

Fiber-Reinforced Nanopigmented Poly(methyl methacrylate) as Improved Denture Base

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ABSTRACT: Nanopigmented and fiber-reinforced poly (methyl methacrylate) (PMMA) were synthesized for denture bases, by incorporating E-glass fibers, flock fibers, or polyethylene fibers into the PMMA powder formulation to improve the flexural behavior and porosity; decreasing the *Candida albicans* adherence and being noncytotoxic. The commercial acrylic resin, Lucitone 199 was used as a control group. Scanning electron microscopy analysis was performed to the PMMA particles and the reinforcing fibers. Flexural strength increased by adding E-glass fibers in the PMMA powder as compared to flock and polyethylene fibers. The reinforced PMMA with flock fibers showed the lower porosity even smaller than Lucitone 199. The syn-

thesized PMMA and the fiber reinforced nanopigmented PMMA groups reduced significantly the *C. albicans* adherence when compared to the commercial acrylic resin. All the tested groups were found to be nontoxic materials after being in contact with mouse fibroblast culture during 24 h, showing that these novel nanostructured composites are suitable for producing adequate and nontoxic reinforced materials with antimicrobial properties for dentistry applications. © 2012 Wiley Periodicals, Inc. *J Appl Polym Sci* 126: 289–296, 2012

Key words: denture bases; acrylic resin; reinforcing fibers; *Candida albicans* adherence; cytotoxicity

INTRODUCTION

Fractures in poly(methyl methacrylate) (PMMA) dentures occur mainly after 3 years in service,¹ since several factors can induce the denture bases failure, such as occlusal disharmonies, overload, repeated chewing stress, handling, and mechanical impacts caused by accidents and so on.² In spite of the current developments in polymer technology, PMMA is still used in 95 % of thermopolymerized acrylic resins for denture bases, because of its color, durability, solubility, and biocompatibility properties.^{3,4} Thence, it is important to enhance the flexural behavior of denture base resins,^{5,6} for instance, incorporating reinforcing fibers during the processing of dentures.⁷ Previous *in vitro* studies claim that the reinforcing fibers used in denture bases allow an increase in the mechanical properties, as well as avoiding crack propagation.^{8,9} Dentures must be able to withstand high impact forces in addition to normal chewing forces,¹⁰ thus some commercial acrylic

resins for denture bases named their products as “high impact” or “impact resistance” like Lucitone 199 (Dentsply).¹¹

Polyethylene fibers are chemically inert with good and high impact resistance, because of its enormous global production (~ 60 million tons annually) it is a very cost-effective product, which has gained popularity and makes a perfect choice for the PMMA reinforcement. Moreover, glass fibers have been the fibers of choice for reinforcing denture base polymers because of the well-documented improvements in flexural properties and fatigue resistance,¹² but it is necessary to carry out a silanization process to modify the glass fibers surface, generating a chemically active surface through silane functional groups and creating covalent bonds with the PMMA.^{13,14} This modification of the glass fiber surface has an extra important advantage, because water sorption decreases significantly, as compared to glass fibers without silanizing.¹⁵ The flock fibers or rayon fibers are obtained from cellulose-based disulfide and, due to its different colors availability, they are used in the Maxillofacial Prosthetic Clinic in the Dentistry Faculty at the Universidad Nacional Autónoma de México for the soft tissue implants and in ocular prostheses.¹⁶ Polyethylene fibers and flock fibers are not treated or silanized since their polymeric nature allows the PMMA binding.

Denture stomatitis is a common form of oral Candidiasis presented among complete or partial

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TABLE I
Nanopigments, Reagents, Reinforcing Fibers and a Commercial Acrylic Resins used in the Study

Product	Manufacturer	Batch number
Titanium Oxide (TiO ₂)	González Cano and Company S.A., C.V. (México)	R-F9400
Iron Oxide (Fe ₂ O ₃)	González Cano and Company S.A., C.V. (México)	R-4511
Benzofenone-2	Sigma (St. Louis, MO)	34156
Methyl methacrylate	Sigma (St. Louis, MO)	MKBC5616
Benzoyl peroxide	Sigma (St. Louis, MO)	01720DH
Trimethoxysilil prophyilmethacrylate	Sigma (St. Louis, MO)	115K0058
Gelatine	Knox, Unilever (México)	0114ABAP
Acetic acid	J.T. Baker (USA).	
E-glass fibers	VVG, Fibers and Resins (México)	
Polyethylene fibers	Plastics, Sonora (México)	
Flock fibers	Navi Packs, S.A. (México).	
Lucitone 199	Dentsply (York, PA)	20122

denture wearers, and it is defined as an inflammation of the palatal mucosa in contact with the denture.¹⁷ *Candida albicans* is an opportunistic pathogen in human oral cavity and some predisposing factors such as systemic diseases, immunosuppressive drugs, xerostomia, or poorly fitting and porous dentures result in fungal infections.¹⁰

It is difficult to avoid pathogenic microorganism's adherence to the surface of the dental materials, even though the important efforts made.¹⁸ An outstanding feature of *C. albicans* is its ability to form biofilms and adhere to the surface of biotic and abiotic materials.¹⁰ The condition begins with *Candida* biofilm growth onto the denture–mucosa interface. Biofilm growth progresses over the denture surface, leading to inflammation of the denture-exposed palatal mucosa.¹⁹ It has been reported the antimicrobial effect of titanium and iron oxide nanoparticles in other materials, but not the effect on denture bases acrylic resins, so that, in this work a mixture of TiO₂ and Fe₂O₃ particles were used as pigments to obtain the PMMA pink-like gum color.

Accordingly, the objective of the present study was to synthesize *in situ* PMMA with TiO₂ and Fe₂O₃ nanoparticles as pigments and to incorporate different reinforced fibers, evaluating their physicochemical behavior, *C. albicans* adherence, and cytotoxic effect. The fundamental hypothesis was that using reinforcing fibers in the PMMA, the physical–mechanical properties could increase, presenting lower porosity and *C. albicans* adherence besides being a nontoxic polymer.

MATERIALS AND METHODS

Materials used are summarized in Table I.

PMMA synthesis

PMMA was synthesized by the suspension polymerization technique using methyl methacrylate as monomer

(200 g), benzoyl peroxide as initiator (1 % w/w), and gelatin (2.5 % w/w) as suspending agent. The reaction was carried out in a five neck flask using reflux and nitrogen atmosphere for 2 h. In the last 30 min of the reaction, 0.01 % w/w of Fe₂O₃ (70–299 nm) and 0.01 % w/w of TiO₂ (50–225 nm) nanoparticles were suspended in water and incorporated into the reaction system to obtain a pink polymer.²⁰ The resulting particles were carefully washed and dried at 60 °C for 24 h.

Control group

The commercial acrylic resin Lucitone 199 was used as control group.

Five grams of Lucitone 199 were used to separate the containing fibers using a mesh number 400.

The acrylic resin Lucitone 199 contains 1 % of fibers and microscopic characterization of the fibers was performed.

Reinforcing fibers

Three types of fibers were incorporated in the synthesized PMMA powder formulation: E-glass fibers, polyethylene fibers, or flock fibers (3, 3, and 0.5 mm in length). The E-glass fibers were silanized prior to use.

Glass fibers silanization

The E-glass fibers were cut at 3 mm in length and wetted in 1 % aqueous solution of methacryloxypropyltrimethoxysilane for 24 h. After that period of time, the E-glass fibers were dried at 60 °C for 24 h.

Spectroscopy

To confirm the silanization procedure, the E-glass fibers were analyzed by Fourier transformed infrared (FTIR) spectroscopy, conducted in a Bruker Vector 33 Instrument by the transmittance technique. The FTIR spectra were obtained at room temperature, in the region between 400 and 4000 cm⁻¹.

Scanning electron microscopy

Scanning electron microscopy (SEM) observations of polymer particles (PMMA and Lucitone 199), E-glass, polyethylene, flock, and Lucitone 199 fibers were carried out with a JSM-6060LV scanning electron microscope (JEOL, Peabody, MA). The samples were vacuum-coated with gold.

Viscosimetry

Dilute PMMA and Lucitone 199 (without fibers) solutions were made in toluene. The viscosities were measured using an Ubbelohde1C capillary viscometer. The test was performed at 25 °C and the viscosity average molecular weight (M_v) was calculated using the Mark-Houwink-Sakurada equation.²⁰

Sample preparation

Samples were prepared by mixing PMMA powder with 1 % (w/w) of each kind of fibers, then the mixture was incorporated to MMA (3 : 1; powder : liquid ratio), with 1 % of benzoyl peroxide as initiator and packed into metallic flasks at the dough stage. The thermopolymerization was conducted by immersing the flasks in water at 70 ± 1 °C for 90 min followed by a second immersion in boiling water for 30 min. Lucitone 199 samples were prepared according to the manufacturer's directions using the acrylic resins as received. Four experimental groups were obtained: (1) PMMA + E-glass fibers (PMMA + GF); (2) PMMA + flock fibers (PMMA + FF); (3) PMMA + polyethylene fibers (PMMA + PF); and (4) Lucitone 199.

Flexural behavior

Ten samples ($65 \text{ mm} \times 10 \text{ mm} \times 2.5 \text{ mm}$) of each acrylic resin group were prepared according to the ISO-1567.²¹ The samples were loaded to failure in a Universal Testing Machine (INSTRON®, Norwood, MA, USA) and the three-point bending test was performed at a cross head of 0.5 mm/min. The flexural modulus (E) and flexural strength (S) were calculated through the formulas $S = 3PL/2bh^2$ and $E = FL^3/4\delta bh^3$, where P is the load at break, b and h are the width and thickness of the samples, L is the length between supports (10 mm), δ is the maximum deflection of the center of the beam, and F is the slope of the tangent to the initial straight-line portion of the load-deflection curve.

Fracture surface

After the flexural test, the fractured segments were covered with gold and axially observed by SEM to

evaluate the fracture surface to assess surface quality and porosity.

Porosity

Ten samples ($30 \text{ mm} \times 10 \text{ mm} \times 2.5 \text{ mm}$) per group were initially weighted and placed into silica gel desiccators. Every 24 h, sample's weight was recorded until constant mass (± 0.0005 g). Internal porosity (V_{ip}) of each sample was calculated with the equation $W_a = (d_r - d_a)(V_{sp} - V_{ip})$, where W_a is the sample's weight (g), d_r is the acrylic resin density (1.198 g/cm^3), d_a (0.00123 g/cm^3) is the local air density at 21 °C and 585 mmHg, V_{sp} is volume of samples and V_{ip} the volume of internal porosity (cm^3).

Candida albicans adherence

C. albicans ATCC 90026 (American Type Culture Collection, Manassas, VA) was cultured overnight in yeast broth (Sigma-Aldrich, St. Louis, MO, USA). Three sterilized resin discs ($10 \text{ mm} \times 2 \text{ mm}$) in each acrylic group ($n = 12$) were placed into 24-well sterile culture plates (Nunc) with 500 μL of 1×10^6 yeast suspension. After a 24-h incubation period at 37 °C, nonadherent cells were removed from samples by washing for 10 min under sonication. Attached fungi were extracted by adding 1.0 mL of benzalconium chloride for 15 min, and then the luminescent ATP measurement (Bac Titer-Glo, Promega, Madison, WI, USA) was performed to determine the number of viable cells adhered to acrylic resins. The luminescence values were recorded in a 20/20 luminometer (Turner Biosystems, Sunnyvale, CA, USA) at wavelength of 590 nm emission. Relative luminescence intensity, in 10-s integration periods, was measured in triplicate.

Cytotoxic assay

Three acrylic samples ($10 \text{ mm} \times 2 \text{ mm}$) in each group ($n = 12$) were sterilized with ultraviolet irradiation for 5 min. NIH-3T3 mouse embryonic fibroblast-like cells were exposed to samples and proliferation was assessed by measuring reductase enzymatic activity by transformation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into a colored reduced form.²² The cell line was grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Invitrogen, Carlsbad, CA) supplemented with 10 % bovine fetal serum (Gibco) and 100 U/mL penicillin-streptomycin in 95 % humid, 5 % CO_2 atmosphere at 37 °C. For cytotoxicity experiments, culture medium was prepared following the ISO10993-5 specifications,²³ then 1×10^4 cells were sown in 24-well sterile plates (Nunc-Thermo Fisher Scientific, Roskilde, Denmark) and incubated in these extraction media for

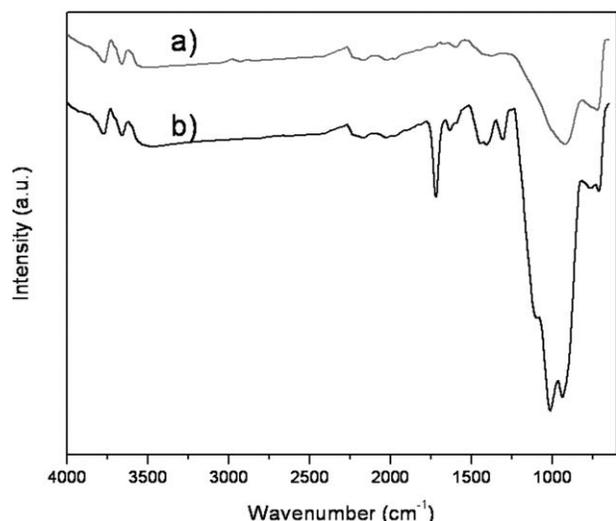


Figure 1 FTIR spectra of (a) nonsilanized and (b) silanized E-glass fibers.

24 h. The samples were placed in contact with the cells and incubated for 24 h at 37 °C. After that, the acrylic resins were removed and the cell viability was measured by the MTT assay following the manufacturer instructions (Sigma). The absorbance was measured in a microplate reader (Bio-Rad 680) at a wavelength of 655 nm. Cell cultures with no resins were used as controls. Each experiment was run in triplicate.

Statistical analysis

One-way ANOVA ($p < 0.05$) and Tukey's *post hoc* test were applied to the flexural modulus, flexural strength, porosity, *C. albicans* adherence, and cytotoxicity results.

RESULTS

Spectroscopy

FTIR E-glass fibers and silanized E-glass fiber spectra showed the characteristic stretching bands of the Si—OH and Si—O—Si bonding in 900 and 1100 cm^{-1} , but the silanized glass fibers presented the ester carbonyl C=O stretching vibrations at 1200 cm^{-1} , proving the silanization interaction (Fig. 1).

Morphology of fibers and polymers particles

Figure 2(a,b) shows that E-glass and flock fibers are similar in width and shape; they are single fibers around 12 and 15 μm in diameter, while the polyethylene and Lucitone 199 fibers [Fig. 2(c,d)] are formed by many joined thin fibers (3–5 μm) forming a bundle of 25–30 μm in diameter. Figure 2(e,f) presents spherical particles for the synthesized nanopig-

mented PMMA and Lucitone 199, with particles size between 5–120 μm and 12–150 μm , respectively.

Molecular weight

The synthesized PMMA and Lucitone 199 powders were subjected to viscometry testing using toluene as solvent. The obtained values of the molecular weight distribution were 36 and 22×10^5 g/mol for the pink PMMA and Lucitone 199, respectively.

Flexural behavior and porosity

In the flexural modulus there was no significant statistical difference ($p < 0.05$) between all groups, but in the flexural strength PMMA with E-glass fibers and Lucitone 199 showed the higher value ($p < 0.05$) compared to the PMMA with polyethylene and flock fibers. The porosity values showed that incorporating flock fibers reduced the porosity percentage (Table II), although all the synthesized polymers with different fibers showed lower porosity than Lucitone 199 ($p < 0.05$).

The SEM images in Figure 3 correspond to the fracture surfaces of the polymers after the flexural test. The PMMA with E-glass fibers [Fig. 3(a)] and Lucitone 199 [Fig. 3(d)] showed similar structure without voids and with similar surface. PMMA with flock fibers [Fig. 3(b)] and PMMA with polyethylene fibers [Fig. 3(c)] showed that fibers pulled-out from crack surface and voids corresponding to the pulled-out fibers of the other half of the samples.

Candida albicans adherence and cellular compatibility

Figure 4(a) shows a statistically significant difference ($p < 0.05$) between the experimental PMMA groups with different kinds of fibers and Lucitone 199, the one with more than the 50 % of *C. albicans* adherence. The higher light relative units (LRU's) value indicates the higher *C. albicans* adherence in the acrylic resin surfaces.

An enzyme metabolic assay, reflected viability of cultured cells and showed no toxic effect on the exposed cell population, so all the reinforced PMMA have a biocompatibility behavior similar to that of the commercial acrylic resin [Fig. 4(b)].

DISCUSSION

The synthesized PMMA particles, using gelatin as suspension agent, showed spherical shape and similar particle size as compared to the commercial acrylic resin. Previous studies reported that using gelatin during the PMMA synthesis as suspension agent produces spherical particles and it is easy to remove for washing.²⁴

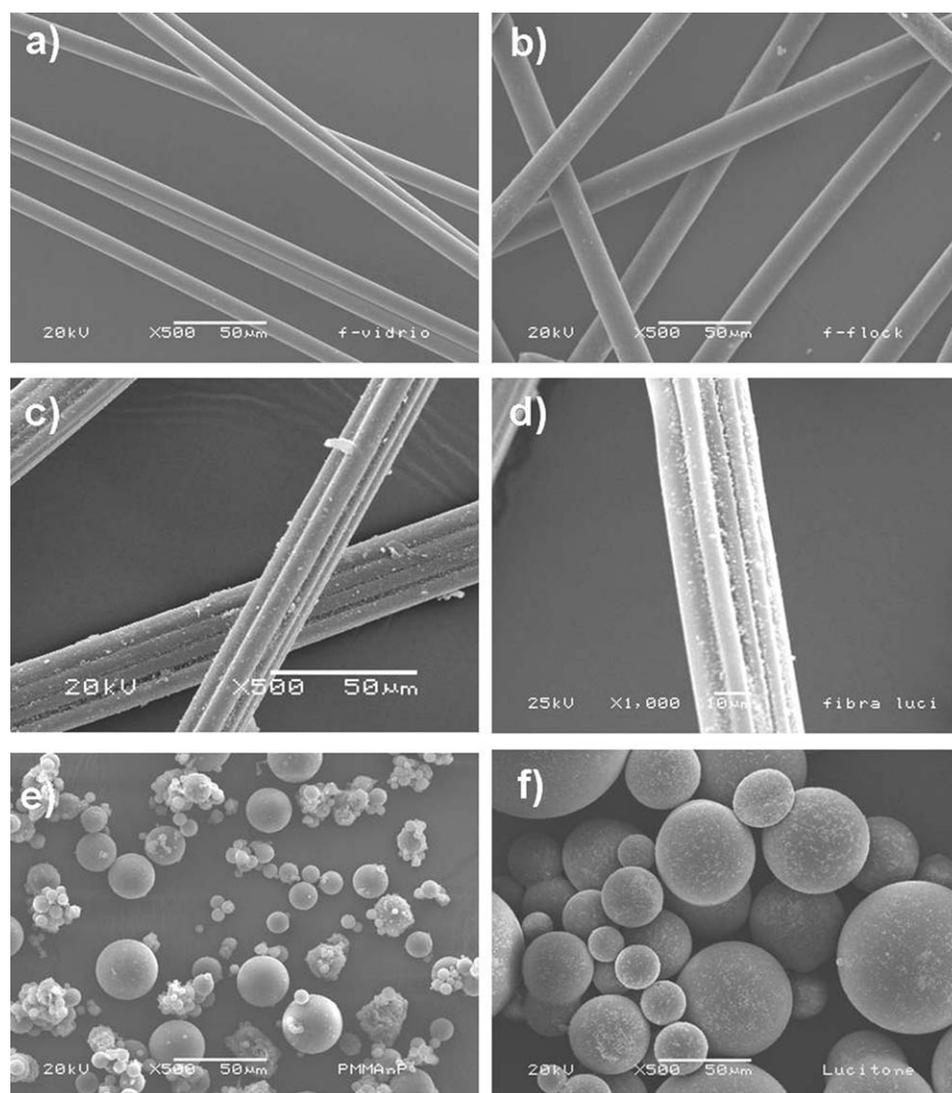


Figure 2 Scanning electron micrographs of (a) E-glass fibers, (b) flock fibers, (c) polyethylene fibers, and (d) Lucitone 199 fibers. Particle polymer images for (e) synthesized PMMA and the commercial acrylic resin (f) Lucitone 199.

It has been reported the synthesis of PMMA with metal-oxide nanopigments to yield a pink gum-like acrylic resin with high-molecular weight, lower porosity, and *C. albicans* adherence, but with no improved flexural behavior.²⁰ As aforementioned, in this study, the same pink PMMA was synthesized and fiber reinforced in order to improve the mechanical properties, considering that the fracture

of the prosthesis is common, which means a time consuming and costly problem for patients.¹ The reinforcing fibers and the nanopigments did not interact because TiO_2 and Fe_2O_3 nanopigments were *in situ* integrated to the PMMA particles during the suspension polymerization process; and the reinforcing fibers were incorporated to the pink PMMA by manual mixing before the sample preparation.

Glass fibers have good aesthetic characteristics and can chemically bond to denture base resins through a silane treatment,⁵ and flexural properties and wear resistance are improved.²⁵ In the present study, the results of FTIR spectroscopy confirmed the chemical silane integration to the structure of the E-glass fibers assuring the better integration of the fibers into the PMMA composition.

According to other researches, the best reinforcing agents are considered to be nonwoven bundle filaments of 10–20 μm diameter of high-density

TABLE II

Mean Values of the Flexural Behavior and Porosity Test

Group	Elastic modulus (GPa)	Flexural strength (MPa)	Porosity (%)
PMMA + GF	2.6 (0.3)	79.2 (0.4)	4.2 (0.7)
PMMA + FF	2.5 (0.3)	76.4 (0.3)	3.9 (0.5)
PMMA + PF	2.4 (0.2)	76.4 (0.3)	4.6 (0.8)
LUCITONE 199	2.5 (0.2)	78.2 (0.2)	6.8 (1.0)

Standard deviation in parenthesis (S.D.).

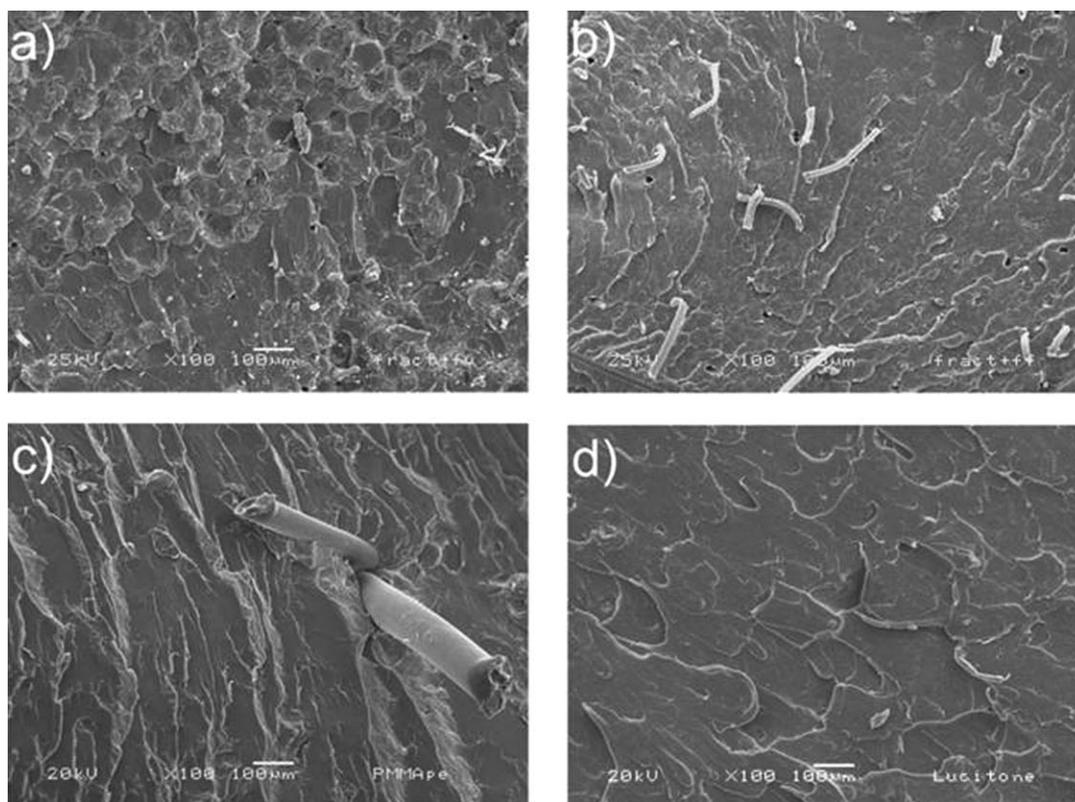


Figure 3 Fracture surface observations after the flexural behavior tests. (a) PMMA with E-glass fibers, (b) PMMA with flock fibers, (c) PMMA with polyethylene fibers, and (d) Lucitone 199.

polyethylene or polypropylene fibers combined with custom-made composite resin pontics.²⁶ In this study, the SEM observations showed that polyethylene and Lucitone 199 fibers are a bundle of fibers, unlike the flock and E-glass fibers which consisted of a monofilament.

It is very difficult to place accurately the reinforcement fibers at the desired position in the denture base resin.²⁷ Continuous unidirectional fiber-reinforced composites are anisotropic and provide superior reinforcement; despite they are difficult to

place in the correct position in the denture.^{25,28} It has been reported the use of chopped fibers with distribution in all directions (isotropic) providing comparable properties than the polymers with anisotropic fiber distribution,²⁵ so, in the present work fibers were mixed with the PMMA powder to obtain a random-oriented fibers distribution in the matrix of the processed polymer. The properties of the final products are related to the kind of fibers, the length of the fibers, and the concentration of fibers. It was found that reinforcing a commercial acrylic resin

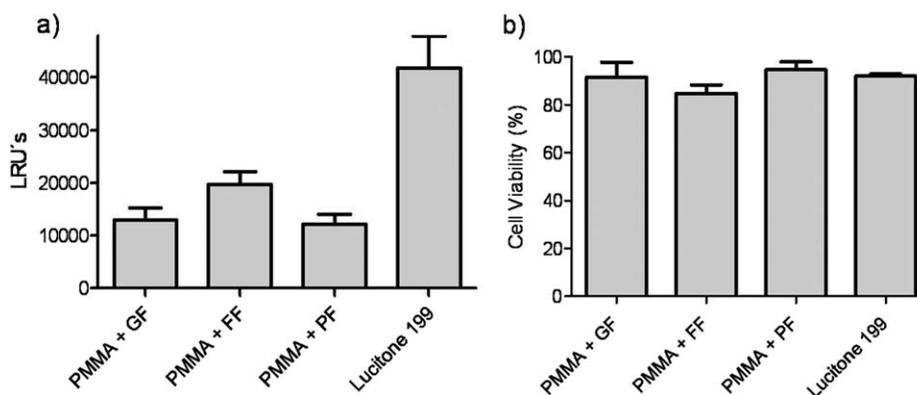


Figure 4 (a) Luminescence assay results of *C. albicans* adherence onto PMMA reinforced with E-glass, flock or polyethylene fibers, and Lucitone 199. (b) Biocompatibility of PMMA fiber reinforced assessed through the MTT metabolic assay in 3T3 fibroblast-like cell line.

adding glass fibers (1 %; 4 mm) improved the flexural modulus and the transverse strength.²⁵ In the present research, the PMMA powder was reinforced with E-glass fibers (3 mm), polyethylene (3 mm), and flock fibers (0.5 mm) using 1 % of fibers in all groups. The comparison was carried out with Lucitone 199, a commercial high impact acrylic resin with high impact containing organic pink fibers. The flexural modulus was similar in all groups, but the flexural strength was significantly higher when the E-glass fibers were used, it can be justified because E-glass fibers further a previous silanization process that achieved a chemical interaction with the PMMA molecule resulting in higher resistance.²⁸ Therefore, denture base resins reinforced with polyethylene fibers lack adequate strength and there were no publications about denture base reinforcements with flock fibers.

In this study, the results of Lucitone 199 are according to previous publications in flexural strength value (78.2 ± 2 MPa) and in the appearance of the fracture surface of showing a layered pattern.²⁹ Further studies are to be conducted using different percentage of reinforcing fibers with E-glass, polyethylene, and flock fibers to verify whether the flexural strength increases.

The porosity was lower when experimental (E-glass, polyethylene, or flock) fibers were used in the PMMA formulation compared with Lucitone 199 resulting in a good quality for the synthesized PMMA, because significant porosity can severely weaken the acrylic resin prosthesis making it ideal for the accumulation of species such as *C. albicans*.³⁰

The pink PMMA showed higher-molecular weight than Lucitone 199 which is probably attributable to the interaction of metal oxides with organic compounds during the synthesis of polymers. The results of the present study indicate that the higher the molecular weight of the acrylic resin, the lower the porosity. The porosity and the *C. albicans* adherence of the PMMA were not affected by adding different reinforcing fibers and all experimental PMMA groups showed lower values compared with the commercial acrylic resin.

The first interaction leading to plaque formation is the microbial adherence to surface prosthetic materials; herein in this work *C. albicans* was cultured under aerobic conditions to obtain a cell suspension and incubated with the acrylic samples assessing the attachment of *C. albicans* to the fiber reinforced PMMA. The inability of current antifungal therapy to cure denture stomatitis emphasizes the importance of treatment methods directed toward reducing initial fungal attachment to oral surfaces, including mucosa and denture surfaces.³¹ Some researchers have tried to incorporate antibacterial components into dental materials, hoping to reduce the microorganism adherence, but it could not produce an everlasting effect

and may affect some physical and mechanical performance of the dental materials.¹⁸ The present results showed decreased *C. albicans* adherence in the three experimental tested groups compared with Lucitone 199; this behavior was not different among the three types of fibers, so it can be associated the antimicrobial activity to the incorporated nanopigments during the *in situ* PMMA synthesis. The TiO₂ and Fe₂O₃ nanopigments are metallic oxide particles with photocatalytic activity and with reported antimicrobial effect because of their crystal structure of rutile and anatase (TiO₂) and hematite (Fe₂O₃).³²

One of the biocompatibility criterion is that the material shows nontoxic effect to cells. *In vitro* cytotoxicity tests are required steps in the screening of new materials for use *in vivo*. The MTT test method based on an evaluation of the mitochondrial function after exposure to potential toxic substances was selected for use in the present study. Biological properties of dental materials are important in relation with their clinical use, because in some clinical situations, the fibers may be covered only by a thin layer of polymer or come directly in contact with oral tissue causing hypersensitivity reactions.³³

As shown in Figure 4(b), cells incubated for 24 h with the PMMA groups indicated that the new nanopigmented and reinforced PMMA and Lucitone 199 were devoid of toxicity.

According to the hypothesis of the study, the results demonstrated that nanostructured metal-oxide coloring additives, E-glass fibers, polyethylene fibers, and flock fibers are suitable means to produce adequate physical-mechanically and nontoxic reinforced acrylic resin materials with antimicrobial properties for dentistry applications. Further studies on the reinforced denture bases are encouraged for future prosthodontics developments.

CONCLUSIONS

This work pointed out the potential of fiber reinforcement on the development of an acrylic resin for denture bases. The flexural strength was notably improved using E-glass fibers when compared to the commercial acrylic resin. Reduced porosity and *C. albicans* adherence were showed in all the fiber-reinforced nanopigmented PMMA groups. Nontoxic effect was demonstrated in all the experimental and commercial acrylic resins tested by the cytotoxicity assay.

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