Original Article

Influence of the Surface Energy of Different Brands of Polymethyl Methacrylate on the Adherence of *Candida albicans*: An *In Vitro* Study

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ABSTRACT

Objective: The objective of this study was to evaluate the influence of the surface energy of different brands of polymethyl methacrylate (PMMA) on the adherence of *Candida albicans* ATCC 10231 in an *in vitro* study. **Materials and Methods:** The study had an *in vitro*, longitudinal, and comparative experimental design. The following groups were made: (1) Vitacryl versus controls (water, dimethyl sulfoxide, glycerol, diethylene glycol, and formamide); (2) Triplex versus the same controls; (3) Vitacryl versus Triplex (surface energy); and (4) Vitacryl versus Triplex (adhesion per cell/field). Adhesion was measured in the area of each field magnified 10 × 10, and with an increase in magnification to 40 × 10, very dense colonies of 0.152 mm^2 were observed. **Results:** The surface energy of Vitacryl and Triplex was 40.3 ± 0.3 and 39.5 ± 0.3 N/m, respectively, showing statistically significant differences ($P < 0.001$). On the contrary, in relation to the adhesion per cell/field of *C. albicans*, Vitacryl presented 15.7 ± 1.1, whereas Triplex had 16.7 ± 2.3, with no significant differences ($P = 0.058$). **Conclusion:** In relation to the adhesion per cell/field of *C. albicans*, there was no evidence of significant differences between Vitacryl and Triplex.

Keywords: *Adherence, Candida albicans, polymethyl methacrylate, surface energy*

Introduction

Biomaterials are biological materials used to replace some lost living tissue. A biomaterial is a pharmacologically inert and biocompatible component in a living system.[1-3] These components restore the functions of the tissues of the lost body, and therefore, it is important to understand the relationships between the functions and properties of these biological materials.[3,4]

Along history, various materials have been used in the manufacture of denture bases, including wood, bone, ivory, metals, and numerous polymers such as polymethyl methacrylate (PMMA), polystyrene, polyamide, epoxy resin, polycarbonate, and vulcanite. The selection of the different specific materials is based on availability, cost, physical properties, aesthetic qualities, and handling characteristics. From different materials used, PMMA shows the best properties, and as a result, it currently dominates the field of denture bases.[5-7]

One of the physical properties of denture bases that has been studied is surface energy, which directly influences the amount of microorganisms, such as bacteria and fungi, which the surface of a biomaterial can retain.

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Surface energy can determine the presence or absence of adherence of microorganisms. It is composed of the sum of the intermolecular forces that are on the surface of a body, that is, it corresponds to the degree of attraction that the surface of one material exerts on another. For example, when a body has a high surface energy, it has a high tendency to attract itself, while the adhesive has a low possibility of surface tension.[2-4] In the search for new substances and/or biomaterials, it is also important to control microorganisms such as *Candida albicans* in the oral cavity.[8-11]

Therefore, the objective of this study was to assess the influence of the surface energy of different brands of PMMA on the adherence of *C. albicans* ATCC 10231 in an *in vitro* study.

Materials and Methods

Sample size

The study had an *in vitro*, longitudinal, and comparative experimental design, and was carried out in the Microbiology Laboratory of the Faculty of Dentistry of the Universidad Nacional Federico Villarreal, Lima, Peru. The independent variable was the surface energy of the acrylic resins, and the dependent variable was the adherence of *C. albicans* ATCC 10231. The sample size was obtained by the means comparison formula using Stata 15 software (College Station, TX, USA). Each brand of PMMA had an $n = 18$.

Group allocation

The following four groups were formed:

Group 1: PMMA Vitacryl versus controls (water, glycerol, formamide, diethylene glycol, and dimethyl sulfoxide) [Figure 1]
Group 2: PMMA Triplex versus controls (water, glycerol, formamide, diethylene glycol, and dimethyl sulfoxide)
Group 3: PMMA Vitacryl versus PMMA Triplex (surface energy) [Figure 2A and B]
Group 4: PMMA Vitacryl versus PMMA Triplex (adhesion per cell/field)

Polymethyl methacrylate preparation

PMMA was prepared from a wax master model with dimensions of (15 × 10 × 1 mm). Each type of acrylic was processed according to the manufacturer's specifications. The procedures known as packaging, polymerization, muffling, and demuffling were applied, for which the same type of plaster was used, and the entire procedure was carried out after calibration. The brands of acrylic resins were: Vitacryl (A. Tarrillo Barba, Lima, Peru) and Triplex (Ivoclar Vivadent, Ellwangen, Germany). Eighteen samples were prepared for each type of acrylic.

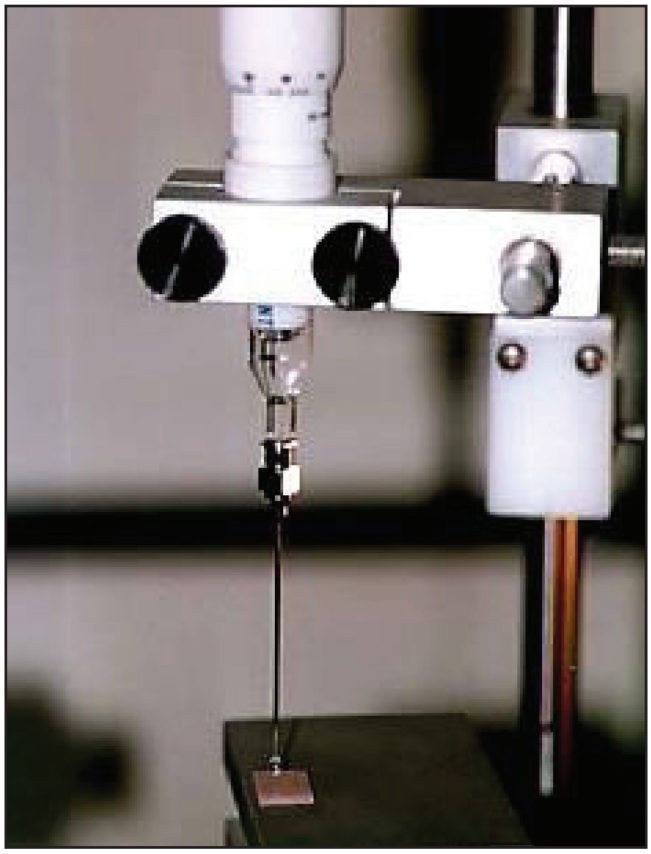


Figure 1: Micropipette installed on the contact goniometer

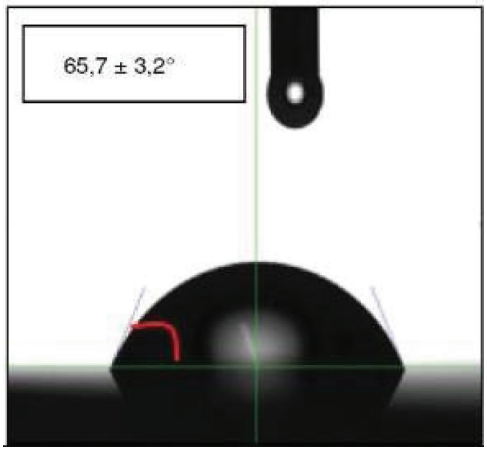
The samples were examined using a magnifying glass (×50), and those with superficial defects or pores were discarded, for both brands of acrylics.[1,2,4]

Suspension preparation

C. albicans strain ATCC 10231 was reactivated in 0.5 mL of Sabouraud dextrose broth and incubated for 24 h at 37°C. On completion of the growth period, it was verified that *C. albicans* was in the yeast phase, cultured on Sabouraud dextrose agar. The separation of the yeasts from the culture broth was carried out by centrifugation at 1200 rpm for 10 min, and then washed with 10 cc of phosphate-buffered saline (PBS). After centrifugation of the tubes with the cultures, the broth was removed and the pellet was left, and 0.5 mL of PBS was added to this. After three washings with PBS, an optical density of 0.5 was achieved according to the McFarland scale standard, which corresponds to 1.5×10^8 cells/mL.[1,7]

Incubation of polymethyl methacrylate samples

The samples of both brands were kept in distilled water for 17 days, renewing the water daily, to achieve saturation. One polymer sample per tube was placed, and 2 mL of yeast suspension was added. After verifying that the sample was completely covered, the samples were incubated for 2 h at 37°C. At the end of the incubation period, the samples were removed from



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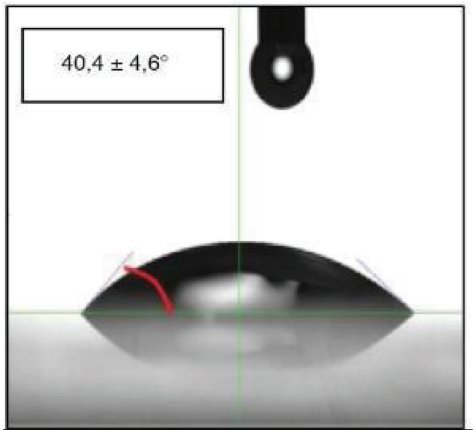

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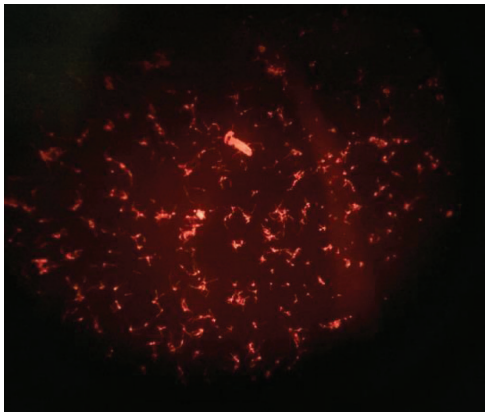
Figure 2: (A) Contact angle between glycerol and a sample of Vitacryl. (B) Contact angle between formamide and a Triplex sample



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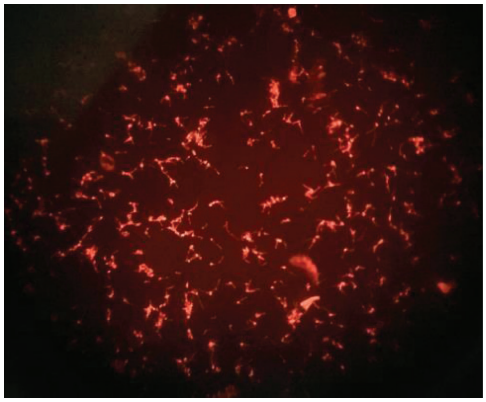

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Figure 3: (A) *Candida albicans* cells in the Vitacryl sample, stained with 0.003% acridine orange, viewed under a fluorescence microscope (×10). (B) *C. albicans* cells in the Triplex sample, stained with 0.003% acridine orange, seen under a fluorescence microscope (×10)

the incubator and rinsed twice with 25 mL of sterile PBS for 1 min by gentle manual shaking. This process allows the elimination of the yeasts not adhered to the polymer samples.[1,4,6]

Evaluation of microbial adhesion

Once the samples were dry, the yeasts were stained with acridine orange at a concentration of 0.003%. The samples were placed in petri dishes adding 15 drops of the acridine orange solution to the surface and staining for 10 min, and then allowed to dry. Nine fields were counted on each sample, the areas selected for the count were evenly distributed on the surface of the sample. The area of each field was magnified 10 × 10, and with an increase in the magnification to 40 × 10, very dense colonies of 0.152 mm^2 were observed[7,8] [Figure 3A and B].

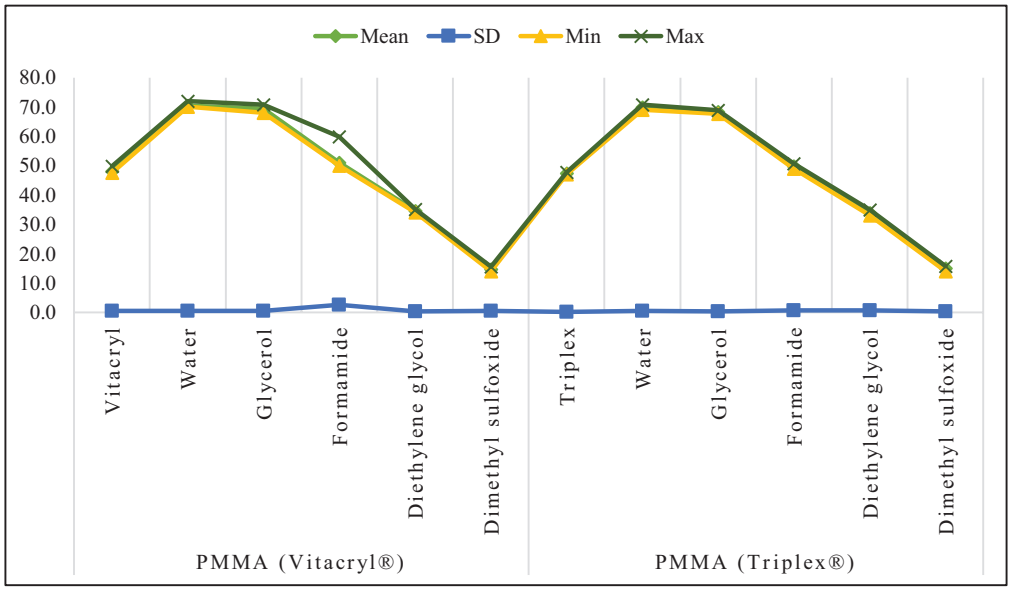
Statistical analysis

Descriptive analysis used measures of central tendency and dispersion (mean, standard deviation, minimum, and maximum). Normality was determined using the Shapiro–Wilk test. Subsequently, the Student's *t* test was performed because of the homogeneity of the data. Finally, to determine correlations among numerical variables, linear regression was used. All the analyses were evaluated with a significance level of $P < 0.05$. All the data were analyzed using Stata 15 software.

Results

The angle between the surface and the Vitacryl was 48.1° ± 0.5° in the group, whereas the diethylene glycol and dimethyl sulfoxide control groups showed the lowest angulation with 34.6° ± 0.3° and 14.6° ± 0.5°, respectively. On the contrary, the distribution in the PMMA (Vitacryl) group was not normal in the chemical substances Vitacryl, water, and formamide ($P < 0.05$) [Graph 1] [Table 1].

In the PMMA (Triplex) group, the angle between the surface and the Triplex was 47.3° ± 0.2°. As in the previous group, the control chemicals, diethylene glycol



Graph 1: Comparison of angle between the surface of polymethyl methacrylate Vitacryl and Triplex versus different chemical experimental substances

Table 1: Angle evaluation between the surface of PMMA Vitacryl and Triplex versus different chemical substances

Groups	Chemical substances	Mean	SD	Min	Max	*P*
PMMA (Vitacryl)	Vitacryl	48.1	0.5	47.6	49.8	0.000
	Water	70.4	0.5	70.0	71.9	0.004
	Glycerol	69.3	0.6	68.0	70.7	0.251
	Formamide	51.0	2.5	50.0	59.9	0.000
	Diethylene glycol	34.6	0.3	34.1	35.1	0.387
	Dimethyl sulfoxide	14.6	0.5	14.0	15.6	0.633
PMMA (Triplex)	Triplex	47.3	0.2	47.0	47.7	0.916
	Water	69.9	0.6	69.0	70.8	0.134
	Glycerol	68.3	0.3	67.7	68.9	0.986
	Formamide	49.8	0.6	49.0	50.7	0.150
	Diethylene glycol	33.9	0.6	33.1	34.9	0.524
	Dimethyl sulfoxide	15.0	0.4	14.1	15.7	0.894

PMMA = polymethyl methacrylate

*Shapiro–Wilk test

Level of significance ($P < 0.05$)

All values are reported in grades

and dimethyl sulfoxide, had the lowest angulation with 33° ± 0.6° and 15.0° ± 0.4°, respectively [Graph 1] [Table 1].

The surface energy of PMMA (Vitacryl) and PMMA (Triplex) was 40.3 ± 0.3 and 39.5 ± 0.3 N/m, respectively, showing statistically significant differences ($P < 0.001$).

In relation to the adhesion per cell/field of *C. albicans*, PMMA (Vitacryl) presented 15.7 ± 1.1, whereas PMMA (Triplex) had 16.7 ± 2.3, with these differences being nonsignificant ($P = 0.058$) [Table 2].

Finally, linear regression analysis showed that in the PMMA group (Vitacryl), for each unit of surface energy in N/m, the adhesion per cell/field of *C. albicans* can be expected to increase by a statistically significant mean of 0.112 ($P < 0.001$) [Graph 2]. In contrast, in the PMMA group (Triplex), the $P = 0.329$ suggested that changes in the predictors are not associated with changes in response [Table 3].

Discussion

Biomaterials are artificial substances (polymers, ceramics, and metals, among others), used in living organisms to modify or replace some tissue, organ, or function. It is desirable to have a biomaterial with surfaces that are not adherent to bacterial or fungal cells, as these colonies are responsible for the pathogenesis of an organism. Knowledge of the characteristics

Table 2: *In vitro* evaluation of the surface energy and adhesion per cell/field of *Candida albicans* according to the polymethyl methacrylate type Vitacryl and Triplex

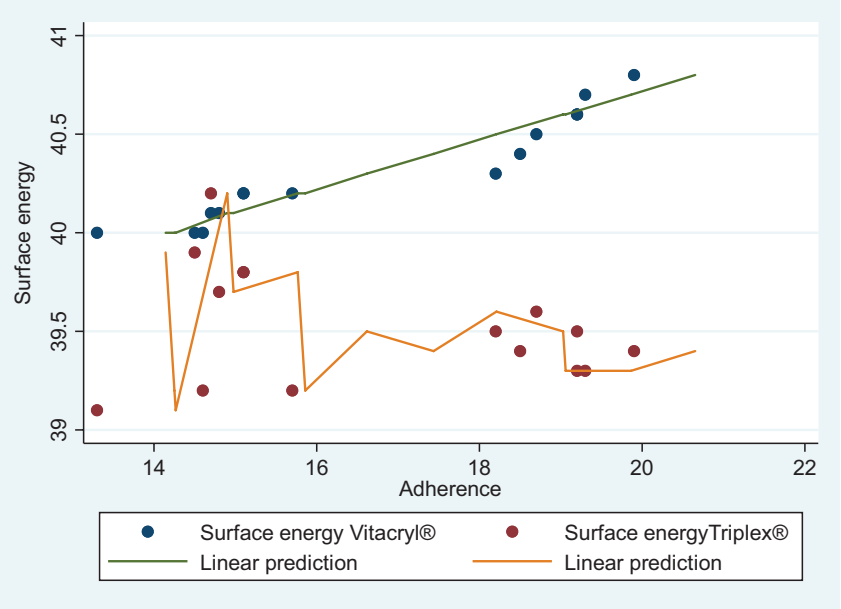
Groups	Variables	Mean	SD	Min	Max	95% CI		*P**	*P***
PMMA (Vitacryl)	Surface energy	40.3	0.3	40.0	40.8	40.1	40.4	0.200	0.001
PMMA (Triplex)	Surface energy	39.5	0.3	39.1	40.2	39.3	39.6	0.696	
PMMA (Triplex)	Adhesion per cell/field	15.7	1.1	14.0	17.2	15.0	16.2	0.050	0.058
PMMA (Vitacryl)	Adhesion per cell/field	16.7	2.3	13.3	19.9	15.4	17.9	0.050	

PMMA = polymethyl methacrylate, CI = confidence interval, SD = standard deviation, Min = minimum, Max = maximum

*Shapiro–Wilk Test, **Student's *t* test

Level of significance ($P < 0.05$)

All values are recorded in N/m



Graph 2: Regression scatter plot and linear prediction of surface energy and adhesion per cell/field of *C. albicans* according to the polymethyl methacrylate type Vitacryl and Triplex

Table 3: Linear regression of surface energy and adhesion per cell/field of *Candida albicans* according to the type of polymethyl methacrylate Vitacryl and Triplex

	Surface energy	Coefficient	Standard error	*P*	95% Confidence interval	
PMMA (Vitacryl)	Adhesion per cell/field	0.112	0.010	0.000	0.090	0.134
PMMA (Triplex)	Adhesion per cell/field	−0.036	0.600	0.329	−0.113	0.040

that influence the adherence of these colonies must be taken into account in the choice and processing of biomaterials. One of these characteristics is the surface energy of microorganisms and biomaterials, as this is directly proportional to the retention of cells present between one and the other. In this sense, it is necessary to know which type of acrylic resin (PMMA) has the least adherence in relation to *C. albicans.*[1-3,12] The essential characteristic that all biomaterials must fulfill is biocompatibility, which is understood as the quality of not inducing toxic or harmful effects to the biological systems in which they operate, and performing an appropriate host response in a specific situation.

The characteristics of the immune system are such that when a foreign body is introduced into the body, it tends to reject or even attack it. Biocompatibility has a compatibility index, which indicates whether or not this material is suitable for use in a living being, taking into account its application.[5-7,12,13]

The association of *C. albicans* with prosthetic stomatitis is important but is not a determining factor for the appearance of disease. Studies determining the microorganisms present in the plaque associated with prosthetic stomatitis have shown that depending on the degree of disease, the presence of *C. albicans* is

more or less frequent. The main causes of prosthetic stomatitis are trauma, infection, and allergy to the components of the prosthesis. However, studies such as that by Budtz-Jörgensen explained that the appearance of the disease was determined by multiple factors, one determining factor being the systemic condition of the individual.[14] The adhesion of *C. albicans* to the surface of PMMA is very complex due to the large number of factors involved. For this reason, some *in vitro* studies carried out to date often report contradictory results. For example, this is the case with respect to the role of saliva, although it is accepted that saliva plays a protective role in relation to prosthetic stomatitis.[15]

In a study on the relationship between the amount of saliva and the *C. albicans* count in individuals with prosthetic stomatitis, Navazesh *et al.*[16] found no significant relationship between these two variables. This study was of note as it evaluated a variable not usually determined in most *in vitro* studies such as the present. This variable is the adhesion between fungi and the prosthesis. Indeed, some studies have described greater adhesion of *C. albicans* in PMMA samples treated with saliva.[17]

The diversity of methods used in the literature makes it difficult to compare results even when using the same system, as there is no established protocol, and each author or work group uses the *C. albicans* strain that seems most suitable for their study. The same occurs with respect to yeast concentrations, incubation times, and temperature, as well as the procedure to eliminate nonadherent yeasts or the staining method. Another problem related to methodology is the choice of the appropriate staining system for the yeast count per field. Using a self-curing type of transparent PMMA, polymer samples thin enough to allow yeast counting by transmission light microscopy and the use of a dye can be obtained.[4,18,19]

In this study, the relationship between the surface energy of PMMA and the adhesion of *C. albicans* from Triplex and Vitacryl was analyzed, showing that for each unit of surface energy in N/m, the adhesion per cell/*C. albicans* field significantly increased by a mean of 0.112 ($P < 0.001$). This high correlation has also been found in some studies, when comparing the surface energy of two different *Candida* (*albicans* and *tropicalis*) with their adherence to thermoset resins.[6-8]

It should be noted that when viewing of the control samples (*C. albicans* in an acridine orange–stained petri dish) was performed under the fluorescence microscope, *C. albicans* was observed in the form of yeast, whereas, on the contrary, in the experimental samples (*C. albicans* in acrylic plate stained with acridine orange), cells in the form of pseudohyphae were observed. None of the previously mentioned studies have reported this finding. *C. albicans* shows great adhesive capacity to not only host cells but also to inert materials such as acrylic resins, largely due to the chemical and structural characteristics of the cell walls, aided by the characteristics provided by the medium.

The main limitation of this study was that as there was no established protocol for the process of sample preparation, incubation, staining, and colony counting, the processes were performed based on experimental methodology. Although PMMA sample making measurements are based on the dimensions of a conventional test tube and on the manufacturer's specifications, there is no standard for polishing. The polishing of conventional total or partial prostheses is carried out differently for the external and the internal faces. The internal part must be the most adapted to the palate mucosa, and thus, the polishing criterion should be respected for studies on acrylic resin samples in subsequent *in vitro* studies. Another limitation was that when the acrylic resin samples are submerged in the medium during the incubation procedure, they should be suspended by some device, as, if they are allowed to submerge completely in the broth, the plate will sink to the bottom of the container, which will allow only one side of the sample to be in contact with the culture broth, whereas the other side will be in direct contact with the container.

According to the results of this study, it is recommended that the incubation process should use a shaker that provides sufficient movement to ensure that the cells are suspended and in uniform contact with the acrylic resin sample. Regarding staining, the location and use of liquid acridine orange was one of the greatest difficulties encountered when performing the present investigation. As this is not essential for the staining of *C. albicans* colonies, it is recommended to use another type of dye. In addition, acridine orange stains use viable and nonviable cells, which can be used in subsequent investigations related to that already described, such as cell counting.

The results of this research have a direct impact on the stomatognathic system. One of the most frequent alterations is the loss of dental pieces producing partial or total edentulism, leading to the need for oral rehabilitation treatments using partial or complete prostheses. In both cases, the base material for these prostheses is thermosetting acrylic resin. The lack of adequate clinical and technical management and the failure of patients to comply with medical instructions, as well as a possibly suppressed immune system, can lead to the development of opportunistic diseases such as subprosthetic stomatitis that is directly related to the colonization of different pathogens including *C. albicans*.

In addition to the aforementioned factors, there are acrylic resin factors derived from each manufacturing company, which can act directly on a greater or lesser retention of bacterial plaque. Although in this study, samples were not obtained from individuals with prosthetic stomatitis, the results and the microphotographs of the cultures may be useful for future comparison with the studies by the aforementioned authors.

Conclusion

In conclusion, the PMMA group (Vitacryl) showed that for each surface energy unit in N/m, the adhesion per cell/field of *C. albicans* can be expected to significantly increase by a mean of 0.112. In contrast, in the PMMA group (Triplex), the *P* value suggested that changes in the predictor are not associated with changes in response. Significant differences were found between the surface energy of PMMA (Vitacryl) and PMMA (Triplex). Finally, in relation to the adhesion per cell/field of *C. albicans*, no significant differences were found between PMMA (Vitacryl) and PMMA (Triplex).

Acknowledgements

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Financial support and sponsorship

Not applicable.

Conflicts of interest

There are no conflicts of interest.

Author contributions

Study conception was by BC and AMM; data collection by BC and AMM; data acquisition and analysis by FMT, DAT, and RM; data interpretation by RM, FMT, and MPG; and manuscript writing by FMT, MPG, AMM, and DAT.

Ethical policy and institutional review board statement

This project was exempted from ethical approval as it was an experimental *in vitro* study.

Patient consent statement

Not applicable.

Data availability statement

The data that support the study results are available from the corresponding author (Dr. Frank Mayta-Tovalino, e-mail: fmaytat@ucientifica.edu.pe) on request.

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