Chapter 3

New Trends for the Processing of Poly(Methyl Methacrylate) Biomaterial for Dental Prosthodontics

Ma. Concepción Arenas-Arrocena,
Liliana Argueta-Figueroa, René García-Contreras,
Omar Martínez-Arenas, Berenice Camacho-Flores,
María del Pilar Rodriguez-Torres,
Javier de la Fuente-Hernández and
Laura S. Acosta-Torres

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Abstract

Rehabilitation of masticatory function in patients with absent teeth with removable dentures is an established form of treating partial or complete dentition in edentulous patients. The developments in recent decades with dental implants dominate current dental research. However, medical contraindications, a negative attitude toward implants, or financial limitations on the part of the patients limit their universal applicability, so the rehabilitation with dental prostheses still makes up a significant portion of everyday clinical practice. Conversely, removable dentures are used in the critical conditions of the oral cavity. There are about 500 strains of microorganisms in the mouth, which form the biofilm in an acidic environment causing several issues, such as denture stomatitis, deterioration of the periodontal status of the remaining teeth, or carious lesions in the supporting teeth. Therefore, it is very important to choose a suitable material for the prosthesis. Poly(methyl methacrylate) (PMMA) is an acrylic resin usually used with a long tradition for prosthetic purposes. The aim of this chapter is to present the trends for the processing of PMMA. It includes the chemical synthesis, conventional thermal processing of this acrylic resin, the new processing technique assisted with ultrasound, the antibacterial effect on PMMA with nanoparticles, and the cytotoxicity, genotoxicity, and mutagenesis of this material.

Keywords: thermal polymerization, acrylic resin, biomaterial, polymer, dental materials, acrylic resin

1. Introduction

The dynamic development of new multidisciplinary areas has a direct impact over the possible treatments and the rehabilitation of the dental function. Teeth rehabilitation with removable denture prosthesis is an established form of treating both partial and complete dentition in edentulous patients [1]. The developments in recent decades with dental implants dominate the current dental research, not only medical contraindications but also a negative attitude toward implants [2] and economic limitation [3] are the major disadvantages for their universal applicability, so the rehabilitation with dental prostheses still makes up a significant portion of everyday clinical practice [4].

The PMMA material revolutionized the preparation techniques used so far since Walter Wright introduced the acrylic resin as the denture base material in 1937 [5]. The acrylic resin became the preferred material for making denture bases, due to its ability to overcome many of the deficiencies of the materials used at that time [6].

Conversely, removable dentures are used in critical conditions of the oral cavity. There are about 500 microorganisms in the mouth, which produce a biofilm in an acidic environment causing several diseases [7], such as denture stomatitis [8], deterioration of the periodontal status of the remaining teeth [9], or carious lesions in abutment teeth [10]. Therefore, it is very important to choose a suitable material for dental prosthesis.

Poly(methyl methacrylate) (PMMA) is an acrylic resin usually used with a long tradition for prosthetic purposes [11]. It can be classified as chemically or thermally polymerized material depending on the factors that initiate the reaction. For dental prosthesis, thermally polymerized materials are used and the heat can be generated by hot water bath or microwave energy [12]. It was suggested that residual monomer concentration is the most important parameter in the determination of the final properties of the PMMA for dental prosthesis [12, 13]. It was found that in the chemical structure of PMMA, the alpha methyl groups tend to remain in the outer layer surface, whereas the methylene groups are in the inner layer of the PMMA surface, which gives an idea of the arrangement of the polymer [13]. In other words, PMMA has exhibited moderate cytotoxicity in bulk material and polymerized form [14, 15].

The aim of this chapter is to present the trends for the processing of PMMA, including the chemical synthesis, conventional processing (thermal polymerization), the new technique of thermal polymerization assisted with ultrasound, the antibacterial effect on PMMA with nanoparticles, and biocompatibility (cytotoxicity, genotoxicity, and mutagenesis).

2. Poly(methyl methacrylate) (PMMA): synthesis, morphology, and physical properties

Acrylic acid ($C_3H_4O_2$) gives rise to the so-called acrylic, where the poly(methyl methacrylate) (PMMA) is the most important thermoplastic in this group, which is commercially known as Plexiglas, Lucite, and Perspex [16].

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PMMA is an amorphous polymer formed by the polymerization of MMA monomer carried out using different mechanisms [free radical vinyl polymerization, anionic polymerization, group transfer polymerization (GTP), or atom transfer radical polymerization (ATRP)] [16–20]. The bulk or solution (homogeneous polymerization) and emulsion or suspension (heterogeneous polymerization) techniques are used to obtain PMMA [18, 20–22]. Among them, suspension polymerization is a good route to produce PMMA with high molecular weight (36,100), high yield (83%), and a polydispersity of 2.4 (polydispersity index: Mw/Mn) [18].

2.1. Suspension polymerization

Hoffman and Delbruch developed suspension polymerization in 1909 for the first time [23]. In this technique, the initiator and the monomer are miscible with each other (**Figure 1**) and it involves droplet formation by the initiator/monomer (polymerizing phase) dispersed into water (oil/water system), where the volume ratio of monomer about 0.5 or less is suggested [22]. Water works as a heat-transfer agent and a dispersion medium, which improves the reaction rate and the yield in the polymerizing phase. To prevent settling or creaming, the suspension polymerization was kept under stirring during polymerization. In this polymerization, the addition of a soluble stabilizer in water [gelatin, clay or clay derivative, cellulose derivatives, water-soluble polymers such as poly(vinyl alcohol) (PVA) or starch] helps to prevent the breakup of droplets or avoids the droplet from adhering to each other [20–22, 24]. This process could be assisted with low temperature or ultrasonic waves [20, 24–27].

2.1.1. Spherical microparticles: effect of stabilizer agent on size

Suspension polymerization is adequate technically to obtain PMMA spherical microparticles with controlled sizes ranging from 5 to 1000 μm [22]. Alginate stabilizer produces microparticles from 5 to 80 μm (**Figure 2a**), whereas microparticles below 30 μm are obtained with gelatin stabilizer (**Figure 2b**) as previously reported [24–26]. These sizes are within the range of commercial PMMA (10 to 100 μm) used for prosthodontics (**Figure 2c** and **d**). Therefore, polydisperse particles could influence the surface roughness of PMMA without affecting their mechanical properties [28].

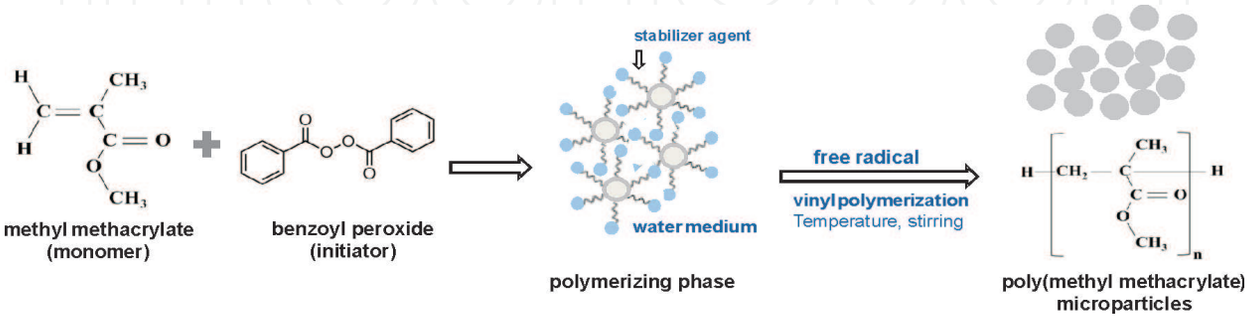

Figure 1. Suspension polymerization of MMA monomer. In the first step, the initiator, benzoyl peroxide interacts with the monomer in water in order to form emulsion oil/water, where the volume of water is twice as that of the monomer. Soluble water-stabilizer helps to obtain smooth and controlled size spherical microparticles.

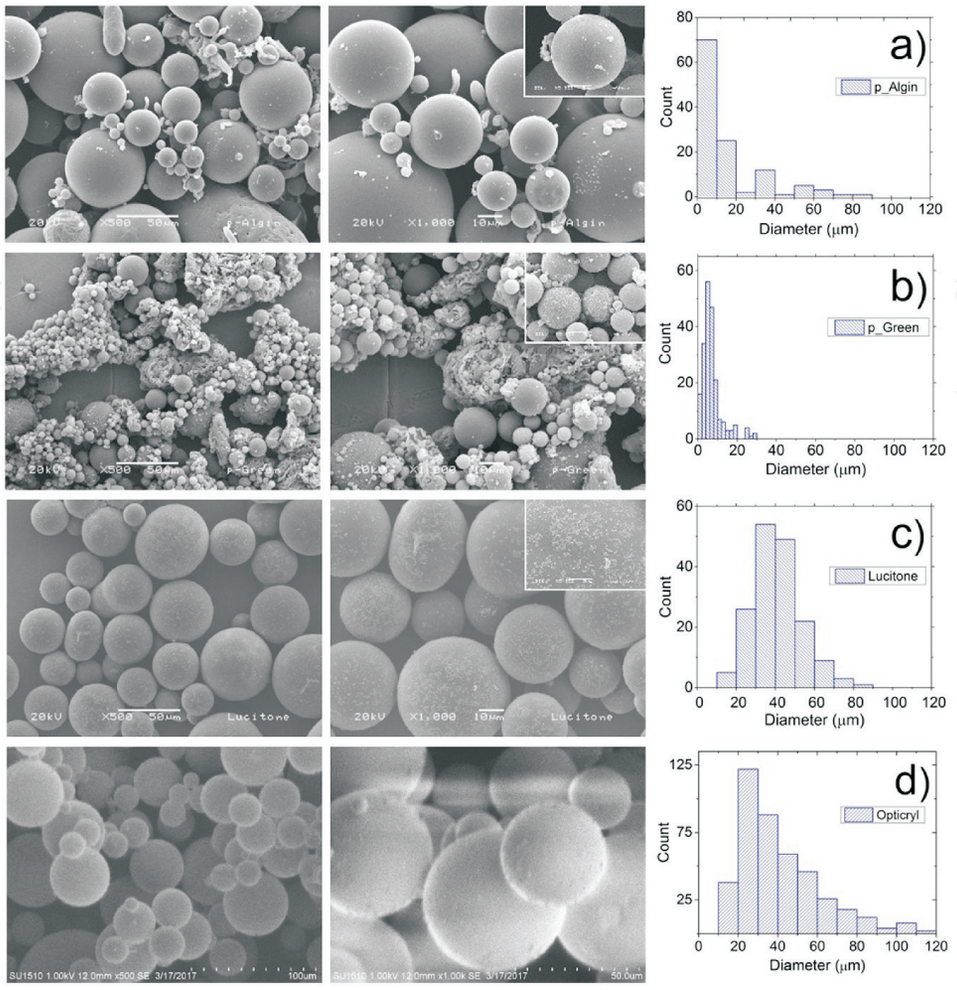

Figure 2. Experimental PMMA microparticles obtained by suspension polymerization with (a) alginate or (b) gelatin stabilizer agents. Commercial PMMA microparticles: (c) Opticryl®, (d) Lucitone® used for prosthodontics.

2.2. Physical properties

PMMA has different characteristics and properties, such as chemical stability, hardness, stiffness and high transparency, resistance in atmospheric conditions and greater impact resistance than glass, and thermal and acoustic insulation. **Table 1** enlists the physical properties of PMMA [17, 29].

Properties	Values
Relative molecular mass	100.12
Elastic modulus	2.4–3.1 GPa
Tensile strength	80 MPa
Flexural strength	140 MPa
Elongation at break	2–5%
Volatility	3.87 kPa at 20°C
Stability	Highly inflammable vapor, lower explosive limit 2.1 vol%

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Properties	Values
Glass transition temperature (T_g)	100–130°C
Fusion temperature	200°C
Density	1.2 g/cm^3
Refractive index	1.49
Water absorption	30 mg

Table 1. Properties of poly(methyl methacrylate) [17, 29].

These properties are important for the final application, such as optical device, airplane windows, lenses, covers, automotive taillight, dental articles, and bioengineering [29]. Also, PMMA is a material widely used daily in dental practice, such as dental prosthesis for edentulous patients [24, 26]. For this particular application, PMMA (experimental or commercial acrylic resin) must be processed by heat, which can be generated by hot water bath or microwave energy [12].

3. Thermal polymerization processing of PMMA

The denture bases made up of acrylic PMMA resin, which is in contact with the oral mucosa of the patient is a critical aspect for biocompatibility in contact with tissues. The PMMA resin was chosen due to this important adequate processing technique [14]. Polymerization of PMMA by water bath and microwaves are the most commonly used processing techniques for making denture bases [12]. The water bath and microwave polymerization techniques produce a material with reduced porosity and irregularities on the PMMA surface. Independent of the processing method, the PMMA surface exhibits some defects (pores, cracks, and irregularities) that are produced at the time of its elaboration [30, 31]. These defects can be excellent reservoirs for fungi and opportunistic bacteria, besides decreasing the elastic modulus and flexural strength [12, 13, 32].

Over the years, the water bath processing technique has been the most widely used due to its ease of handling and cost effectiveness. But, the residual monomer content and porosity have been suggested as the most significant reasons for the reduced flexural strength [33]. It has been accounted the unfavorable thermal gradient produced during the processing technique. In the water bath processing technique, the benzoyl peroxide (initiator) was activated by heating the water to a very high temperature, which leads the polymerization reaction by crosslinking methyl methacrylate moieties. At this point, the methyl methacrylate particles begin to boil by creating porosities in the denture base resin [34]. As the reaction progresses, heat is liberated and cannot escape easily as the water surrounding the flasks is being heated as well. Thus, an unfavorable thermal gradient was created [35].The residual monomer inside the polymeric mass can negatively influence the physical and mechanical properties of the materials due to its plasticizing action [36]. On the other hand, during the microwave polymerization, monomer molecules move in a high-frequency electromagnetic field [37]. The microwaves cause the methyl methacrylate molecules within the acrylic resin to orient themselves in the electromagnetic field at a frequency of 2450 MHz [38], and numerous

polarized molecules are flipped over rapidly and generate heat due to molecular friction [39]. Numerous intermolecular collisions are promoted, causing a rapid internal heating in which energy was immediately absorbed by the resin regardless of the thermal conductivity of the materials involved in the processing of the prosthesis [40]. This warming occurs rapidly and homogeneously and thereby transfer of heat from the water bath to the resin inside the flask occurs faster in this method [41].

There are several studies to compare the flexural strength and elastic modulus values of PMMA using water bath and microwave polymerization [13, 35, 36, 41–43]. In most cases, the results of microwave polymerization did not differ from those obtained with water bath, independent of the acrylic resins used [41, 43]. However, in some studies, water bath technique showed higher flexural strength than microwave processing technique [44]. On the contrary, in other studies, a statistically higher flexural strength was found for microwave-processed denture resins [45, 46]. Other researchers did not find a significant difference in porosity between microwave polymerization and conventional water bath cycles [39, 47, 48]. In contrast, the other work reported that heat polymerization technique presents lower mean porosity values than microwave-polymerization method [31]. Both processing techniques produced PMMA material with divergent properties. Therefore, new processing techniques for PMMA are needed to reduce the amount of residual monomer and porosity and to increase its physical strength.

3.1. Thermal polymerization assisted with ultrasound

The most widely used heat-curable acrylic material to make dental bases and temporary restorations is PMMA. A disadvantage of this acrylic resin is the residual monomer which remains in the polymer even after its polymerization is finished [49]. Several attempts were made to find a better strategy in order to prevent the presence of residual monomer. For example, the effects of temperature, time, initiator concentration, curing environment, water bath or microwave oven, pressure, and mixing ratio (polymer:monomer) have been investigated [32].

The first effort to employ ultrasound for the acceleration of conventional chemical reactions [50] by Richards and Loomis was reported in 1927. A lot of interest has been attracted for the use of ultrasound toward the development of synthetic routes in a variety of areas of chemistry, chemical production, and materials science [27, 51]. It is possible to generate chemical changes in consequence of acoustic cavitation while more powerful ultrasound at a lower frequency is applied to a system. During cavitation, bubble collapse produces intense local heating, high pressures, and very short lifetimes. These transient and localized hot spots drive high energy toward completing chemical reactions faster [52]. Besides, the physical effect of the medium on the wave was referred to low power or high frequency ultrasound [53].

In previous studies, Charasseangpaisarn and Wiwatwarrapan [49, 54] found that the use of an ultrasonic treatment at several frequencies reduced the presence of residual monomer in acrylic resins. For example, heat-polymerized MMA by the immersion in water at 50°C for 10 min at 40 kHz reduced the residual monomer. They have concluded that sonication could reduce the amount of residual monomer in acrylic resins. According to the authors, the ultrasonic treatment could enhance the extraction rate of the residual monomer from the resin and could cause postpolymerization of the residual monomer.

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3.1.1. Influence of frequency and power of ultrasonic waves on the flexural strength and elastic modulus

The method of denture processing is directly related to the physical properties of the acrylic resins. One of those properties is Young's modulus, also known as elastic modulus. That is defined as capacity of a body to deform to the application of stress and strain after removing the body recovers its original shape. It can be assumed that the relationship between the increased effort and increased deformation is constant [55]. Flexural failure of denture base of PMMA is considered to be the main form of clinical failure [56]. The dental prostheses are subjected to various conditions such as forces during chewing, drastic changes of temperature and humidity, and acidic environment in the oral cavity. Therefore, it is important that prosthetic materials possess an adequate elastic modulus [42]. The elastic modulus can be determined by indentation techniques. However, the correct use of these techniques requires knowing their limitations in order to avoid misinterpretation.

Experimental results about the elastic modulus and flexural strength (ISO20795-1:2008 *Part 1: Denture base polymers)* of commercial acrylic resin (Opticryl®) indicate that the thermopolymerization assisted with ultrasound is a good option for the processing of PMMA. Commercial acrylic resin (Opticryl®) specimens (n = 25) were prepared according to the technical sheet with a volume ratio of monomer to polymer (1:6). For the processing condition by ultrasound waves, two frequencies and powers were used at 80°C of water bath for 1 hour: 37 or 80 kHz and 50 or 100%, respectively, in order to obtain four experimental groups (**Table 2**). Water bath and microwave technical processing were considered to be the control groups. The results of the elastic modulus and flexural strengths are given in **Figure 3** and **Table 2**.

For statistic comparison among the groups, Kruskal-Wallis test and Mann-Whitney *U*-test were used for analyzing the data. These tests were used because not all groups had a normal distribution as shown by the Shapiro-Wilk normality test (see **Table 3**).

Kruskal-Wallis test showed that there are significant differences among the groups (for elastic modulus, p = 0.006 and for flexural strength, p = 0.018). Mann-Whitney *U* test was conducted

Group	Thermopolymerization	Frequency (kHz)/ power (%)	Elastic modulus (MPa)	Flexural strength (MPa)
1	Ultrasonic	37/50	1710.38 ± 429	58.63 ± 13.8
2	Ultrasonic	37/100	1730.75 ± 335.13	58.16 ± 10.64
3	Ultrasonic	80/50	1488.86 ± 80.02	51.05 ± 9.61
4	**Ultrasonic**	**80/100**	**1828.08 ± 363.67**	**62.14 ± 12.92**
5	Water bath	–	1744.40 ± 441.85	60.57 ± 14.91
6	Microwave	–	1466.12 ± 428.39	54.15 ± 17.13

Specimens processed by water bath and microwaves are considered the control groups.

Table 2. Elastic modulus and flexural strength of specimens processed by ultrasound at 80°C: 37 or 80 kHz and 50 or 100% of power under constant temperature of water (80°C).

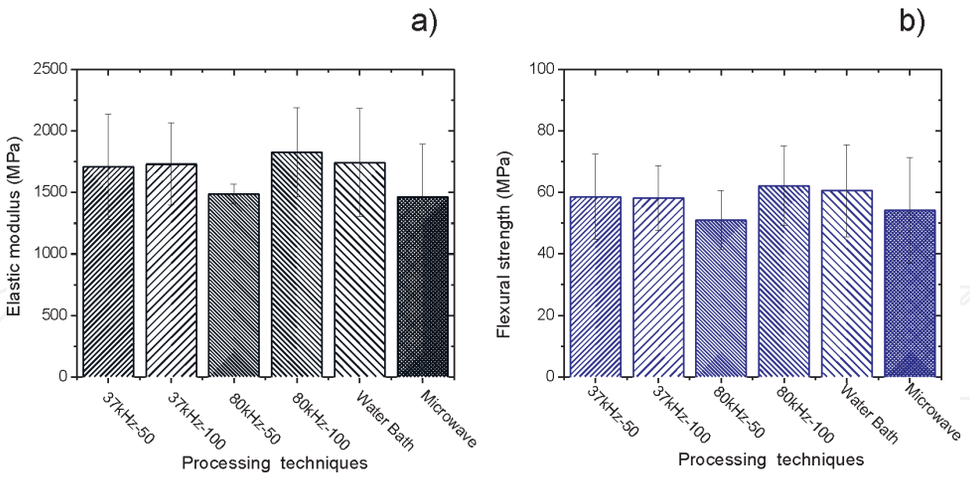

Figure 3. Results of elastic modulus and flexural strength of commercial Opticryl resin polymerized by ultrasound. Control groups were processed by water bath and microwave energy.

Mechanical properties	Frequency (kHz)/ power (%)	Shapiro-Wilk statistic analysis		
		Statistic value	Degrees of freedom	*p*-Value
Elastic modulus	37/50	0.940	32	**0.076***
	37/100	0964	38	**0.261***
	80/50	0.844	26	0.001
	80/100	0.922	35	0.017
	Water Bath	0.967	19	**0.711***
	Microwave	0888	21	0.020
Flexural strength	37/50	0.893	32	0.004
	37/100	0.962	38	**0.225***
	80/50	0.924	26	0.056*
	80/100	0.932	35	0.032
	Water bath	0.955	19	**0.472***
	Microwave	0.919	21	**0.083***

*The data follow a normal distribution if $p \geq 0.05$.

Table 3. Results of Shapiro-Wilk normality test.

among groups in all possible combinations to determine the differences among groups. The results are shown in **Table 4**.

The specimens processed at 80 kHz and 100% of power (group 4) exhibited the highest values with an elastic modulus of 1744.40 ± 441.85 MPa and a flexural strength of 60.57 ± 14.91 MPa. However, the flexural strength values were not statistically significant compared to those processed by the water bath and microwave, respectively (**Figure 3**). But, with regards to

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Comparison among groups	Elastic modulus		Flexural strength	
	Value of the test statistic	*p*-Value	Value of the test statistic	*p*-Value
1–2	563,000	0.596	535,000	0.389
1–3	321,000	**0.137***	269.500	**0.022***
1–4	439,000	0.129	452,500	0.177
1–5	276,000	0.585	274,000	0.559
1–6	242,000	0.087	287,000	0.373
2–3	322,000	**0.019***	273,000	**0.003***
2–4	563,000	0.260	579,000	0.342
2–5	341,000	0.735	310,000	0.388
2–6	255,000	**0.023***	363,000	0.569
3–4	227,000	**0.001***	213,000	**0.001***
3–5	163,000	**0.054***	153,000	**0.031***
3–6	262,000	0.814	252,000	0.653
4–5	312,000	0.710	322,000	0.849
4–6	187,000	**0.002***	284,000	0.158
5–6	130.000	0.60	152,000	0.198

*Statistical significance, $p \leq 0.05$.

Table 4. Comparison among groups using Mann-Whitney *U*-test.

the elastic modulus, a highly significant difference among the specimens of group 4 and the specimens processed with microwave (group 6) was found. Hence, these results indicate a better performance of the PMMA processed by ultrasound in 80 kHz and 100% of power in comparison to that by microwave processing.

In addition, it is further noted that the acrylic resins processed at 80 kHz and 50% of power (group 3) had significantly lower values compared to the other experimental groups, for both the elastic modulus and the flexural strength. No statistically significant differences in the elastic modulus and flexural strength among groups (water bath and microwave) were found. Therefore, it was concluded that these two methods have similar results. From these results, it seems that the power is more important than the frequency of ultrasound for better results in the processing of PMMA.

Spearman correlation test was performed in order to determine if the elastic modulus values and flexural strength values are correlated. It was found that a weak correlation existed since the correlation coefficient between the elastic modulus and flexural strength was 0.618 ($p \leq 0.001$). Since the coefficient is a positive value, the increasing elasticity modulus value also increases the flexural strength.

In summary, the best conditions for higher values of both elastic modulus and flexural strength correspond to the specimens processed at 80 kHz and 100% of power (group 4). The processing of PMMA with water bath or microwave processing generated similar values for elastic modulus and flexural strength. Ultrasound can be used to process the acrylic resin (Opticryl®) as an alternative technique for PMMA processing with similar results to those obtained using water bath or microwave processing (control groups). The correlation coefficient between the elastic modulus and the flexural strength indicates a weak correlation but statistically significant association between these two variables. The sign of the coefficient is positive, this means that as the values of the elastic modulus increase, those of the flexural strength also increase.

4. Biological properties of PMMA

4.1. Antimicrobial activity

As mentioned above, the current techniques for processing base denture produce porosities, which allow bacterial colonization [30, 31]. One way to approach this issue is the covering up of the PMMA surface. Since the introduction of nanoparticle-based antimicrobial agents, these have generated really huge interest. Diverse mechanisms for explaining the activity of antimicrobial agents have been discussed, such as the release of ions from the nanoparticle surface, the internalization through cell wall, the production of reactive oxygen species [57], and the destruction of cell wall by the nanometric pillars on the surfaces, among others [58]. For instance, the wing surfaces of insects such as dragonflies and cicadas exhibit a texture that is formed by nanopillars, which are very effective against certain type of pathogenic microorganisms [58, 59]. The possibility of developing surfaces that have antibacterial effects quickly became the subject of study [60].

The characteristics of the surfaces of certain objects make them excellent places for proliferation of pathogenic microorganisms and thereby prevent the bacterial adhesion. The main characteristics of polymer surfaces related to microbial adhesion are chemical composition and topography [61].

Different surface modifications have been suggested to reduce the adhesion of pathogenic microorganisms. At present, one of the most effective methods is the surface modification with metallic antibacterial agents such as silver, copper, and zinc oxide at nanometric scale [62, 63]. It has been demonstrated that the oxidized state on surfaces (through electrochemical anodization) shows a significant decrease of some bacterial strains present in the oral cavity and bacteria involved in the process of biofilm formation [64].

Besides, polymeric glycol-based coatings have been proposed in order to immobilize the molecules on the surface of the substrate. Thus, this prevents bacterial adhesion. The modification of the surface topography generates an unfavorable surface chemistry for the adhesion of certain microorganisms and therefore the colonization of surfaces [65, 66].

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The arrangement of polymeric coatings with antibacterial agents such as nanoparticles has been studied. The best alternative is that the nanoparticles have to be contained in the polymer matrix, so that their release acts at the level of biofilm formation [67]. According to the type of antimicrobial agent and disposition on the surface, it may offer more than one function eradicating an acute infection and even providing extended periods of suppression of bacterial proliferation [68, 69]. The action can be differentiated depending upon its mechanism in passive coatings (coatings that prevent bacterial adhesion), contact-killing coatings, and active coatings with the ability to release the antibacterial agent incorporated [70]. A coating includes different antimicrobial agents, such as moieties, nanoparticles, and antibiotics for specific pathogens [66, 71]. Silver nanoparticles as a cover on PMMA decrease the roughness from 566.7 nm (without nanoparticles) to 104.08 nm (with nanoparticles) (**Figure 4**). On the other hand, Ziad *et al*. found that Nystatin modifies the roughness of PMMA so that this could influence the antifungal agents on the PMMA surface [66].

These results show that PMMA with antimicrobial agent are potentially useful for their application in dentures for the future. Not many studies have been carried out and there is still scope for further study in this area.

In addition to the antibacterial effect, PMMA-metal oxide nanoparticles have been synthesized with the purpose of improving PMMA's flexural strength as well [63]. With this aim, several works have been carried out by incorporating TiO_2 nanoparticles and assessing the dependence of the flexural strength on the TiO_2 nanoparticle concentration. It was observed that by increasing the concentration of nanoparticles, the flexural strength of PMMA value increases. In some cases, better flexural strength value was found in comparison with PMMA alone [72, 73]. Studies on the improvement of tensile strength concluded that increasing the TiO_2 nanoparticle concentration provided better tensile strength up to some concentration and then the strength decreases [74]. Recently, Totu *et al*. developed a PMMA-TiO_2 material with improved antibacterial activity, for manufacturing 3D-printed dental prosthesis [75].

Other metal oxide nanoparticles that have also been used for their integration to PMMA are the iron dioxide nanoparticles [25]. These nanoparticles improved the antimicrobial and mechanical properties of the acrylic resins. Nanopigmented particles incorporated into PMMA also have been shown to be non-cytotoxic (against fibroblast *in vitro*) and to exhibit good physical

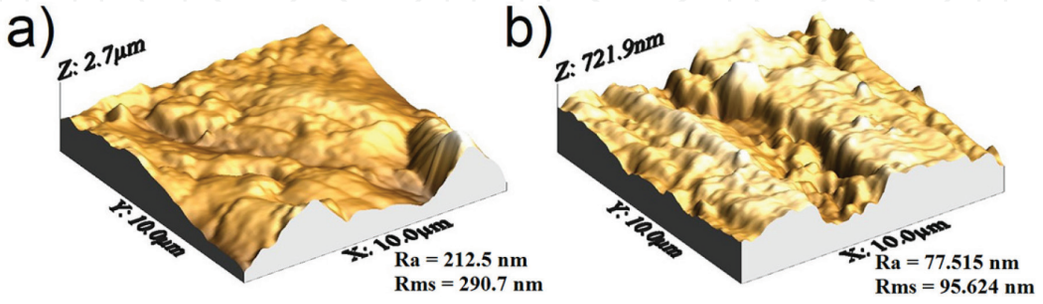

Figure 4. Surface roughness of (a) PMMA uncoated and (b) coated with silver nanoparticles by spin coating.

and mechanical properties as well [24]. In both the cases, specimens exhibited good mechanical and physical properties and were not non-cytotoxic showing similar appearance to commercial acrylic resins.

4.2. Biocompatibility

One of the most important factors that distinguish biomaterials is its ability to exist into or in contact with tissues of the human body without inducing any collateral effect, where both biomaterials and tissues coexist, and the biocompatibility may be compromised.

Biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific situation [76].

The biocompatibility of a material depends on the type of material, where it is placed, and the function it is expected to perform. Therefore, a biocompatible material elicits an acceptable tissue response when tested or used in a specific tissue under certain conditions, including the health status of the patient [77]. It is important to understand the paradigms of biocompatibility by the determination of which chemical, biochemical, physiological, physical, or other mechanisms, under specific conditions, associated with contact between biomaterials and cells or tissues of the body. The interactions of materials that are in direct contact with the human body depends on the characteristics of the host such as age, sex, general health and current disease, physical mobility, lifestyle features, and pharmacological status [78]. Thus, the major features influenced in the host and generic host response (implanted or in contact with tissues) of biomaterials are enlisted in **Table 5**.

On the other hand, PMMA-based acrylic resin has been broadly used as a dental material, especially in denture base processing due to its favorable working characteristics, processing ease, accurate fit, stability in the oral environment, and superior aesthetics with inexpensive equipment. Despite these excellent properties, there is a need for improvement in the biological aspects of biocompatibility. This section is oriented to summarize the different methods of PMMA biocompatibility alone and enriched or modified with different biomaterials in contact with cells and implantation in animal bodies highlighting the type of cells or animal test, period of incubation or implantation, method for analysis, and results. The incorporated studies are recent publications indexed at MEDLINE/PUBMED based on a systematic review.

4.2.1. Test methods

Testing for cytocompatibility depends on the site of use and the duration of exposure. Biomaterials or other associated products do not have to exhibit the same compatibility as materials that are placed permanently into the tooth structure, used as implants into bone or soft tissues, or used in dentures and dental or orthodontic appliances. All the tests are usually conducted sequentially, with shorter term, *in vitro*, or less expensive screening testing, and involve the use of animals. If a material is not showing biocompatibility based on initial studies, it may be better to eliminate it from consideration for further testing for certain applications [79]. The biocompatibility concerns and the testing methods have been discussed for over 40 years. However, new issues and new testing possibilities must be considered for innovating dental materials and evaluate the response of cells to medical materials at the cellular

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Variables that could influence the host response	Characteristics of the generic host response to biomaterials
Bulk material composition, micro- (or nano)-structure, morphology	Protein adsorption and sorption characteristics
Crystallinity and crystallography	General cytotoxic effects
Elastic constants	Neutrophil activation
Water content, hydrophobic–hydrophilic balance	Macrophage activation, foreign body giant cell production, granulation tissue formation
Macro-, micro-, nano-porosity	Fibroblast behavior and fibrosis
Surface chemical composition, chemical gradients, surface molecular mobility	Microvascular changes
Surface topography and energy	Tissue/organ-specific cell responses (e.g., osteoclasts and osteoblasts for bone, endothelial proliferation)
Surface electrical/electronic properties	Activation of clotting cascade
Corrosion parameters, ion release profile, metal ion toxicity (for metallic materials)	Platelet adhesion, activation, aggregation
Degradation profile, degradation product form, and toxicity (for polymeric materials)	Complete activation
Leachables, additives, catalysts, contaminants, and their toxicity (for polymeric materials)	Antibody production, immune cell response
Dissolution/degradation profile, degradation product toxicity (for ceramic materials)	Acute hypersensibility/anaphylaxis
Wear debris release profile	Delayed hypersensitivity
	Mutagenic response, genotoxicity
	Reproductive toxicity
	Tumor formation

Table 5. Biomaterial variables that could influence the host response [80].

and subcellular levels such as cell proliferation or death in contact with materials [80]. These protocols and methods of interpretation may be used to enhance the information given in the National and International standards.

4.2.1.1. Cell culture testing

The most common and initial evaluation of a new material is by placing the material or an extract of the material into a suitable laboratory cell culture and by observing any changes in the cells over a period of hours to a few days [81]. These tests are performed on primary cell cultures or established cell lines (commercially available), which allows comparison of testing performed for different materials using nearly identical cloned cells. The use of PMMA acrylic base denture has been widely investigated in culture cells alone and enriched with different materials. The enlisted publications in **Table 6** was searched at MEDLINE/PUBMED with the following keywords: "Cytotoxicity AND acrylic resins," "cytotoxicity AND denture

Author	PMMA modification	Culture cells	Assays	Culture time	Results
Herman et al. [82]	PMMA, monomer modified with DABCO (DC16, DC16F, DC18, C6DC16) and conjugated monomers (DC11MAF and C2DC11MAF) at 1, 2, or 3%	Periodontal ligament cells (PDL) and gingival fibroblast (HGF)	BioTek Synergy2 fluorescent	24 h	DABCO components exhibit intermediate to high cytotoxicity and DC11MAF exhibited the lowest toxicity against PDL and GF
Song et al. [83]	PMMA with chitosan (0.50, 1, 2, 3 mg/ml)	Mouse fibroblast cells (L929)	MTT	0, 24, 48, 72 h	No significant difference in cell proliferation between conventional resin and the chitosan quaternary ammonium salt modified
Liu et al. [84]	PMMA-PEI (polyethyleneimine) nanoparticles	Kupffer cells (KCs) primary culture	Cell Counting Kit-8 assay Fluorescence Western blot	6, 12, 24, 48 h	Exhibit survival fraction higher than 90%. These results suggested that the PEI-PMMA/miRNA-complexed NPs had low cytotoxicity to KCs
da Silva et al. [85]	N1 acrylic (MMA polymer, dibutyl phthalate), ethyl acrylate pigments), Poli-Côr (color R2, MMA polymer, dibutyl phthalate, ethyl acrylate, around 1.5% of various organic and inorganic pigments), Clássico (MMA monomer, topanol)	Human conjunctiva cell line (CCL-20.2).	MTT ELISA RT-PCR	72 h	Non-cytotoxic based on cell proliferation. Resin with pigment showed significant increase of IL6
Carlsson et al. [86]	PMMA-based bone cement-Osteopal V modified with castor oil and linoleic acid	Human osteoblast-like Saos-2 cells (HPACC)	AlamarBlue assay Fluorescence	24 h	*In vitro* cytotoxicity appeared somewhat affected by the castor oil and linoleic acid additions
Jiao et al. [87]	PMMA enriched with 15% of N-acetyl cysteine (NAC)	Human dental pulp cells	Extracts preparation and MTT	3, 7 days	The addition of NAC remarkably improved biocompatibility of PMMA resin

Author	PMMA modification	Culture cells	Assays	Culture time	Results
Jang et al. [88]	Heat-polymerized acrylic resin (Paladent 20), thermoplastic acrylic resin (Acrytone and Bio Tone)	Human gingival fibroblasts (HGF) primary cell culture	EZ-Cytox Enhanced Cell Viability Assay Kit Cell attachment (FE-SEM)	1, 6, 10 days	The three types of denture base showed low cytotoxicity in cell viability assay Thermoplastic acrylic resin showed the similar cell attachment but more stable attachment than conventional heat-polymerized acrylic resin
Sahin et al. [89]	PMMA enriched with 2-hydroxyethyl methacrylate (HEMA) and isobutyl methacrylate (IBMA) at 2, 3, and 5%	Mouse fibroblast cells (L929)	Agar overlay test	24 h	Only IBMA showed no cytotoxic effect at low concentrations while HEMA showed cytotoxic effect in the injection-molded resins
Yu [90]	PMMA enriched with calcium phosphate cement (CPC) at 3:1, 2:1, 1:1, 1:2, 1:5, 1:10, 1:15, and 1:20	Osteoblastic progenitor cells (MC3T3-E1)	CCK8 detection reagent	24 h	Bone cement extracts had no effect on the relative MC3T3-E1 cell growth rate, and the toxic reaction was level 1 (75–99%)
Retamoso et al. [91]	Self-curing acrylic resin of different colors: clear, pink, blue, and green	Mouse fibroblast cells (L929)	Dye-uptake technique	24, 48, 72, 168 h	Supernatants evaluation of the color of resin proved not to influence material cytotoxicity
Brochu et al. [92]	PMMA cement (Palcos R bone cement) enriched with microencapsulated 2-octyl cyanoacrylate (OCA); extracts solutions	Human osteosarcoma cells (MG63)	Click-iTEdUAlexa Fluor 488 kit for fluorescence	24, 48, 72 h	Cell proliferation and viability were not significantly different from each other, whereas extracts from OCA were moderately toxic to cells
dos Santos et al. [93]	Acrylic resin (OrtoCril) chemical and mechanical polishing and without polishing	Mouse fibroblast cells (L929)	Dye uptake	24, 48, 72, 168 h	With the increase of cell viability, from the 72 h, there was no significant difference among the groups

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Author	PMMA modification	Culture cells	Assays	Culture time	Results
Son et al. [94]	Scaffolds were fabricated by electrospinning using polycaprolactone (PCL) blended with PMMA; extracts solutions	Human osteosarcoma cells (MG63)	MTT Western blot	1, 5, 7 days	PCL/PMMA blends are suitable for osteoblast cell proliferation
Jiang et al. [95]	PMMA particles	Bone marrow stromal cells	MTT CytoTox 96 ELISA RT-PCR.	1, 3, 5, 7 days	PMMA did not stimulate cell proliferation effect even at low doses (0.63mg/ml) and the particles appeared to exhibit certain cytotoxic effect at high concentration (3 mg/ml)
Neves et al. [96]	Ethanol treatment postpolymerization of PMMA, extracts test from heath treatment and conventional	Human adult dermal fibroblast cells	MTT LDH assay	24 h	Specimens showed significant reduction on cytotoxicity compared to immersion in hot water
Tencomnao et al. [97]	PMMA core/polyethyleneimine (PEI) shell magnetic nanoparticles	Human neuroblastoma (LAN-5)	MTT	24 h	The viability of LAN-5 cells after transfection was in the range of 80–100%
Acosta-Torres et al. [98]	PMMA enriched with Silver nanoparticles (AgNPs)	NIH-3T3 mouse embryonic cells	MTT BrdU assay	24, 72 h	Non-cytotoxic material
Tay et al. [99]	Lucitone 550-HR Soft-Liners: Ufi-Gel P-Silicon Dentuflux-AR Trusoft-AR Dentusoft-Tissue conditioner Water storage time after polymerization	Mouse fibroblast cells (L929)	H-thymidine incorporation assay	24 h	Trusoft, lucitone 550 showed slightly cytotoxic effect Dentuflux showed moderate cytotoxic effect when materials were stored in water non-cytotoxic effect Thermal treatment did not reduce the cytotoxicity effect of the acrylic-based soft lines

Author	PMMA modification	Culture cells	Assays	Culture time	Results
Ebrahimi Saravi et al. [100]	AR-Futura Gen AR-GC Reline Hard HR-Meliodent	Mouse fibroblast cells (L929)	MTT ELISA	1 h, 24 h, 1 week	Cytotoxicity of Futura Gen, GC Reline Hard and Meliodent resins failed to show any significant reduction from 24 h to one week. The lower the incubation periods, the higher the cytotoxicity
Regis et al. [101]	MMA MUPB (monomer methacryloyloxyundecylpyridinium bromide)	Mouse fibroblast cells (L929)	MTT	48 h	High concentration of MMA (1g/L) reduces cell viability. MUPB exhibit less cytotoxicity than MMA
Trubiani et al. [102]	Tokuyama Rebase Fast II-AuR IvoclarProbase Cold-AuR Coldpack Tooth Acrylic-AuR polished or unpolished	Human gingival fibroblast (HGF)	MTT ELISA Western blot	24, 48, 72 h	Polishing procedure can reduce the cytotoxicity
Cochis et al. [103]	Paladon 65-HR precoated with biosurfactant	Mouse fibroblast cells (L929), human keratinocytes	MTT	–	Surfactants on resin for prosthetic devices were non-cytotoxic

Table 6. Cytotoxicity test of PMMA alone or modified.

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base resins," and "cytotoxicity AND oral prosthesis." Inclusion criteria were: *in vitro* studies published from 2012 to 2017, free full text, and published in English evaluating the PMMA and its components, considering cytotoxicity activity, type of material tested, kinds of cells used, period of incubation, assay executed, and results of the biocompatibility. Two reviewers read the selected studies, and their information was analyzed and discussed. **Figure 5** shows the flow chart of search strategy and the total number of studies included.

4.2.1.2. Mutagenicity testing

A concern for any material used in medicine is that long-term exposure to a material can lead to neoplastic changes in cells adjacent to it. Most materials are known to be acceptable based on a history of use, but changes in formulations and innovation of new materials are necessary to re-execute testing. Small animal *in vivo* mutagenicity studies allow screening of materials in development and reduce the use of research animals. Mutagenicity studies (also called genotoxicity studies) involve looking for changes in cells and cellular DNA in the forward or reverse directions. In forward mutation studies, normal cells are exposed to the test material and the resultant cells or animal tissues are evaluated for signs of mutation. Just a few studies have been tested for genotoxicity between PMMA and culture or small number of animal evaluations has been conducted. **Table 7** summarizes the investigations performed between mutagenicity and PMMA. The keywords used for search strategy at MEDLINE/PUBMED were as follows: "Genotoxicity AND acrylic resin," "genotoxicity AND polymethylmethacrylate resin,"

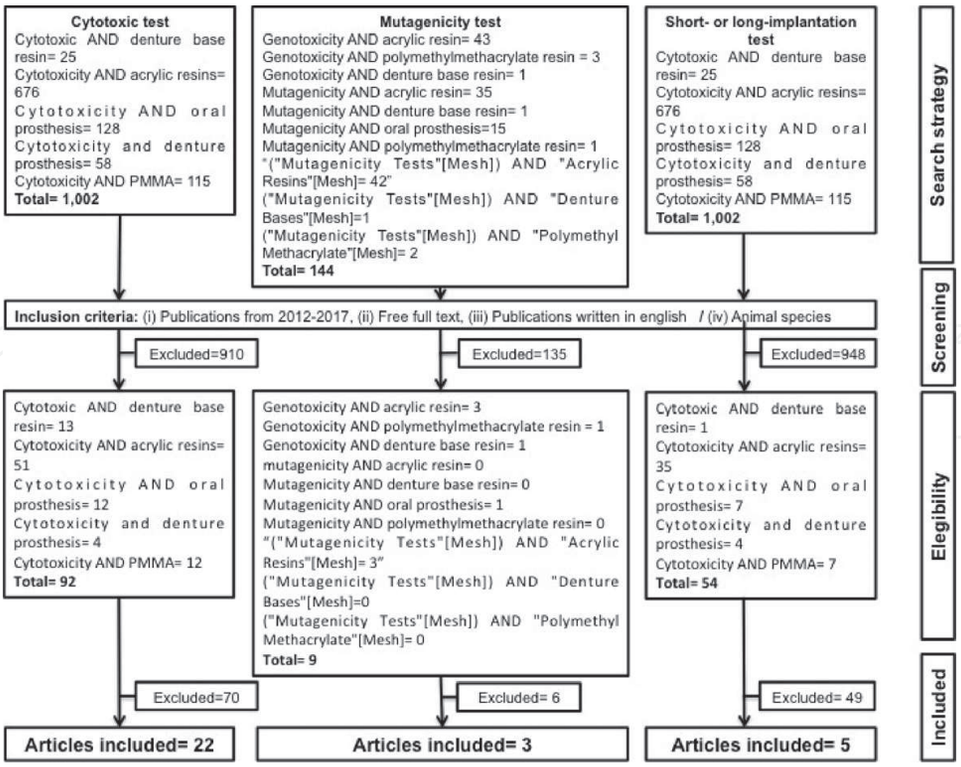

Figure 5. Search strategy flow chart. Source: Direct.

Author	PMMA modification	Culture cells or animal test	Assays	Culture time	Results
Azhar et al. [104]	Methyl methacrylate (MMA) detected in dental lab technicians	Buccal mucosa scrapes (epithelial cells)	Papanicolaou staining Buccal Micronucleus Cytome (BMCyt) assay	Exposure to MMA time of **d**ental lab technicians during their professional career	No significant differences in the incidence of dental lab technicians and control group
Araújo et al. [105]	Methyl methacrylate (MMA) vapor by simulating standard occupational exposure of 8 hours per day	Male Wistar rats	Stained with Giemsa staining (Micronucleus test)	1–5 days	MMA was genotoxic when measured after 1 day of exposure but was not evidently genotoxic after 5 days
Acosta-Torres et al. [98]	PMMA enriched with Silver nanoparticles (AgNPs)	NIH-3T3 mouse embryonic cells	MTT BrdU assay Comet assay	24, 72 h	Non-cytotoxic material

Table 7. Mutagenicity test evaluation of PMMA alone and modified with different materials.

"genotoxicity AND denture base resin," "mutagenicity AND acrylic resin," "mutagenicity AND denture base resin," "mutagenicity AND oral prosthesis," and "mutagenicity AND polymethylmethacrylate resin." The inclusion criteria were: publications from 2012 to 2017, free full text. Only a few studies are reported in literature, a further MeSH term search was executed "("Mutagenicity Tests"[Mesh]) AND "Acrylic Resins"[Mesh], ("Mutagenicity Tests"[Mesh]) AND "Denture Bases"[Mesh], and ("Mutagenicity Tests"[Mesh]) AND "Polymethyl Methacrylate"[Mesh]. **Figure 1** shows the flow chart of search strategy and the total number of studies included.

4.2.1.3. Short- or long-term injection or implantation studies

Several different tests may be conducted to provide information on the effects of relatively short-term exposure to materials or their extracts. These tests include systemic injection, intracutaneous injection for irritation, and short- and long-term implant studies ranging from 24 hours to as long as 90 days or years [80]. Certain materials have the potential to cause local inflammation of tissues. A special case of long-term implantation studies involves the lifetime bioassay performed for investigation of carcinogenicity. These studies are usually performed in several hundred rats and mice to look for differences in tumor formation as a result of exposure to the test material [79]. These studies allow screening out of candidate materials that may not be suitable for further testing. **Table 8** shows the results of search strategy at MEDLINE/PUBMED. The search strategy was previously described in cells tested with the incisive criteria of animal test. **Figure 5** shows the flow chart of search strategy and the total number of studies included.

Author	Type of material	Animal model	Implantation time	Analysis	Results
Carlsson et al. [86]	PMMA-based bone cement-Osteopal V modified with castor oil and linoleic acid	Male Sprague-Dawley rats	1, 4, 12 weeks	Flow cytometry Histological analysis	No differences could be found in the *in vivo* response to these PMMA-based cements
Tsuji et al. [106]	Coating with titanium dioxide (TiO_2) nanoparticles	Hamster oral mucosa irritation test, a guinea pig skin sensitization test and a rabbit intracutaneous test	Irritation test: 24 h Skin sensitization: 2 days Intracutaneous test: 24, 48, and 72 h	Histological analysis	The PMMA coated with TiO2 NPs does not cause irritation or sensitization of the oral mucosa, skin, or intracutaneous tissue and is therefore good
Liu et al. [84]	PMMA-PEI nanoparticles	C57/BL6 mice	6 hours	Western blot NF-κB P65 protein levels in liver tissue	PMMA-PEI NPs could induce targeted transfection (34.7%)
Yu [90]	PMMA enriched with calcium phosphate cement (CPC) at 3:1, 2:1, 1:1, 1:2, 1:5, 1:10, 1:15, and 1:20	SD rats bone defect	4 weeks, 15 weeks	X-ray Histological observation	Except for the PMMA group significant degradations appeared in both the CPC/PMMA group (50%; 1:1) and CPC group. Enhanced the bone cell growth
Son et al. [94]	Scaffolds were fabricated by electrospinning using polycaprolactone (PCL) blended with poly(methylmethacrylate) (PMMA)	Sprague Dawley rats; skull defects and PCL/PMMA implantation	1 and 2 months	Micro CT Histological observation	Bone formation was observed on the 7/3 PCL/PMMA scaffold within 2 months

Table 8. Short- or long-term exposure test of PMMA alone or modified.

4.2.2. Acrylic resin cytotoxicity

Different methods are used for cytotoxicity, mutagenicity, and short- or long-term implantation analysis in the literature. Among them, the most common is the MTT test [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] and a histological evaluation. In the case of MTT, the method quantifies the mitochondrial succinate dehydrogenase enzyme activity and measures the conversion of water-soluble tetrazolium salt in insoluble blue formazan by spectrophotometry. This test is an excellent marker of cell survival because it evaluates cellular respiratory activity [100, 107].

The cytotoxicity of PMMA is correlated with the polymerization methods, temperature, the cycle of polymerization, and acrylic resin storage time can influence the monomer quantity and the material cytotoxicity [95, 100]. Based on the polymerization method, acrylic resin can be classified as heat-polymerized, microwave-polymerized, light-polymerized, and autopolymerized MMA. The latter being the most commonly used in dental practice [15]. The autopolymerized resin exhibited higher cytotoxicity level than heat-polymerized resin after 1 and 24 h of incubation [100]. The experiment performed of PMMA alone in contact with HGF showed similar results of dose-dependent cytotoxicity. It is important to use polished acrylic resins for clinical applications. The unpolished acrylic resin showed cell growth reduction, and an increase in pro-inflammatory cytokines were caused by the tested material [93, 102].

Postpolymerization heat treatments, such as water bath or microwave irradiation, have been suggested in order to reduce the quantity of autopolymerized acrylic resin residual monomers. The PMMA that was immersed into water showed a reduction in MMA monomer elucidation [88, 96].

Several substances such as chitosan [83], PEI (polyethyleneimine) nanoparticles [84], 15% of N-acetyl cysteine (NAC) [87], calcium phosphate cement (CPC) [90], acrylic resin of different colors [91], (bone cement) enriched with microencapsulated 2-octyl cyanoacrylate (OCA), extracts solutions [92], scaffolds fabricated by electrospinning using polycaprolactone (PCL) [94], core/polyethyleneimine (PEI) shell magnetic nanoparticles [97], silver nanoparticles (AgNPs) [98], and Paladon 65-HR precoated with biosurfactant were also evaluated for cytotoxicity. The authors observed reduction in cytotoxicity and increase in biocompatibility from non-cytotoxic (cell viability higher than 75%) to slightly cytotoxic (cell viability ranging from 50 to 75%)

On the other hand, DABCO (DC16, DC16F, DC18, C6DC16) and conjugated monomers (DC11MAF and C2DC11MAF) at 1, 2, or 3% [82], base bone cement-Osteopal V modified with castor oil and linoleic acid [86], 2-hydroxyethyl methacrylate (HEMA) and isobutyl methacrylate (IBMA) at 2, 3, and 5% [89], and MUPB (monomer methacryloyloxyundecylpyridinium bromide) [101] showed cytotoxic effect from moderately cytotoxic (cell viability ranging from 25 to 50%) to severely cytotoxic (cell viability lower than 25%).

Only three studies were reported about the genotoxicity test where the exposure of occupation time did not show difference from patients without continuous exposure or cell culture [98, 105]. It is necessary to include genotoxicity assay for further investigations of potential biomaterials in dental practice.

Several studies have been carried out at short- or long- term implantation with PMMA enriched or coated with different materials as base bone cement-osteopal V modified with castor oil and linoleic acid in rats [86], coating with titanium dioxide (TiO_2) nanoparticles for hamster oral mucosa irritation and guinea pig skin sensitization and intracutaneous rabbit implantation [106], enriched with CPC in rats [90], scaffolds of electrospinning using polycaprolactone (PCL) implantation in rats [94], no toxic effect or histological findings were observed, even a regeneration was perceived. By contrast, the use of PMMA-PEI nanoparticles injected in mice induces significant toxicity by the detection of protein levels in liver tissue.

Acrylic resin cytotoxicity is associated with the presence of residual monomer in the polymerization process. The monomers change cell morphology and function that can reduce their viability. Since acrylic resins are widely used in dental practice, an acceptable biocompatibility is desirable. Considering that the majority of studies reported acrylic resin toxicity responses, further studies with different assessment methods are necessary for the development of biocompatible materials.

In summary, there exist different methods to evaluate acrylic resin cytotoxicity, genotoxicity, and short- or long-term implantation with the MTT method and histological evaluation being the most common tests. In conclusion, there is no non-cytotoxic acrylic resin evidently available in the dental market. Regarding the methods of polymerization, the autopolymerized resin is more cytotoxic and toxic than heat-polymerized resin. The cytotoxic and toxicity effect is dose dependent and is directly correlated with the residual amount of monomer leachable and induce the inflammatory reactions of tissues in contact with the acrylic resin. It is suggested that a water or ethanol bath after polymerization of acrylic resin could decrease the cytotoxic and toxicity activity against oral cells and tissue.

5. Remarks and perspectives

The particle size differences could influence the roughness surface of PMMA without decreasing their mechanical properties. Furthermore, studies should continue to determine that issue in detail.

Ultrasound can be used to process the acrylic resin (Opticryl®) as an alternative technique for PMMA processing with similar results to those obtained using water bath or microwave processing (control groups).

The addition of antifungal agents on PMMA surface or into PMMA, such as moieties, metallic or metallic oxide nanoparticles, and antibiotics, could be useful for the inhibition of specific pathogens such as *Candida albicans* for prosthodontic denture.

Residual monomer leach induces cytotoxic and inflammatory reactions of oral cells and tissues in contact with the acrylic resin. It is suggested that water or ethanol bath after polymerization of acrylic resin could decrease the cytotoxic and toxicity activity against oral cells and tissues. But ultrasonic waves are a good option for both, for thermopolymerization of PMMA and at the same time to reduce the residual monomer.

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Author details

Ma. Concepción Arenas-Arrocena*, Liliana Argueta-Figueroa, René García-Contreras, Omar Martínez-Arenas, Berenice Camacho-Flores, María del Pilar Rodriguez-Torres, Javierde la Fuente-Hernández and Laura S. Acosta-Torres

*Address all correspondence to: carenas@enes.unam.mx

National School of Higher Education Unit Leon, National Autonomous University of Mexico, Guanajuato, Mexico

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