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Longitudinal Evaluation of Ethyl Cyanoacrylate Adhesives on *Candida albicans* Biofilm

Evaluación Longitudinal de Adhesivos de Cianoacrilato de Etilo en Biopelículas de *Candida albicans*

Helena Sandrini Venante[1]; Ana Paula Chappuis Chocano[1]; Rodrigo Moreira Bringel da Costa[1]; Mariana Domingues Pordeus[1]; Jade Laísa Gordilio Zago[2] & Vinícius Carvalho Porto[3]

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ABSTRACT: The aim of this study is to evaluate the effect of two ethyl cyanoacrylate-based adhesives on the growth of *Candida albicans* biofilms on a heat-polymerized resin, after 7, 14, and 30 days of exposure. Ninety circular (10 × 2 mm) heat-polymerized resin specimens were equally divided into three groups: control, conventional ethyl cyanoacrylate (ECAc), and ethyl cyanoacrylate gel (ECAg). Two layers of 50 μL of each material were applied to the respective groups. *C. albicans* SC5314 strain was activated and standardized to 10^7 cells/mL^{-1}. Specimens were immersed in 1 mL of artificial saliva and deposited in 1 mL fungal suspension, washed, and immersed in 1 mL of RPMI for 7, 14, and 30 days. The medium was changed at 48-hour intervals. The final suspension was diluted (10^{-1} to 10^{-4}) and deposited on Sabouraud dextrose agar for 48 h at 37 °C. After this period, the colonies were quantified using the CFU/mL calculation. Data were evaluated using one-way ANOVA and Tukey's test for post-hoc analysis (P=0.05). It was observed that both adhesives significantly reduced (P<0.05) biofilm formation compared to the control at all evaluated periods. In conclusion, an immediate and long-term inhibitory effect on *C. albicans* biofilm formation was observed.

KEY-WORDS: complete denture, ethyl cyanoacrylate, *Candida albicans*, denture stomatitis, acrylic resin.

INTRODUCTION

Polymethylmethacrylate (PMMA) has been used for the fabrication of complete denture bases for many decades. It has advantages such as ease of handling and repair, low cost, low weight, and acceptable aesthetics (Fathi *et al*., 2017; Shakeri *et al*., 2019). However, one of the major disadvantages of PMMA is the material porosity and surface roughness, which combined with the oral environment, allows for pathogenic biofilm attachment to the denture base. Thus, an acidic environment favorable to the growth and development of yeasts such as *Candida albicans* is created, leading to oral infections such as denture stomatitis (DS) (Dhir *et al*., 2007; Albin-Ameer *et al*., 2020).

The treatment of DS commonly involves systemic and topical medication, repackaging, hygiene, and disinfection instructions (Cuéllar-Cruz *et al*., 2012). However, systemic drugs can cause nephrotoxic and hepatotoxic effects (Samet *et al*., 2007). Topical agents such as nystatin and miconazole are widely used for the treatment of DS. However, they have an unpleasant taste that prevents the daily use of the complete denture. Moreover, their use prolongs treatment as the drug must be in direct contact with the mucosa (Lalla & Dongari-Bagtzoglou, 2014). Prolonged use of disinfectants such as sodium hypochlorite 1 % and chlorhexidine digluconate 2 % can cause damage to the physical properties of acrylic

[1] PhD. Department of Prosthodontics, Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, Brazil.
[2] DDs Student. Department of Prosthodontics, Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, Brazil.
[3] Associate Professor. Department of Prosthodontics, Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, Brazil.

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resin, thus decreasing its longevity (Felton *et al*., 2011; Peracini *et al*., 2017). Hence, studies are now focused on new approaches to improve the denture base surface in order to control and/or prevent biofilm formation without compromising oral health and material properties.

Authors have reported the coating of PMMA surfaces (Ali *et al*., 2013; Távora *et al*., 2019) with cyanoacrylate-based adhesives as beneficial against *C. albicans* development. Ali *et al*. studied the effects of octyl-cyanoacrylate adhesives coated on plates of heat-cured acrylic resin and reported complete inhibition of fungal adhesion. Among cyanoacrylate adhesives, ethyl cyanoacrylate has shown promising results against *C. albicans*. It stands out because of its low cost and bactericidal effect. Távora *et al*. studied *C. albicans* growth on relining acrylic resin coated with ethyl-cyanoacrylate (Superbonder) and verified that this adhesive reduced the initial fungal development.

In this context, the possibility of applying cyanoacrylate-based adhesives as coatings on acrylic resins was considered, thus modifying its surface properties, reducing roughness, and increasing hydrophobicity (Távora *et al*.). However, no study has assessed its long-term efficacy. Therefore, the inhibitory effect of two ethyl cyanoacrylate adhesives on *C. albicans* biofilm was evaluated after 7, 14, and 30 days in this study.

MATERIAL AND METHOD

Fabrication of specimens. A total of 90 circular specimens (10 × 2 mm) were fabricated using a heat-polymerized acrylic resin (Vipi Cril Plus, Vipi Indústria, Comércio, Exportação e Importação de Produtos Odontológicos, Pirassununga, SP, Brazil) following the manufacturer's instructions. Subsequently, one surface of each specimen was roughened using a polishing machine (PFL, Fortel Indústria e Comércio, São Paulo, SP, Brazil) with 120-grit sandpaper (Norton Abrasivos, São Paulo, SP, Brazil) for 15 s. The surface roughness of all specimens was measured using a roughness tester (Surftest SJ-301, Mitutoyo Corporation, Kanagawa, Japan). Readings were taken at four different positions on the roughened surface (Rahal *et al*., 2004) and standardized to 2-3 μm to simulate the inner surface of a complete denture (Silva *et al*., 2016). Subsequently, the specimens were immersed in 2 mL of distilled water at 37 °C for 48 h to allow the release of the residual monomer (ISO 1567, International Organization for Standardization, 1988) and were further sterilised using ethylene oxide (Acecil, Central de Esterilização de Comércio e Indústria Ltda., Campinas, São Paulo, Brazil) (Almeida *et al*., 2018). Finally, the specimens were randomly distributed into three groups: control or without coating (GC), coated with conventional ethyl cyanoacrylate (ECAc, G1), and coated with ethyl cyanoacrylate gel (ECAg, G2) (Table I).

Surface treatment of specimens. The experimental specimens were coated with 50 μL of adhesive (ECAc or ECAg), which was equally distributed across the surface using a disposable brush tip (Disposable Brush Tips/60, 3M ESPE, St Paul, Minnesota, USA). Afterwards, the specimens were dried at room temperature for 40 min. This procedure was repeated to obtain a second layer of coating (Távora *et al*.).

Yeast strain and growth conditions. Initially, *C. albicans* (strain SC5413) were incubated in tryptic soy broth (TSB) (Accumidia manufactures Inc., Lansing, MI, USA) with 1 % chloramphenicol (Quemicetina Succinato, Carlo ErbaR, Milano, Mi, Italy) at 30 ºC for 24 h under aerobic conditions. Afterwards, the suspension was centrifuged at 5000 rpm for 10 min at 22 °C. Cells were harvested and washed with phosphate-buffered saline (PBS, pH 7.2) and standardised to 1×10^7 cell/mL^{-1} (Almeida *et al*.).

Biofilm development. Specimens were exposed to 1 mL of artificial saliva for 2 h at 37 °C using a 24-well tissue culture plate (Cell Culture Plate, Nest Biotech Co., Ltd., China) (Silva *et al*.). Subsequently, the specimens were immersed in 1 mL of the previously standardized *C. albicans* cell suspension and incubated for 90 min at 37 °C at 75 rpm. The non-adherent cells were removed from the specimens by washing with 1 mL of PBS. Finally, the specimens were immersed in 1 mL of Roswell Park Memorial Institute solution (RPMI-1640, GibcoR, Grand Island, NY, USA) for 7, 14, and 30 days at 37 °C at 75 rpm. During these periods, the medium was changed at 48-hour intervals (Almeida *et al*.).

Viable cell count (CFU/mL). For all groups, three independent experiments were performed to measure the number of viable *C. albicans* cells adhering to the specimens. After each period (7, 14, and 30 days), the specimens were removed from the culture plate and washed with PBSin another 24-well tissue culture

plate. Afterwards, the biofilm was removed from the surface of the specimens with a sterilized cell scraper and the cells were stored in 1 mL of PBS, and the suspension was serially diluted (10^{-1} to 10^{-4}). Subsequently, 50 μL of these dilutions were inoculated into a Sabouraud agar plate and incubated for 48 h at 37 °C. After this period, the mean values obtained indicated the growth of biofilms in CFU/mL and all colonies were counted and expressed as CFU/mL (Almeida *et al*.).

Statistical methods. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23. Additionally, to compare biofilm formation between groups and their correlation with coating material performance, a one-way ANOVA test was used, followed by the Tukey's correction test for multiple comparisons (P=0.05).

RESULTS

According to the results, cyanoacrylate adhesives showed a marked inhibitory effect on microbial biofilm formation with statistically significantly lower values compared to the control group: ECAc (P=0,00) and ECAg (P=0,00) (Table I).

Considering the evaluated periods, the cyanoacrylate groups did not show statistically significant differences. However, the control group showed a significant increase in the colony count during the 14- and 30-day periods (P<0.05) (Table I).

Table I. Mean values and Standard Deviation (SD) in number of Colony-Forming Units (CFU) of *Candida albicans* over the evaluation periods of 7, 14 and 30 days.

Groups	Periods	CFU/mL
GC	7	1445.71 ± 452.65 (10^3) aA
	14	4794.29 ± 997.78 (10^3) bA
	30	4854.29 ± 1134.53 (10^3) bA
ECAc	7	32.86 ± 17.28 (10^3) B
	14	38.29 ± 18.49 (10^3) B
	30	290 ± 129.31 (10^3) B
ECAg	7	22.57 ± 18.17 (10^3) B
	14	180.29 ± 97.33 (10^3) B
	30	466 ± 383.21 (10^3) B

GC (control group), ECAc (ethyl-cyanoacrylate), and ECAg (ethyl-cyanoacrylate formulation gel). Different lowercase letters at the end of the lines indicate a statistical difference between the experimental periods (P<0.05). Different capital letters at the end of the lines indicate a statistical difference between the groups (P<0.05).

DISCUSSION

The cyanoacrylate adhesives evaluated in this study have been successfully used in surgical procedures, both in medicine and dentistry (Thakeb *et al*., 1995; Pascual *et al*., 2016) for the control of hemorrhages (Al-Bawardy *et al*., 2016). Recently, cyanoacrylates and fibrin biopolymers have also been studied for use as denture coating adhesives (Ali *et al*.; Soon *et al*., 2015; Távora *et al*.).

This study established that ECAc and ECAg showed promising long-term results in inhibiting *C. albicans* biofilm formation. These findings agree with other studies reported in the literature. Távora *et al*. verified the lower adhesion of *C. albicans* to acrylic resin treated with different cyanoacrylate adhesives, including conventional ethyl cyanoacrylate and formulation gel. Similarly, Ali *et al*. highlighted the inhibitory effects of octyl cyanoacrylate coated prostheses on *C. albicans* biofilms after 24 h of exposure. This may be associated with the changes in surface energy and roughness (the main factors for the development of *C. albicans* biofilm on dentures) of acrylic resins that represent niches for *C. albicans* biofilm adhesion. Moreover, the cyanoacrylate adhesives tested in this study contained the compound methyl 2-cyanoacrylate (C5H5NO2), which could be another important factor in inhibiting *C. albicans* biofilm development (Soon *et al*.).

In addition, some authors attribute this microbial reduction to the release of formaldehyde and cyanoacetate compounds during its degradation. These by-products could trigger antifungal activity because of their cytopathic effect (Chen *et al*., 2007; De Melo *et al*., 2013) that tends to decrease after 14 days and remains low for 28 days (Soon *et al*.).

The degradation of cyanoacrylates is related to the length of the side chain; the smaller the side chain, the faster the degradation (Tseng *et al*., 1990; Trott, 1997; Sohn *et al*., 2016). In a previous study, the inhibitory effect of two short-chain adhesives was evaluated, with evidence indicating that they degrade rapidly. Thus, it was suggested that with the decrease in the cytopathic reaction, *C. albicans* could adapt or colonize the surface as the coating gradually degraded. However, this was not observed in the present study, as the development of the *C. albicans* biofilm significantly reduced for up to 30 days after the application of ethyl cyanoacrylate coating.

The cyanoacrylate groups presented no statistically significant difference at all evaluated periods, although the ECAc presented a slightly better performance. This may be associated with the low viscosity of the ECAc liquid, which seeps in filling up the porosities and cracks, thus providing a smooth and regular surface.

The results obtained in this study are promising as cyanoacrylate adhesives showed long-term inhibitory effects on *C. albicans* biofilm formation; however, the lack of information regarding their resistance to biological changes and sustainability against chemical and mechanical challenges (commonly caused during the disinfection of a complete denture) warrants further investigation.

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RESUMEN: El objetivo del estudio fue evaluar el desarrollo microbiano en superficies de resina acrílica de termopolimerización, acondicionadas previamente, con adhesivos a base de etil cianoacrilato después de 7, 14 y 30 días de exposición en biofilm de *Candida albicans*. Noventa muestras circulares (10 x 2 mm) de resina acrílica de termopolimerización se dividieron por igual en tres grupos: control, etil cianoacrilato convencional (ECAc) y etil cianoacrilato en gel (ECAg). Se aplicaron dos capas de 50 µl de cada adhesivo en cada muestra. Simultáneamente, se activó la cepa *C. albicans* SC5314 y se estandarizó a 10^7 células/ml^{-1}. Las muestras fueron sumergidas en 1 mL de saliva artificial por dos horas y luego se depositó 1 mL de suspensión fúngica por una hora. En seguida cada muestra se lavó y se sumergió en 1 mL de RPMI durante 7, 14 y 30 días, con cambios del medio a cada 48 horas. La suspensión final se diluyó (10^{-1} a 10^{-4}) y se depositó en agar dextrose Sabouraud durante 48 h a 37 ° C. Después de este período, las colonias se cuantificaron mediante el cálculo de UFC / mL. Los datos obtenidos se evaluaron por medio del test ANOVA-one way y la prueba de Tukey para el análisis post-hoc (p = 0,05). Se observó que ambos adhesivos redujeron significativamente (P<0,05) la formación del biofilm de *Candida albicans* al ser comparados con el grupo control en todos los períodos evaluados. Los adhesivos a base de etil cianoacrilato poseen un efecto inhibidor de biofilm de *Candida albicans* de hasta 30 días, al ser aplicados dos veces en resinas acrílicas de termopolimerización.

PALABRAS CLAVE: Dentadura completa; cianoacrilato de etilo; *Candida albicans*; estomatitis por dentadura postiza; resina acrílica.

REFERENCES

Al-Bawardy, B.; Gorospe, E. C.; Saleem, A.; Buttar, N. S. & Wong Kee Song, L. M. Outcomes and predictors of rebleeding after 2-octyl cyanoacrylate injection in acute gastric variceal hemorrhage. *J. Clin. Gastroenterol., 50(6)*:458-63, 2016.

AlBin-Ameer, M. A.; Alsrheed, M. Y.; Aldukhi, I. A.; Matin, A.; Khan, S. Q.; Abualsaud, R. & Gad, M. M. Effect of protective coating on surface properties and *Candida albicans* adhesion to denture base materials. *J. Prosthodont., 29(1)*:80-6, 2020.

Ali, A. A.; Alharbi, F. A. & Suresh, C. S. Efectiveness of coating acrylic resin dentures on preventing Candida adhesion. *J. Prosthodont., 22(6)*:445-50, 2013.

Almeida, N. L. M.; Saldanha, L. L.; da Silva, R. A.; Pinke, K. H.; da Costa, E. F.; Porto, V. C.; Dokkedal, A. L. & Lara, V. S. Antimicrobial activity of denture adhesive associated with Equisetum giganteum- and Punica granatum-enriched fractions against *Candida albicans* biofilms on acrylic resin surfaces. *Biofouling, 34(1)*:62-73, 2018.

Chen, W. L.; Lin, C. T.; Hsieh, C. Y.; Tu, I. H.; Chen, W. Y. & Hu, F. R. Comparison of the bacteriostatic effects, corneal cytotoxicity, and the ability to seal corneal incisions among three different tissue adhesives. *Cornea, 26(10)*:1228-34, 2007.

Cuéllar-Cruz, M.; Vega-González, A.; Mendoza-Novelo, B.; López-Romero, E.; Ruiz-Baca, E.; Quintanar-Escorza, M. A. & Villagómez-Castro, J. C. The effect of biomaterials and antifungals on biofilm formation by Candida species: a review. *Eur. J. Clin. Microbiol. Infect. Dis., 31(10)*:2513-27, 2012.

De Melo, W. M.; Maximiano, W. M. A.; Antunes, A. A.; Beloti, M. M.; Rosa, A. L. & de Oliveira, P. T. Cytotoxicity testing of methyl and ethyl 2-cyanoacrylate using direct contact assay on osteoblast cell cultures. *J. Oral Maxillofac. Surg., 71(1)*:35-41, 2013.

Dhir, G.; Berzins, D.; Dhuru, V.; Periathamby, R. & Dentino, A. Physical properties of denture base resins potentially resistant to Candida adhesion. *J. Prosthodont., 16(6)*:465-47, 2007.

Fathi, H. M.; Benonn, H. A. & Johnson, A. Nanocryl coating of PMMA complete denture base materials to prevent scratching. *Eur. J. Prosthodont. Restor. Dent., 25(3)*:116-26, 2017.

Felton, D.; Cooper, L.; Duqum, I.; Minsley, G.; Guckes, A.; Haug, S.; Meredith, P.; Solie, C.; Avery, D.; Chandler, N. D.; *et al*. Evidence-based guidelines for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists. *J. Am. Dent. Assoc., 20 Suppl 1*:S1-S12, 2011.

Lalla, R. V. & Dongari-Bagtzoglou, A. Antifungal medications or disinfectants for denture stomatitis. *Evid. Based Dent., 15(2)*:61-2, 2014.

Pascual, G.; Sotomayor, S.; Rodríguez, M.; Pérez-Köhler, B.; Kühnhardt, A.; Fernández-Gutiérrez, M.; San Román, J. & Bellón, J. M. Cytotoxicity of cyanoacrylate-based tissue adhesives and short-term preclinical in vivo biocompatibility in abdominal hernia repair. *PLoS One, 11(6)*:e0157920, 2016.

Peracini, A.; Machado Andrade, I.; Oliveira, V. C.; Macedo, A. P.; Silva-Lovato, C. H.; Pagnano, V. O.; Watanabe, E. & Paranhos, H. F. O. Antimicrobial action and long-term effect of overnight denture cleansers. *Am. J. Dent., 30(2)*:101-8, 2017.

Rahal, J. S.; Mesquita, M. F.; Henriques, G. E. & Nóbilo, M. A. A. Surface roughness of acrylic resins submitted to mechanical and chemical polishing. *J. Oral Rehabil., 31(11)*:1075-9, 2004.

Samet, N.; Laurent, C.; Susarla, S. M. & Samet-Rubinsteen, N. The effect of bee propolis on recurrent aphthous stomatitis: a pilot study. *Clin. Oral Investig., 11(2)*:143-7, 2007.

Shakeri, F.; Nodehi, A. & Atai, M. PMMA/double-modified organoclay nanocomposites as fillers for denture base materials with improved mechanical properties. *J. Mech. Behav. Biomed. Mater., 90*:11-9, 2019.

Silva, M. J.; de Oliveira, D. G.; Marcillo, O. O.; Neppelenbroek, K. H.; Lara, V. S. & Porto, V. C. Effect of denture-coating composite on *Candida albicans* biofilm and surface degradation after disinfection protocol. *Int. Dent. J., 66(2)*:86-92, 2016.

Sohn, J. J.; Gruber, T. M.; Zahorsky-Reeves, J. L. & Lawson, G. W. Comparison of 2-ethyl-cyanoacrylate and 2-butyl-cyanoacrylate for use on the calvaria of CD1 mice. *J. Am. Assoc. Lab. Anim. Sci., 55(2)*:199-203, 2016.

Soon, H. I.; Gill, D. S. & Jones, S. P. A study to investigate the bond strengths of orthodontic brackets bonded to prosthetic acrylic teeth. *J. Orthod., 42(3)*:192-9, 2015.

Távora, F. F. F.; Chocano, A. P. C.; de Oliveira, D. G.; Pereira, J. R.; Almeida, R. S.; Neppelenbroek, K. H. & Porto, V. C. Beneficial effects of ethyl-cyanoacrylate coating against *Candida albicans* biofilm formation. *Braz. Dent. J., 30(3)*:266-71, 2019.

Thakeb, F.; Salama, Z.; Salama, H.; Abdel-Raouf, T.; Abdel-Kader, S. & Abdel-Hamid, H. The value of combined use of N-butyl-2-cyanoacrylate and ethanolamine oleate in the management of bleeding esophagogastric varices. *Endoscopy, 27(5)*:358-64, 1995.

Trott, A. T. Cyanoacrylate tissue adhesives. An advance in wound care. *JAMA, 277(19)*:1559-60, 1997.

Tseng, Y. C.; Tabata, Y.; Hyon, S. H. & Ikada, Y. In vitro toxicity test of 2-cyanoacrylate polymers by cell culture method. *J. Biomed. Mater. Res., 24(10)*:1355-67, 1990.

Corresponding author:
Helena Sandrini Venante
Department of Prosthodontics
Bauru School of Dentistry
University of São Paulo
Alameda Dr. Otávio Pinheiro Brizola, 9-75
17012-901 Bauru
São Paulo
BRAZIL

E-mail: helenavenante@gmail.com