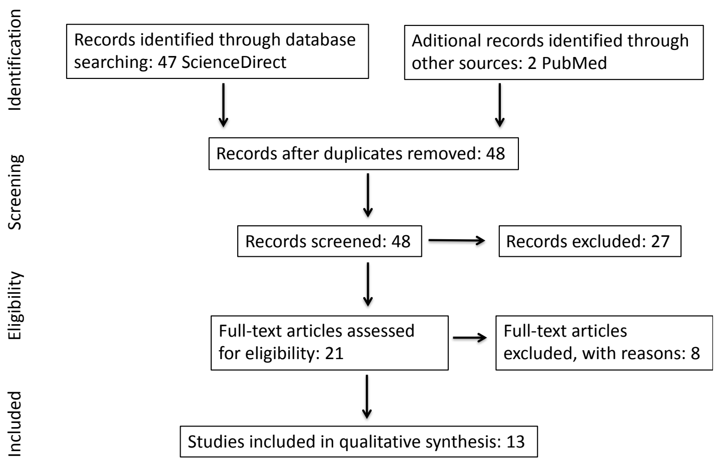

Figure 1. PRISMA flow chart.

3. Synthesis Methods

The reported methods in this review show that mixing AgNPs in monomer is the most frequently used method for synthesizing PMMA–AgNP composites independently of any kind of synthesized material (bone cement or denture base material). Another reported method includes the use of solvents and specialized equipment to form PMMA modified with silver nanoparticles, such as dimethyl-formamide, oleic acid, N-methyl pyrrolidone and chloroform. This is done using a high-speed sonicator, as well as techniques such as cluster beam deposition and pulsed laser deposition. Methods reported are depicted in Table 1.

Table 1. Synthesis methods, biocompatibility tests, antimicrobial tests, evaluation of material properties and application of PMMA-AgNPs.

Author, Publication Year	Synthesis Method	Biocompatibility Test	Antimicrobial Effect	Material Properties	Application
Sodagar et al., 2012 (16)	Mixing powder and monomer. AgNPs were synthesized by adding silver nitrate and isopropyl alcohol to monomer	No	No	Flexural strength	Acrylic resins for dentures
Acosta Torres et al., 2012 (1)	Mixing MMA monomer with AgNP suspension	Cell viability test (MTT)	Microbial adherence	Flexural strength, flexural modulus, SEM imaging	Acrylic resins for dentures
Ghaffari et al., 2014 (15)	Mixing acrylic resin powder and nano-silver in an amalgamator	No	No	Tensile strength	Acrylic resins for dentures
Prokopovich et al., 2014 (9)	Encapsulating AgNPs with oleic acid and mixing with PMMA	Cell viability test (MTT)	Microbial adherence	Compression test, AFM, Inductively coupled plasma mass spectrometry (ICP-MS)	Bone cement
Lyutakov et al., 2015 (11)	Dissolving $AgNO_3$ with n-methyl pyrrolidone and mixing with PMMA solution in chloroform	No	Agar diffusion method	AFM, FTIR	Films for dental use
Pooyan Makvandi et al., 2015 (17)	Stirring AgNP dispersion in monomer	No	No	UV spectrophotometry, DSC, TGA, FTIR, swelling test, flexural strength	Acrylic resins for dentures
Elashnikov et al., 2106 (13)	Mixing MMA monomer with AgNP suspension	No	Inhibition zone test	Nanofibers made by electrospinning. SEM and TEM imaging	Nanofibers
Hanif et al., 2016 (3)	Cluster beam deposition technique	No	No	AFM, TEM	Acrylic resins for dentures
Petrochenko et al., 2017 (7)	Pulsed laser deposition (PLD)	Cell viability test (MTT), necrosis detection by flow cytometry	Dynamic contact conditions test	SEM, inductively coupled plasma mass spectrometry (ICP-MS)	Bone cement
Nunes de Souza et al., 2018 (10)	Mixing MMA monomer with AgNP suspension	No	Quantification of biofilm biomass formation using Crystal Violet staining method; XTT for metabolic activity	Films made using casting method. TGA, SEM imaging, NMR	Films for dental use

Table 1. *Cont.*

Siddiqui et al., 2018 (12)	Mixing PMMA in DMF and adding $AgNO_3$ solution	No	Inhibition zone test	UV-visible absorption, FTIR, TEM, FE-SEM	Acrylic resins for dentures
Slane et al., 2018 (8)	Mixing monomer with AgNPs with ultrasonic homogenizer	Cell counting with automated cell counter	Kirby–Bauer disk diffusion assay	DMA, TGA, DSC, SEM imaging	Bone cement
Wekwejt et al., 2018 (2)	Mixing monomer with AgNP suspension	No	Inhibition zone test	Compression test, contact angle test, SEM imaging	Bone cement

The "Material Properties" column refers to the properties measured or tested in the PMMA–AgNP composites that claim to have dental applications. In this column, we also show different characterization methods. The aim of the table is to show different approaches that researchers used to synthetize and characterize these materials, as well as how their different properties, including flexural strength, flexural modulus and tensile strength, were tested. In the "Antimicrobial Effect" column, where we indicate whether reported papers on the use of PMMA and silver nanoparticles in forming materials for dental applications evaluated the antimicrobial effect. As we can see, some authors used silver nanoparticles to improve the mechanical properties of PMMA without taking into account the antimicrobial effect or biocompatibility. This shows us the heterogeneity that exists in the development and evaluation of dental materials, such as those discussed in this review.

The applications of the PMMA–AgNP composites reviewed here are focused mainly on bone cements and acrylic bases for dentures. The most used synthesis method is mixing an aqueous suspension of silver nanoparticles directly on the monomer before mixing the monomer–polymer system. Other synthesis methods, such as cluster beam deposition and pulsed laser deposition, were used to form PMMA–AgNP composites [3,7]. Characterization methods show that the addition of silver nanoparticles modifies the mechanical properties of PMMA depending on the size and concentration used. The size of the silver nanoparticles reported in this review was between 2 and 200 nm, and a spherical shape was most commonly reported by authors. The size and shape depends on the synthesis method. The concentrations of AgNPs used on the materials tested were between 0.01% and 2% in aqueous solutions. An example of silver nanoparticles is shown in Figure 2.

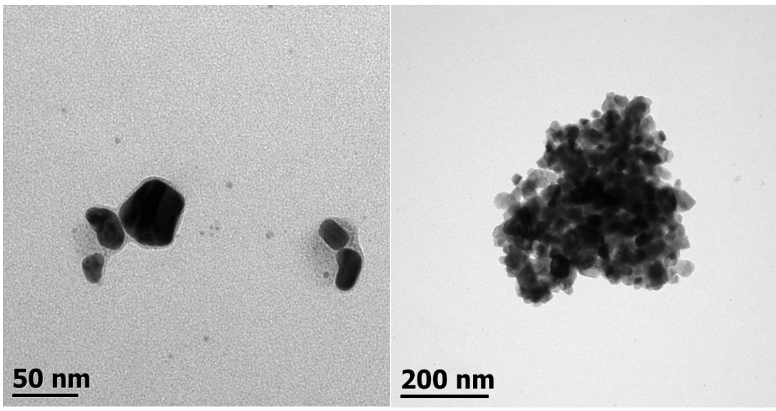

Figure 2. Electronic Transmission Microscope (TEM) images of silver nanoparticles (TEM JEOL JEM-1010 60–80 kV, Digital Micrograph 3.1 digital camera GatanBioscan 1k × 1k). Author´s own images.

4. Biocompatibility

In the present review, only four authors reported biocompatibility tests. Acosta-Torres et al. [1] show that the silver nanoparticles and PMMA-AgNPs are biocompatible materials by using a comet assay to evaluate genotoxicity and using an MTT assay and other testing methods as a cellular immunoassay (named BrdU assay) with a mouse monoclonal antibody. In their results, they find no statistically significant differences when comparing between PMMA discs with and without silver nanoparticles. They report spherically shaped AgNPs in a size range of 10 to 20 nm, and they used OptiCryl (New Stetic S.A., Antioquia, Colombia), a commercial PMMA denture base for forming discs, following the manufacturer's directions. Slane et al. [8] evaluated biocompatibility by cell counting transgenic mouse osteoblast cell lines (TMOb) that were exposed to PMMA–AgNP composite bone cement, and they report no significant effect on the viability of the osteoblast cells. In this study, only cell viability was evaluated, although, as the authors mention, it is important to evaluate other parameters for biocompatibility in vitro, such as cell proliferation, and LDH test for further results. The authors report using the commercially available PMMA bone cement Palacos R (Heraeus Medical GmbH, Werheim, Germany) and AgNPs with a diameter between 30 and 50 nm, functionalized with poly(vinylpyrrolidone) (0.2% wt/wt). Petrochenko et al. [7] and Prokopovich et al. [9] evaluated cell viability in vitro by means of an MTT assay. Petrochenko et al. show a viability decrease in human bone marrow stromal cells (hBMSCs) grown on PMMA–Ag-coated wafers, depending on the laser pulses used when synthesizing PMMA–Ag using the pulsed laser deposition (PLD) method. They report that the use of 20,000 pulses decreased the viability of hBMSCs by 17%, compared with 10,000 and 14,000 pulses. They report AgNPs ranging in size from below 20 to 200 nm. On the other hand, Prokopovich et al. report no cytotoxic effects on an MC3T3-E1 preosteoblast cell line when they compared three different concentrations of silver nanoparticles encapsulated in oleic acid and mixed with a PMMA-based bone cement. AgNPs had an average diameter of 5.3 ± 2.3 nm. These results could indicate that the synthesis methods have affected the biocompatibility of the resultant materials; further studies are mandatory to test this position.

5. Antimicrobial Effect

With regard to antimicrobial properties, the use of silver nanoparticles can be considered a useful approach to promoting an antimicrobial effect. Nunes de Souza et al. [10] report that lower concentrations of silver nanoparticles could affect biofilm formation on PMMA surfaces. Moreover, they mention that the agglomerates of silver nanoparticles were more effective against *C. glabrata* than nanoparticles distributed individually. In the case of *Candida albicans*, the major etiological agent for stomatitis in dental prostheses users, Acosta Torres et al. [1] show less adherence to the surface of PMMA–AgNP-based discs, suggesting an antifungal effect and the possibility that it acts as an anti-adherence agent for microorganisms that colonize surfaces. Lyutakov et al. [11] show that the use of silver-coated polymers is a reliable approach to forming PMMA-based materials to treat bacterial colonization. They mention the release of silver ions as an important mechanism that promotes an antibacterial effect against *Escherichia coli* and *S. epidermidis*, even though the immobilization of silver nanoparticles on the surface achieved effective microbial growth inhibition. Siddiqui et al. [12] indicate that AgNP agglomerates show a slight decrease of the antimicrobial effect, which is observed more at higher concentrations (2 wt %) compared with lower concentrations (1.5 wt %), and well-dispersed nanoparticles. The authors conducted the antimicrobial tests against *P. aeruginosa* and *S. aureus* and they studied the antifungal effect against *Cryptococcus neoformans.* In all cases, concentrations lower than 0.75 wt % had no well-defined inhibition zone when observed in agar plates; however, when using concentrations between 1 and 1.5 wt %, they observed positive results against Gram-negative and Gram-positive bacteria and an antifungal effect. Elashnikov et al. [13] formed light-activated PMMA nanofibers doped with photosensitizer meso-tetraphenylporphyrin (TPP), which has been reported as an effective reactive oxygen species (ROS) producer. TPP inactivates bacterial membranes upon light irradiation. The authors used an Ag/TPP/PMMA solution to prepare films by electrospinning. After this, the films were irradiated with a light-emitting diode light source (110 mW power output, 405 nm center wavelength and a light spot of 10 nm) and a 405 nm emitting laser system with 50 mW output. They evaluated the antibacterial effect using *E. faecalis* and *S. epidermidis.* They reported that the light-activated PMMA nanofibers had a significantly increased inhibition zone compared with non-irradiated nanofibers. Light irradiation leads to the migration and release of AgNPs to the surface from nanofibers or to the formation of AgNP aggregates. The authors suggest that their obtained material is a promising option for the photodynamic inactivation of bacteria. This strategy is known as photodynamic therapy and has been used as a disinfection method in oral cavities [14].

The antimicrobial effect of silver nanoparticles can be induced by the formation of ROS or by releasing silver ions from the material tested, depending on the synthesis method, silver-nanoparticle dispersion and silver concentration in the PMMA matrix. The studies reviewed here show the antibacterial effect of silver nanoparticles against Gram-positive and Gram-negative microorganisms and antifungal effects against different *Candida* species. According to the results of this review, the crucial factor for the antimicrobial function in PMMA is the additive. Some authors report the use of antibiotics to form bone cements that are used in orthopedic surgery to perform hip and joint implants. By itself, PMMA does not have antimicrobial properties, but by adding additives like silver nanoparticles, researchers are looking to design an appropriate material for dental prostheses, bone cement and other applications [2,7–9].

An example of the possible application of silver nanoparticles and poly(methyl methacrylate) to form dental prostheses can be seen in Figure 3.

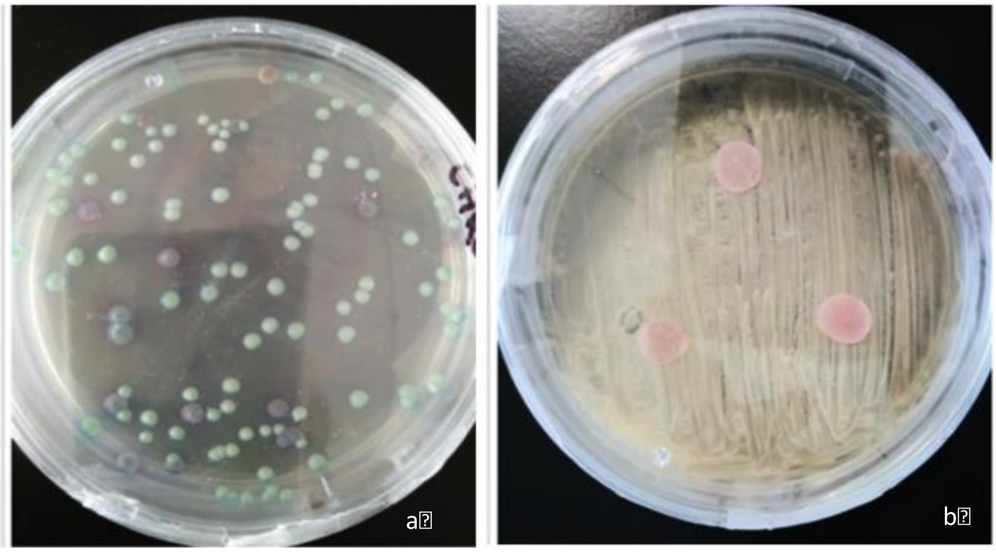

Figure 3. Example of a PMMA–AgNP composite application. (**a**) Culture obtained from a patient's oral mucosa. The green colonies correspond to *Candida albicans* and the purple colonies correspond to *Candida tropicalis* cultured on CHROMagar Candida (CHROMagar, 4, Place du 18-Juin-1940, Paris, France, 75006). (**b**) PMMA–AgNP discs on cell culture plates. With this technique, we could not observe an inhibition zone because AgNPs could not be released from the PMMA discs. Silver nanoparticles embedded in the PMMA act as an agent that inhibits the growth of microorganisms on the surface of the discs. Author´s own images.

6. PMMA–AgNP Composite Properties

PMMA is used in different fields due to properties that enable it to be used in biomedical applications. The use of silver nanoparticles promotes the antibacterial properties of those PMMA-based materials applied in different ways in the biomedical field. In this review, we focused on dental applications, although the use of PMMA–AgNP composites as bone cement is also widely reported (Figure 1).

Ghaffari et al. [15] evaluated the effects of silver nanoparticles on the tensile strength of self-cured PMMA. They reported that the presence of 5% silver nanoparticles significantly decreased the tensile strength of commercial-brand heat-cured acrylic resin Triplex (Ivoclar Vivadent). These results are consistent with those reported by Sodagar et al. [16]. In their study, they observed that the addition of 0.05% silver nanoparticles to the commercially used acrylic resins Rapid Repair and Selecta Plus (Dentsplay, Weibridge, UK) significantly decreased the flexural strength. The authors mentioned that this effect could be due to the silver nanoparticles' dispersion, and they also mention that the use of 0.02% silver nanoparticles increased the flexural strength, but not significantly.

In contrast, a study reported by Pooyan Makvandi et al. [17] reports higher values of flexural strength and modulus, which represents an enhancement of the mechanical properties of PMMA–AgNP acrylic resins. Dental prostheses are made of PMMA due to its better mechanical properties, dimensional stability and minimal inflammatory response. It is a biocompatible polymer and, due to its properties, is a suitable material for use in the patient's oral environment. According to this review, the ideal material for dental applications would be one that is biocompatible, with better mechanical properties (flexural modulus, fracture resistance and compression modulus) and resistance to corrosion, as well as one that provides an antimicrobial and antifungal effect to prevent oral infections.

7. Conclusions

In this review, we present an overview of the reported studies that use PMMA-based materials that were modified with silver nanoparticles for dentures or bone cements. The use of silver nanoparticles as an antimicrobial agent for surface modification to prevent bacterial and fungal adherence can serve as an interesting approach to prevent oral infections in patients. Moreover, the use of silver nanoparticles in bone cements can promote the optimal functioning of total hip prostheses, which are the most used prostheses. Finally, the use of silver nanoparticles can enhance the mechanical properties of PMMA-based materials, depending on the concentration, dispersion and even the synthesis method used on materials under this approach. According to the results of this review, the better strategy for embedding silver nanoparticles into a PMMA matrix seems to be mixing AgNPs with a monomer before the polymerization reaction. This strategy allows for control of some properties in areas that require improvement, such as nanoparticle concentration, shape and dispersion, depending on what kind of material is sought. This synthesis method is easy and simple to perform. Synthesis methods such as cluster beam deposition and pulsed laser deposition require more specialized equipment, which could be less efficient for synthesizing and obtaining the appropriate material. Standardized methods would allow us to compare materials in a more accurate way, and this fact can bring the development of materials with antimicrobial properties closer to their final clinical application in the dental field in the short term. For the success of dental applications at this moment, we need evidence from human clinical trials that this approach works appropriately.

In the near future, the main question that needs to be addressed is whether polymeric materials are the optimal candidates to continue developing dental prostheses from. The efficiency of the production methods should also be questioned. In recent years, with advances in the area of tissue regeneration and with new production methods such as 3D printing, the field of dentistry faces great scientific and technological changes.

The use of PMMA–AgNP-based materials in dental applications can promote better oral health in prosthesis users, helping to improve their quality of life.

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Abbreviations

MMA	Methyl Methacrylate
DMF	Dimethylformamide
SEM	Scanning Electron Microscope
AFM	Atomic Force Microscope
FTIR	Fourier-Transformed Infrared Spectroscopy
UV	Ultra Violet
DSC	Dynamical Scanning Calorimetry
TGA	Thermo-gravimetrical Analysis
TEM	Transmission Electron Microscope
NMR	Nuclear Magnetic Resonance
FE-SEM	Field Emission Scanning Electron Microscope

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