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Objectives: To evaluate the effects of the waterproofing agent on the adhesion of Candida albicans and the superficial properties of prostheticsoft liners, with and without thermopolymerization. Methods: 32 discs of different brands of relining resins (SC and SR) were made. These were randomized into 4 groups (n=8) and submitted to surface roughness tests, profilometry, scanning electron microscopy and goniometry. Another 40 discs of each brand were made to evaluate the adhesion of Candida albicans through the counting of viable cells of Colony-Forming Units (CFU/mL).

Results: The absence of waterproofing liquid caused increased roughness of the material, as well as thermopolymerization, which incorporated pores inside. After 24 hours Candida albicans showed reduced biofilm formation capacity and resin SR with glaze and thermopolymerization showed a reduction in the microorganism count when comparing to the other groups after 24 and 48 hours.

Keywords: *prosthodontics, candida albicans, denture liners.*

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What is the Importance of the Waterproofing Agent on the Surface Properties of Prosthetic Soft Liners?

Barcellos ASP [α], Penteado MM [σ], Alvarenga JA [ρ], Junqueira JC [ω], Melo RM [¥], Valera MC[§], Bresciani E [χ] & Carvalho RF [ν]

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Conclusions: The importance of the application of glaze is associated with the quality of the soft relining resins and the copolymerization does not bring benefits according to the applied tests. Given the fact that it is a porous material, the accumulation of microorganisms is assured, therefore the hygiene of the patient is fundamental.

Keywords: prosthodontics, candida albicans, denture liners.

I. Introduction

Despite the change from a curative to a preventive perspective, a large part of the population faces the consequences of more invasive dental treatments.[1] The absence of teeth is still a reality that influences the quality of life and even the individual's systemic conditions.[2] Several scenarios are found according to region, culture and socioeconomic conditions, but caries and periodontal disease are still frequent and often lead to tooth loss.[1,3] In many places, edentulism is still a public health issue.[4,5,6]

Prosthetic soft liners are often used in clinical routine in patients requiring temporary rehabilitation, such as after extraction and implantation of implants.[7] These are indicated mainly in frames with atrophic ridge or sharp bone resorption, bruxism, xerostomia or presence of antagonist with natural teeth.[8] Given the fact that it is a soft material, it provides greater comfort and ease of adaptation with the prosthesis, which is often an unprecedented condition for the patient.[8, 9, 10] However, it is a material susceptible to dimensional changes over time due to the absorption of water and the increase of surface porosity, resulting in the accumulation of microorganisms and loss of adhesiveness to the acrylic resin of the prosthesis base.[10,11,12]

Many studies on the liner behavior are found[11] mainly in relation to porosity[13], biofilm accumulation[12] development of prosthetic stomatitis and angular cheilitis.[14, 15] However, there is no consensus in the literature about the relationship between increased roughness and the presence of *Candida albicans*.[12, 16]

Aiming to provide greater surface smoothness, lower bacterial colonization and microbiological combat, numerous liners advocate the use of a waterproofing agent after the prosthesis has been relined, but there is no literature report on the effect of this clinical stage, which is often neglected.[12, 14]

This study evaluated the effects of the application of the waterproofing agent on the adhesion of *Candida albicans* and the superficial properties of prosthetic soft liners, with and without thermopolymerization. The null hypothesis is that the use of the waterproofing liquid does not interfere with the adhesion of *Candida albicans*, with and without thermopolymerization.

II. Materials and Methodology

a) Preparation of samples

Two products available in the market were selected to perform the research: SC (Soft Confort, Dencril, Pirassununga, São Paulo, Brazil) and SR (Soft

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Rebase, TDV, Pomerode, Santa Catarina, Brazil). Thirty-two discs (N=32) of each material, with a diameter of 12mm and a height of 2mm, were made from progressive waxing. The wax discs were positioned and kept in a refractory coating gypsum (Bellavest SH Bego, Wilcos, Rio de Janeiro, Brazil) until it was firm. The device was taken to the oven for evaporation of wax (EDG3PS, 1800, São Paulo, Brazil). To obtain the samples, the cocoons were filled by the linershandled according to the recommendation of each manufacturer. Another 40 discs (N=40) of each material were made with the same methodology described, but with a diameter of 6mm and a height of 3mm, for the test of microbiological adherence.

b) Division of groups

Samples of each material were randomized into 4 groups, with n=8 for the evaluation of surface properties and n=10 for the microbiological adherence test.

The following groups were obtained: SCI: Soft Confortresin + Waterproofing agent; SC: Soft Comfort resin without Waterproofing agent; SCT: Soft Confortresin without Waterproofing agent, with thermopolymerization and SCIT: Soft Confort resin + Waterproofing agent, with thermopolymerization; SRI: Soft Rebase resin + Waterproofing agent; SR: Soft Rebase resin without Waterproofing agent; SRT: Soft Rebase resin without Waterproofing agent, with thermopolymerization; SRIT: Soft Rebase resin + Waterproofing agent, with thermopolymerization.

The waterproofing agent was applied twice over the entire surface of the sample, for 30 seconds with 30 seconds between the applications, with the aid of microbrush (FGM, Joinville, SC, Brazil).

The thermopolymerizationwas performed by inserting the samples of each group separately in professional wax heater (Mega Bell, São Paulo, Brazil) in water at 100°C for 10 minutes.

c) Characterization

1. Analysis of roughness

Two samples from each group were submitted to surface roughness test (RA). The specimens were coupled to a condensation silicone cushion Profile (Contene, Bonsucesso, Rio de Janeiro, Brazil) handled according to the manufacturer's recommendations and submitted to RA with 3 measurements set with 120° between them, obtaining an average per sample. The Surftest SJ 400 rugosimeter (Mitutoyo America, Aurora, Illinoins, USA) was calibrated with a cut-off measuring filter of 0.8mm, total length recorded of 3.2mm and a scanning speed of 0.5mm/s. All measurements were performed by a single operator. Statistical analysis was performed using the Shapiro-Wilk test and the T-test.

2. Analysis of surface topography by scanning electron microscope

After Bal-Tec SCD 050 metallization (Bal-Tec, Balzers, Liechtenstein, Germany), two samples from each group were submitted to scanning electron microscopy (Fei, Hillsboro, Oregon, USA) at 100x and 2000x magnification, set at low vacuum and 20kV for high resolution images.

3. Analysis of internal porosity by stereomicroscope

In order to verify the internal porosity of the materials, two samples of each group were cut in half and the interior was observed through a Zeiss binocular stereomicroscope (STEMI2000-C, Carl Zeiss do Brasil Ltda., Rio de Janeiro, Brazil) with colored digital camera, resolution of 4.1 megapixels, optical fiber for better illumination of the specimens, images with 20.5x magnification and field of 10.0mm.

4. Analysis of surface tension

Two samples from each group were submitted to the optical tensiometer (TL 1000, Theta Lite Attention, Lichfield, Staffordshire, United Kingdom) through the sessile drop technique. A syringe (# 1001 Gastight Syringes, 1 mL, Hamilton, Reno, NV, USA) with distilled water was coupled to the equipment and a drop thrown on the surface of the samples. During the first second, the drop undergoes the settlement and after 10 seconds, 30 images per second were captured for 20 seconds. Software One Attension (Biolin Scientific, Lichfield, Staffordshire, United Kingdom) was used to calculate surface energy, with 2 measurements per sample.

d) Colonization of microorganism

1. Adherence of *Candida albicans*

The samples were previously disinfected with 70% alcohol and then sterilized by ultraviolet light, 30 minutes on each surface (totaling 60 minutes), through laminar flow (Grupo Veco, Campinas, SP, Brazil). It placed in centrifuge tubes containing 8 mL of Brain Heart Infusion Broth (BHI, Himedia, Mumbai, India) plus 5% sucrose (Labsynth, Diadema, Brazil) and taken to bacteriological incubator B.D.O. (Eletrolab 101M/3, São Paulo, Brazil) at 37°C for 168 hours, observed every 24 hours for analysis of the turbidity of the medium and possible bacterial growth.

The reference strain *Candida albicans* ATCC 18804 was used for the analysis of biofilm formation on the samples. For activation, the microorganism was cultured in culture medium yeast extract, peptone, dextrose (YPD) in bacteriological incubator B.D.O. at 37°C for 24 hours. Subsequently, the microbial cells were centrifuged at 2000xg (approximately 4000 rpm) for 10 min (MPW Centrifuge, Warsaw, Poland), the supernatant was discarded and the sediment suspended in 6mL of sterile saline solution (0.85% NaCl, Labimpex, São Paulo, Brazil). This procedure was

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repeated. The counting of the number of cells of the suspension was performed in a spectrophotometer (B582, Micronal, São Paulo, Brazil), obtaining a concentration of 10^7 microorganisms/mL, corresponding to wavelength 530nm and optical density of 0.381.

The method described by Vilela*et al.* (2012) with some modifications was used for biofilm assembly. Each sample was inserted into each well of a 24-well cell culture plate (KASVI, Curitiba, Brazil), then 100μL of standard *C. albicans* suspension and 2 mL of BHI broth plus 5% sucrose were added. The plate was incubated with 75 rpm agitation (Chemistry ISSO 9001 - Diadema - Brazil) at 37°C for 24 and 48 hours, with exchange of the BHI broth in 24 hours of biofilm formation.

After the incubation period, the samples were placed in centrifuge tubes containing 10mL of sterile physiological solution (0.85% NaCl) and homogenized for 30 seconds using ultrasonic homogenizer (Sonics Vibra Cell, São Paulo, Brazil) with amplification of 25%, potency of 50W, to break down the microbial aggregates.

Serial dilutions in sterile physiological solution (0.85% NaCl) were performed, seeded in Sabouraud Dextrose Agar (Difco, Detroit, USA) and incubated at 37°C for 24 hours for the calculation of Colony-Forming Units (CFU/mL). For the static analysis, they were transformed into logarithm (Figure 3).

III. Results

With the measurements of the surface roughness, it was observed that the absence of glaze or the thermopolymerization treatment increase the roughness of the relining resin, independent of its brand (Table 1).

SEM images show higher surface homogeneity for the groups that underwent waterproofing (SCI, SCIT, SRI and SRIT). In contrast, the groups without waterproofing (SC, SCT, SR and SRT) have significant irregularities. The thermopolymerization did not interfere in the increase of irregularities (Figure 1).

Through the stereomicroscopic analysis, it was possible to observe significant differences in the internal structure, with a higher presence of pores in the resins submitted to thermopolymerization (SCT, SCIT, SRT and SRIT - Figure 2).

Through the analysis of the surface tension, it was possible to observe that the groups with waterproofing agent had a greater wettability (SCI, SCIT, SRI, SRIT) than the groups without waterproofing agent, except for SRT. Thermopolymerization did not interfere in this aspect (Table 2).

In 24 hours of biofilm formation by *C. albicans*, it was possible to observe a significant difference in the capacity of adhesion of the microorganism to the analyzed materials. The groups SC, SCT and SRIT did not form colonies and the Rebase resin showed greater accumulation of microorganisms (Figure 3).After 48 hours, all groups presented colony formation, but statistically significant difference was only between SC and SRIT, SCI and SRT. (Figure 4)

The results of counting of colonies of *Candida albicans* after 48 hours demonstrate that the Soft Confort resin maintains a pattern of accumulation of microorganism independent of the treatment performed. On the other hand, Rebase resin presented totally different behavior. The groups SRI and SRT reacted in a similar fashion. The effect of the reaction of the glaze with the thermopolymerization (SRIT) was similar to the non-glaze (SR) group, demonstrating that the thermopolymerization did not bring any benefit to the system (Table 3).

IV. Discussion

Although RA is lower when there is a thin film of glaze, the accumulation of microorganisms occurred within the first 24 hours of analysis. Possibly this happens due to the manual process of application of the product, which does not count with a standardization and also by the greater wettability found in the analysis of surface tension in the groups with glaze. According to the SEM, the surface of the resin has less defects, however it is not possible to avoid the penetration of fluids only with the layer of waterproofing agent.

The relining resin in its pure state has larger defects visualized in SEM and also higher RA, probably due to the chemical polymerization process inherent to the material. However, this aspect was not relevant in the process of colonization of microorganisms during the initial 24 hours for Soft confort resin (figure 3), which indicates that the composition of the relining material may initially interfere with the microbial adhesion, since comparing the information available on the package insert of the resins shows that there are different types of initiators and catalysts used and also the monomers present in the waterproofing liquid are different: Soft confort uses ethyl methacrylate and Rebase uses polymethyl methacrylate (PMMA).

The group SRIT also did not present microorganisms in 24 hours, in other words, exclusively for this condition the thermo-polymerization caused chemical reactions that were initially favorable, since this behavior did not remain, as demonstrated by the analysis after 48 hours (figure 4). Despite this significant increase in microorganisms, its low incidence or absence in the first 24 hours is an important factor in post-surgical moments, in which the lower number of microorganisms prevents the development of infections. Given the fact that it is a material that is indicated for use during a provisional period of 1 or 2 months, biofilm formation proves the need to make definitive prostheses and the importance of hygiene as maintenance.

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In terms of the accumulation of microorganisms, for both materials there was no statistical difference due to the absence or different treatments: with glaze and thermopolymerization, only thermopolymerization, only glaze. This allows us to affirm that the absence of waterproofing agent will not interfere in this aspect, but among the strategies proposed, the use of glaze is a fast and agile step during the care of the patients and that can contribute exclusively to the comfort during the use of the relined prosthesis, since the RA is lower (Table 1) and that although the wettability is accentuated (Table 2), there is no statistically significant difference in the microbial analysis (Figure 3).

These results corroborate Kutlu*et al.* (2016)[10], which research it has been shown that the aging process does not always increase the surface roughness of the relining materials and that the increase in porosity may not necessarily lead to the development of *Candida albicans* colonies. In addition, it pointed out other factors involved in the emergence of colonies such as: type of polymerization and acrylic resin composition, surface characteristics, hygiene techniques and period of use of the prosthesis.[17]

Another important property for the adhesion of microorganisms is the hydrophilicity or wettability of the material. The higher the capacity to absorb water, the greater the likelihood of the material adhering to microorganisms and of these being able to develop.[18] The water sorption also interferes in the physicochemical properties of the material, causing it to undergo syneresis and imbibition, thus promoting dimensional alterations.[10,11,12,19] As it was possible to observe in this study through the groups without glaze that visually suffered great dimensional alteration in the thermopolymerization, showing that they absorbed a greater amount of water in relation to the groups that had application of glaze.

From the aspect that the prostheses may transform into microbial niches if there is no adequate hygiene,[10, 12] other researches present the insertion of antimicrobial components in the acrylic resin that help to delay or even avoid the development of colonies.[20] Moreover, many methods of hygiene are proposed to avoid deepening of bacterial plaque and consequently the development of prosthetic stomatitis.[13]

In the attempt to improve the performance of the liners, silicone-based materials were created, materials that have greater chemical stability and do not require the clinical application stage of the glaze. In contrast to the two types of resins, despite the use of the waterproofing liquid in traditional liners, the silicone-based materials are more stable.[7, 11, 21]

The use of the results of the present study in order to predict the clinical behavior of prosthetic soft liners should be done with caution, since the buccal environment presents important differences in relation to the environment used in the study and also because it is a cross-sectional study, without long-term follow-up, which may differ in some properties.

It is possible to conclude that the waterproofing liquid is important for the quality of the soft relining resins, avoiding greater distortions by syneresis and imbibition, in addition to possibly favoring the comfort during the use of the relined prosthesis, since it provides greater superficial smoothness.

It was also possible to verify that the copolymerization did not bring benefits to the system and that the accumulation of microorganisms is inevitable regardless of the absence or presence of glaze, therefore the hygiene of the patient is fundamental to avoid prosthetic problems related to the marked colonization of microorganisms.

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Conflict of Interest

The authors declared that there is no conflict of nterest.

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Table 1: Mean values and standard deviation of mean roughness (Ra) and mean distance between the 05 largest peaks and valleys (Rz)

Group	Ra (Mean ± Standard deviation)	Rz (Mean ± Standard deviation)
SCI	0.28 ± 0.078	3.33 ± 1.59
SC	3.06 ± 0.25	22.45 ± 3.17
SCT	3.7 ± 0.38	33.37 ± 11.97
SCIT	0.64 ± 0.44	5.85 ± 6.77
SRI	0.20 ± 0.06	2.08 ± 0.87
SR	0.95 ± 0.27	9.52 ± 4.23
SRT	0.76 ± 0.23	7.48 ± 3.82
SRIT	0.65 ± 0.47	13.22 ± 13.00

Table 2: Mean values and standard deviation of surface tension

Group	Mean ± Standard deviation (mN/m)
SCI	[a] 46.528 ± 35421.15656
SC	[b] 35.562 ± 10147.68942
SCT	[b] 32.842 ± 1142.684558

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SCIT	[a] 57.288 ± 1694.934955
SRI	[a] 41.700 ± 772.1606051
SR	[b] 29.822 ± 3603.416157
SRT	[a] 45.221 ± 1391.586145
SRIT	[a] 40.470 ± 8152.941187

Table 3: Result of counting colonies of *Candida albicans* after 48 hours

Group	Soft Count	TDV Count
Resin + Glaze	Aa	Aa
ResinwithoutGlaze	A a	AB a
ResinwithoutGlaze + Thermopolymerization	A a	A a
Resina + Glaze + Thermopolymerization	A a	B a

Capital letter represents the comparative in the same column, lowercase letter in the same line

Figure legends

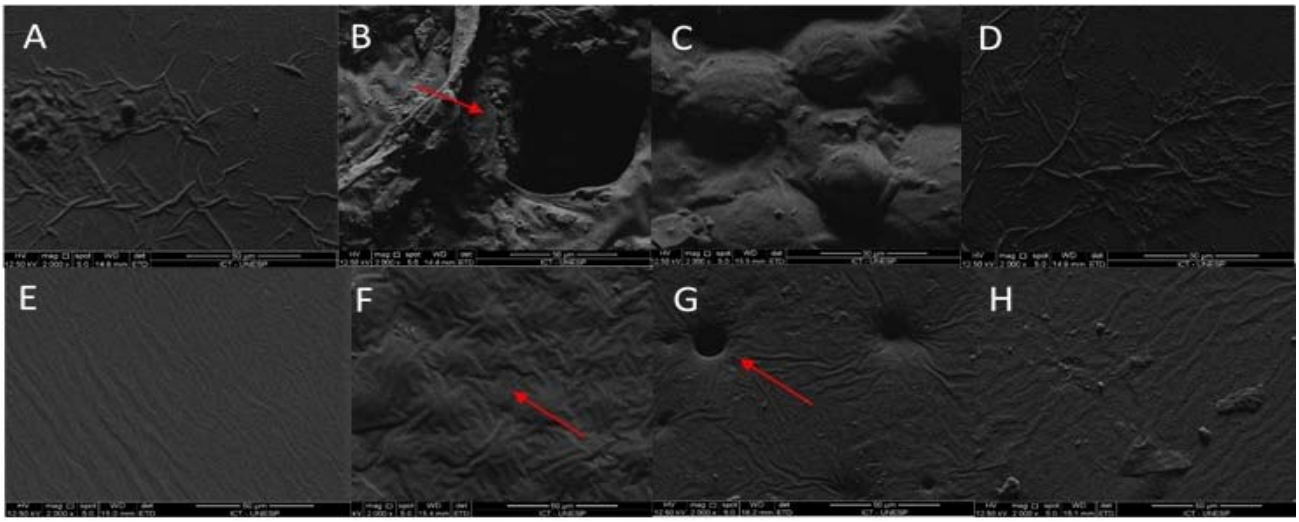


Figure 1: Photomicrograph representative of the surfaces analyzed with 2000x magnification: A-SCI; B-SC; C-SCT; D-SCIT; E-SRI; F-SR; G-SRT; H-SRIT.

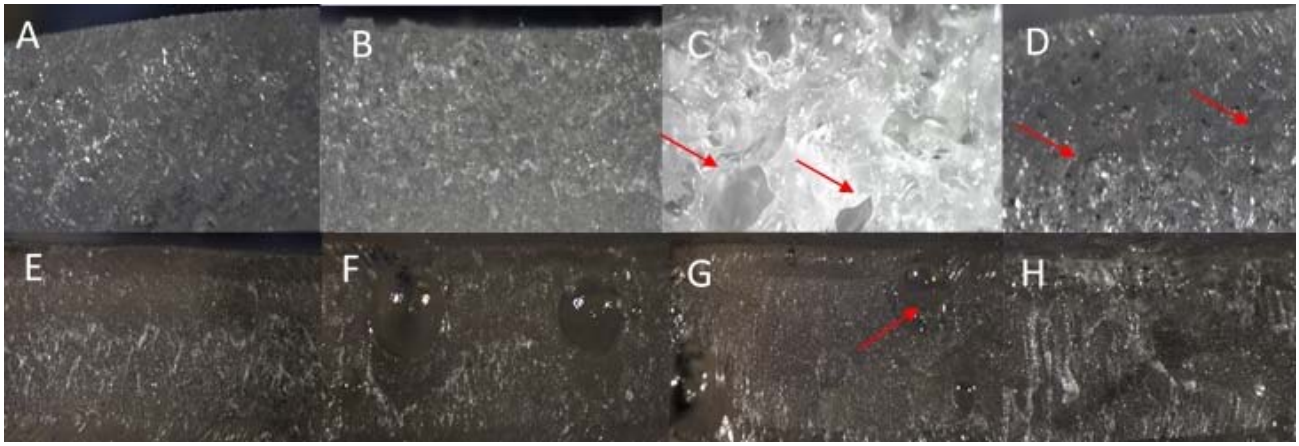


Figure 2: Images representative of sectioned surfaces with 20.5x magnification: A-SCI; B-SC; C-SCT; D-SCIT; E-SRI; F-SR; G-SRT; H-SRIT.

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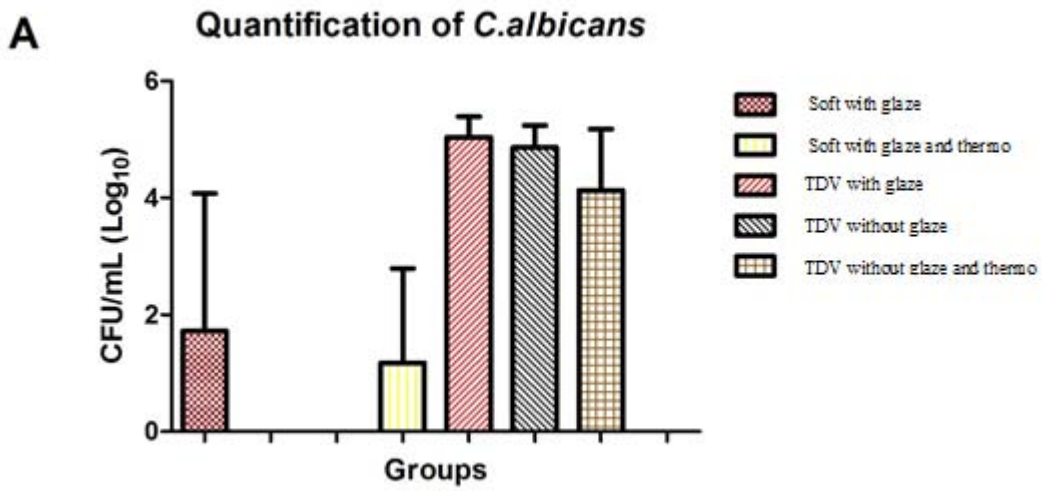


Figure 3: Quantitative analysis of *in vitro* biofilm formation by CFU/mL count: Mean and standard deviation of the number of CFU/mL of *C. albicans* (Log10) for the 24-hour biofilm.

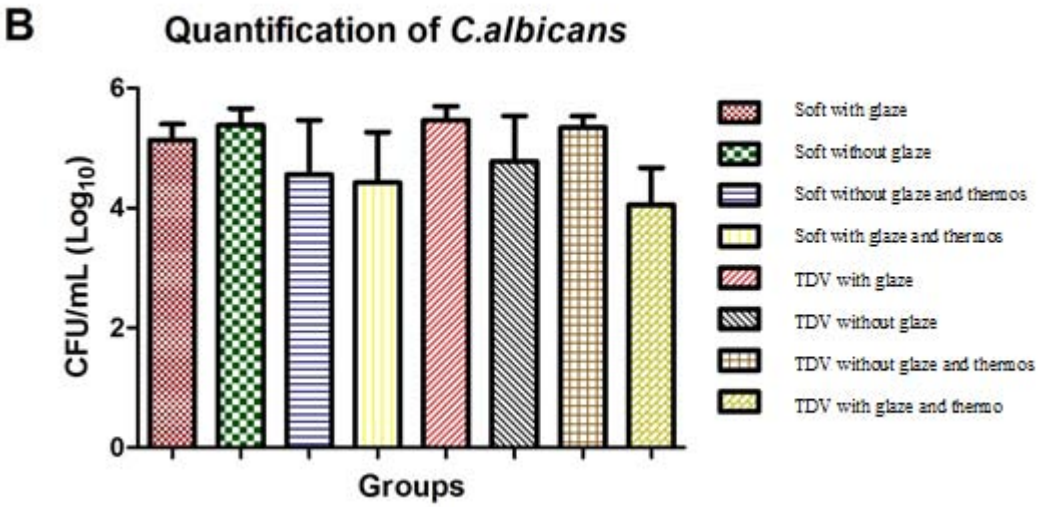


Figure 4: Quantitative analysis of *in vitro* biofilm formation by CFU/mL count: Mean and standard deviation of the number of CFU/mL of *C. albicans* (Log10) for the 48-hour biofilm.

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