

Influence of roughness and surface free energy on adhesion and biofilm formation of *Candida albicans* to polymeric materials

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Abstract

Objectives

Denture-associated candidiasis can lead to severe oral infections and is typically caused by the yeast *Candida albicans* (*C. albicans*). This study examines how the adhesion and biofilm formation of *C. albicans* on polymeric materials is influenced by their roughness, surface free energy (SFE) and saliva coating.

Methods

For this purpose, three standard polymeric materials (polyvinyl chloride (PVC), polytetrafluorethylene (PTFE), polyethylene (PE)) with two roughnesses (treatment A $R_a = 0.2 \mu\text{m}$ and treatment B $R_a = 0.04 \mu\text{m}$) were characterized using profilometry, contact angle measurements, confocal laser scanning microscopy, and scanning electron microscopy. Adhesion and biofilm formation of *C. albicans* (DSM 1386) were quantified by fluorescence staining and confocal microscopy.

Results

Surface free energy (SFE) significantly influenced both initial adhesion and biofilm formation, with lower SFE generally reducing these processes. PTFE (SFE $\approx 17.73 \text{ mJ/m}^2$) exhibited a lower adhesion volume than PE (SFE $\approx 32.59 \text{ mJ/m}^2$). Roughness variations affected only *C. adhesion* by altering SFE and available surface area. Saliva treatment generally decreased adhesion and biofilm formation and also reduced the formation of virulent hyphae.

Conclusion

Material specific SFE had the strongest influence on yeast adhesion and biofilm formation. Therefore, reducing the SFE could help to minimize *C. albicans* adhesion and biofilm formation, and potentially lower the risk of denture stomatitis.

1 Introduction

Candida albicans (*C. albicans*) is a commensal yeast and is typically found on the oral mucosal surfaces of 30% to 70% of healthy individuals [1]. However, it is also an opportunistic pathogen that particularly spreads in immunocompromised individuals, diabetics, the elderly, and denture wearers, where it can cause oral infections [2, 3]. These infections are either directly caused by biofilm formation of *C. albicans* on polymeric prosthesis surfaces or indirectly promoted by bacterial growth via cross-kingdom interactions. As these interactions may in turn enhance the virulence of *C. albicans* [4], polymeric materials are potential reservoirs for adhesion and biofilm formation [5, 6]. The combination of

uncontrolled biofilm growth and a weakened immune system can lead to the development of deep tissue infections [3] and denture stomatitis [7, 8]. Despite the clinical relevance of this issue, the specific influence of different polymeric materials on *C. albicans* adhesion and biofilm formation remains poorly understood.

One of the key factors in *C. albicans* pathogenicity is its ability to rapidly adhere to surfaces in the oral cavity (such as polymeric denture materials) and form colonies [7, 9, 10]. This initial adhesion occurs to the thin layer of adsorbed saliva proteins, called pellicle [11], and is often followed by the development of biofilms - three-dimensional, multicellular agglomerates encased in an extracellular matrix, that contribute to the persistence and virulence of the yeast [12].

One crucial material property influencing *C. albicans* adhesion is surface roughness, often quantified by the arithmetic mean roughness value (R_a), which measures two-dimensional surface texture. Schubert et al. [7] demonstrated that R_a is a reliable predictor of microbial adhesion on material surfaces. Tactile profilometry, a standard technique for assessing surface roughness, has been widely used for this purpose [13]. Modern optical methods, such as confocal laser scanning microscopy (CLSM), offer additional approaches for the visual assessment of topography and surface roughness. Microbial adhesion has commonly been correlated with line roughness, although it has been reported that when surface roughness is lower than a certain threshold, it has no relevant effect on biofilm formation in the oral cavity. Consequently, smoother surfaces do not automatically lead to a reduction in microbial adhesion and growth and microbial adhesion behaviour needs to be characterized for the respective clinical setting [7, 14, 15].

For *C. albicans*, several studies have indicated that increased surface roughness (high R_a values) enhances yeast adhesion [7]. Rough surfaces facilitate increased microbial adhesion due to their larger contact area, which offers more protection against shear forces and promotes greater microbial attachment than smoother surfaces with a smaller contact area [16, 17]. However, there have also been contradictory findings. For example, other studies reported that surface roughness has no measurable impact on *C. albicans* adhesion, indicating the need for further research [18, 19].

Another factor influencing microbial adhesion is surface free energy (SFE), which influences the surface's wettability. It has been shown to affect both *C. albicans* adhesion and biofilm formation [17, 20, 21]. In certain cases, SFE might be more relevant than surface roughness in determining the extent of *C. albicans* adhesion [9, 22]. In contrast, Pereira-Cenci et al. [23] reported that the adhesion of *C. albicans* is influenced by roughness but not by SFE. These discrepancies further support the complexity of the adhesion process and suggest that further studies are required to clarify the roles of surface roughness and SFE in microbial colonization.

To address this knowledge gap, the current study investigates the adhesion and biofilm formation of *C. albicans* on three standard polymeric materials, with a focus on the effects of surface roughness and SFE. Additionally, the influence of pre-incubation in saliva on this interaction is analyzed. We

hypothesized that differences in adhesion and biofilm formation of *C. albicans* can be attributed to the distinct surface properties and surface treatments of the polymeric materials.

2 Materials and Methods

2.1 Study Design

This *in vitro* study on *C. albicans* colonization of polymeric surfaces consisted of two parts: a technical analysis followed (**Figure 1**, blue part) by a biological investigation (**Figure 1**, green part). First, specimens with distinct surface properties were produced by automated surface processing from three polymeric materials. Surface characteristics, such as roughness, surface free energy, and polarity were subsequently examined. Then, the specimens were colonized with *C. albicans* with and without prior exposition to artificial saliva. After different incubation conditions, initial adhesion as well as biofilm formation were quantitatively analysed.

Figure 1: Study design.

2.2 Specimen Preparation and Surface Analysis

2.2.1 Specimen Preparation

Specimens (20 mm in diameter; approx. 2 mm in height) were fabricated from industrially manufactured round bars of three polymeric materials, using a precision cutting device (Accutom-100, Struers, Willich, Germany) and a diamond cut-off wheel for plastic (E0D15, Struers, Willich, Germany). The polymeric materials used were:

- Polyvinyl chloride (PVC, PVC-U, Modulor, Berlin, Germany),
- Polytetrafluorethylene (PTFE, online-plast, Koblenz, Germany), and
- Polyethylene (PE, UHMW-PE, online-plast).

2.2.2 Surface Treatment

To manufacture reproducible and comparable surfaces, the specimens were subjected to an automated grinding process using a precision grinding machine (Tegramin-25, Struers, Willich, Germany) [7]. The specimens were fixed in the slots of specially manufactured specimen holders with double-sided adhesive tape and then the specimen holders were positioned in the cut-outs of the specimen moving head of the polishing machine. Pins then applied a precise contact force to the specimens while both the specimen moving head and the specimen stage rotated in the same direction at a predefined rotational

speed. The polishing parameters were optimized in preliminary tests to ensure consistency for all specimens. For all specimens, a first, “rough” polishing treatment (A) was employed to produce specimens with a roughness of approximately 0.2 μm . In the second treatment, 50% of the specimens underwent a second, “fine” polishing process (B) that produced a roughness of approximately 0.04 μm . A sequential polishing process was used for the fine polishing treatment, to ensure controlled material removal, including the micro-scratches, and to guarantee reproducible results. In total, ten specimens were prepared for each material and surface treatment. The polishing treatments were as follows:

- A: SiC foil #1000, water cooling, 120 rpm, 120 s, with PVC: 5 N, PTFE: 10 N, PE: 20 N; Struers, Willich, Germany

- B: SiC foil #1000, water cooling, 120 rpm, 120 s, PVC: 5 N, PTFE: 10 N, PE: 20 N, followed by SiC foil #2500, water cooling, 120 rpm, 60 s, PVC: 10 N, PTFE: 25 N, PE: 35 N; Buehler, Düsseldorf, Germany

2.2.3 Roughness

To verify the target values of line roughness (R_a and R_z), the generated surfaces were evaluated using a mechanical contact profilometer (CP, LT = 1.7 mm, LC = 0.25 mm, velocity 0.1 mm/s, diamond tip with a 2 μm radius, Perthometer S6P, Perthen Mahr, Göttingen, Germany). Three parallel measurements were made at the centre of each specimen, perpendicular to the macroscopically visible grinding lines.

2.2.4 Surface Free Energy

SFE was determined using the sessile drop method, with a computerized measuring device for the contact angle (in average 23°C; DSA25, Krüss, Hamburg, Germany). Before conducting contact angle measurements, the specimen surfaces were cleaned with ethanol (70%) to eliminate potential influences from contamination. A single drop (drop volume = 4 μL) of two liquids with distinct hydrophobicity, namely deionized water and diiodomethane [21, 24], was applied to the surface of the specimens and the left and right contact angles of the drop were then determined and averaged. SFE, along with its polar and disperse fractions, was calculated with the method outlined by Owens and Wendt [25, 26].

2.2.5 Surface Topography

The topography of the specimens' surfaces was qualitatively assessed using optical imaging techniques. Confocal laser scanning microscopy (CLSM) was performed using a KJ VK-100 (Keyence, Osaka, Japan) with 5 $\times$ magnification and a scanning area of 2000 $\times$ 2824 μm . Automatic laser brightness adjustment was applied. CLSM images were acquired using VK Viewer 1.1.2.174 (Keyence, Osaka, Japan), and a tilt correction was performed for all images. Scanning electron microscopy (SEM) was conducted using a Phenom (FEI, The Netherlands) at 850 $\times$ magnification with a full-view setting. The specimens were mounted on aluminium stubs and coated with a thin layer of electrically conductive

platinum for 50 s to enhance conductivity. For each material and surface treatment, one random specimen was selected for imaging.

2.3 Microbiological Experiments

2.3.1 Yeast Strains and pre-Culture Conditions

C. albicans DSM 1386, obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany), was stored at -80°C as glycerol stocks. For cultivation, yeast cells were grown in medium 186 (3 g/L yeast extract, 3 g/L malt extract, 5 g/L soy peptone, 10 g/L glucose monohydrate; all components from Roth, Karlsruhe, Germany) prepared according to the manufacturer's instructions, but without the addition of agar. This liquid medium was used to establish pre-cultures for subsequent adhesion and biofilm experiments: *C. albicans* was incubated for 24 h overnight at 30°C under continuous agitation (200 rpm) to ensure optimal cell growth.

2.3.2 Artificial Saliva Preparation

Saliva was prepared according to previous protocols [27–31] with 8.5 mg/mL mucin from porcine stomach, 1 mg/mL lysozyme from chicken egg, 100 mg/mL α -amylase from hog pancreas, and 4 mg/mL bovine serum albumin (BSA, all components provided by Sigma-Aldrich, Taufkirchen, Germany). All substances were solved 1:100 in Dulbecco's Phosphate Buffered Saline (PBS) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) [27–31]. After sterilization (mucin solution: autoclaving; BSA and lysozyme solution: 0.22 μ M filter; α -amylase: sterilely weighed; sterilization test), the four solutions were merged and mixed.

To test the sterility of α -amylase prior to preparation of the saliva, 500 μ L of α -amylase dissolved in PBS was incubated in a 6-well plate (Greiner Bio-One, Frickenhausen, Germany) with 15 g/L tryptone soya broth (Oxoid, Basingstoke, Hampshire, United Kingdom) and 186-medium control for 24 h at 37°C. If the media were comparable to the medium without α -amylase, the prepared α -amylase was used for the saliva solution.

2.3.3 Specimen Sterilization and Saliva Pretreatment

Specimens of the test materials (2.2) were fixed in 6-well plates with silicone (DMG, Hamburg, Germany) and sterilized by immersion in alcohol-based disinfectant (Bacillol AF, Hartmann, Heidenheim, Germany) or ethanol (70%) for 10 – 20 min, followed by three washing steps with sterile distilled water.

For the experiments with saliva, the test specimens were incubated with freshly prepared saliva at 37°C for 2 h. Afterwards, saliva was removed and the specimens were washed twice with PBS.

2.3.4 Pre-Stained Adhesion and Confocal Microscopy

Following centrifugation (10 min, 4000 × g), the yeast pre-culture was washed twice with 50 mM Tris-HCl. To prepare the washing solution, Tris (hydroxymethyl)aminomethane (Tris, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) was dissolved in deionized water, and the pH was adjusted to 7.5 using hydrochloric acid (HCl, Carl Roth GmbH & Co. KG, Karlsruhe, Germany). After each washing step, the cultures were centrifuged at 4,000 × g for 10 min. The cell pellet was resuspended in PBS and the optical density was adjusted to 1.0. *C. albicans* cells were subsequently dyed with Live/Dead staining, containing propidium iodide (PI) and SYTO9 (BacLight Bacterial Viability Kit, Invitrogen by Thermo Fisher Scientific, Schwerte, Germany) dissolved in PBS (final concentration: 1 µM) for 30 min under continuous shaking at 30°C [32]. After washing the stained cells with PBS, they were diluted 1:2 with PBS to an optical density (OD₆₀₀) of 0.5.

The cell suspensions were then seeded onto the test specimens as well as directly into the 6-well plates for control purposes, for 1 h at 30°C without agitation. All surfaces were washed twice with PBS before adhering cells were fixed with 2.5% glutaraldehyde (GA, Roth, Karlsruhe, Germany) in PBS for 15 – 20 min. At the end of fixation, the specimens were washed twice and covered with PBS. Adherent *C. albicans* cells were examined using CLSM (Leica TCS SP8, Leica Microsystems, Mannheim, Germany) at 63× magnification. The excitation for the SYTO9 dye was at 488 nm and the emission spectrum was measured from 500 – 550 nm. For the propidium iodide dye, the excitation and emission were at 552 nm and 675 – 750 nm, respectively. Five image stacks were captured for each specimen and control group, with a z-step size of 1 µm. The biofilms were analysed using the surface wizard of the Imaris x64 8.4.2 software package (Bitplane AG, Zurich, Switzerland).

2.3.5 Biofilm Formation and Fluorescence Staining

Yeast overnight cultures were harvested by centrifugation, the 186-medium was renewed and the OD₆₀₀ was adjusted to 0.1. The cells were seeded onto the specimens and reference 6-well plates for control as described above and then incubated for 24 h. Unlike for the adhesion process, incubation for biofilm formation was performed at 37°C in the presence of CO₂ without agitation.

The biofilms were then rinsed twice with PBS and stained with a solution of SYTO9 and PI dyes (1 mM in PBS, BacLight Bacterial Viability Kit) for 30 min. The biofilms were again washed twice with PBS and then fixed with 2.5% GA for 15 – 20 min. At the end of fixation, the biofilms were washed twice, covered with PBS, and then analysed by CLSM as explained above.

2.4 Statistical Analysis

Data analyses were performed with different statistics software for surface parameters (SPSS Statistics 29, IBM, USA) and for microbiological results (GraphPad Prism, Version 10). Surface parameters and

microbiological data were analysed for normal distribution using the Shapiro-Wilk test. Mean values and standard deviations were calculated for R_a (μm), R_z (μm), SFE (mJ/m^2) as well as its polar (mJ/m^2) and disperse (mJ/m^2) fractions. Statistical differences were tested using the non-parametric Kruskal-Wallis test for independent specimens and Dunn's multiple comparison correction. Microbiological data sets included at least $n = 36$ images. Statistical significance was defined with $\alpha = 0.05$.

3 Results

3.1 Surface Properties of Differently Treated Polymeric Materials

3.1.1 Surface Roughness and Surface Free Energy

Initially, after specimen preparations, surfaces were characterized regarding roughness and SFE. **Table 1** displays the mean values and standard deviations of the roughness parameters R_a and R_z as well as SFE and its corresponding polar and disperse fractions. Uniform surface roughness was achieved for both surface treatments, with low standard deviations. For surface treatment A (rough), all materials exhibited a mean R_a value of $0.21 \mu\text{m} \pm 0.04 \mu\text{m}$. For surface treatment B (fine), PTFE and PVC precisely attained a mean roughness value of $R_a = 0.04 \mu\text{m}$, while PE exhibited a slightly higher mean R_a value of $0.05 \mu\text{m}$. For the surface roughness parameter R_z , the results were comparable within the treatment groups but with slightly higher variations. All materials with surface treatment A showed significant differences in both R_a and R_z compared to those with surface treatment B ($p \leq 0.048$; Dunn post-hoc test). For R_z , the difference for PVC, PTFE, and PE was statistically significant in some cases, as indicated in Table 1.

The three materials featured differences in their SFE (**Table 1**). The surface treatments A (rough) and B (fine) mainly influenced the SFE of PVC and PTFE, but not of PE. PTFE (A) showed the lowest SFE, which was significantly lower than for PVC (A and B) and PE (A and B). PVC (B) showed the highest SFE. The dispersive fraction of the SFE followed a similar trend. For the polar fraction of the SFE, all values were within a common range, except PTFE (B), which was significantly higher.

Table 1: Mean values and standard deviations (SD) of the different surface parameters for three polymeric materials after two different surface treatments. The same letters indicate statistically significant differences of the individual parameters within the material and surface treatment groups ($p \leq 0.05$).

material	surface treatment	R _a (μm)	R _z (μm)	SFE (mJ/m ²)	polar (mJ/m ²)	disperse (mJ/m ²)
PVC	A	0.21 (0.04) ^{abc}	1.42 (0.25) ^{abc}	29.81 (5.04) ^{ac}	0.76 (0.69)	29.06 (4.62) ^a
	B	0.04 (0.01) ^{adg}	0.54 (0.09) ^{adf}	37.09 (4.94) ^e	0.32 (0.43) ^c	36.78 (5.15) ^{ce}
PTFE	A	0.21 (0.05) ^{def}	1.41 (0.30) ^{de}	17.73 (3.98) ^{abcd}	0.48 (0.84) ^a	17.25 (3.52) ^{abcd}
	B	0.04 (0.00) ^{beh}	0.48 (0.04) ^{beg}	25.75 (3.75) ^e	1.75 (1.18) ^{abc}	24.00 (3.59) ^{ef}
PE	A	0.21 (0.04) ^{ghi}	1.37 (0.24) ^{fg}	32.59 (2.77) ^b	0.16 (0.23) ^b	32.44 (2.61) ^b
	B	0.05 (0.02) ^{cfi}	0.60 (0.08) ^c	33.68 (3.68) ^d	0.49 (0.45)	32.94 (4.02) ^{df}

3.1.2 Qualitative Surface Topography

Qualitative assessments of the surface topography were performed using two microscopic methods, CLSM and SEM, for differently scaled resolution. CLSM analysis revealed that all surfaces exhibited uniform, crossed grinding marks (**Figure 2**). The maximum height varied only slightly for surface treatment A, with PE showing the highest value (84.1 μm) and PVC the lowest (83.7 μm). For surface treatment B, PE had the highest measured value (79.8 μm) and PTFE the lowest (77.8 μm). The CLSM data showed minor visual differences and the overall roughness values were within a narrow range, which could indicate minimal topographical differences between the surface treatments.

The SEM images provided a more detailed analysis of the surface topography and complemented the CLSM observations (**Figure 2**). Grinding lines were visible on all surfaces, but their appearance clearly depended on the material and surface treatment. For treatment A, PVC showed grinding lines with a crossed structure and isolated areas of brightening, possibly due to material properties or light scattering effects. PTFE displayed isolated dark spots, which were most likely attributed to dust particles on the surface. The SEM images of PE revealed increased elevations on the materials surface. In contrast, treatment B resulted in more homogeneous surfaces across all materials. The bright areas previously visible on PVC (A) were no longer detectable, suggesting a smoothing effect. Additionally, the elevations observed on PE in treatment A were no longer distinguishable, indicating a potential reduction in surface irregularities.

These SEM findings are consistent with the CLSM analysis, in which the surface irregularities were more pronounced in surface treatment A and less pronounced in surface treatment B. The SEM results

confirm these trends in detail and illustrate the material-dependent reaction to the surface treatments.

Figure 2: Exemplary CLSM and SEM images of the three polymeric material surfaces after the different treatments (CLSM: magnification 5'; SEM: magnification 850'; white bar: 140 µm for each image).

3.2 Adhesion of *C. albicans* on the Polymeric Materials with and without Saliva Coating

Following the technical surface analysis, specimens were subjected to one-hour pre-stained yeast cell adhesion with subsequent microscopic evaluation. **Figure 3a** displays the total cell volume on surfaces without saliva coating. Even though the data showed relatively high standard deviation, it is apparent that the cell volume on PTFE (A and B) (with an average volume of less than $1 \times 10^4 \mu\text{m}^3$ per image) was significantly lower than on all other polymeric materials, but almost comparable to the control (well plate bottom; **Figure 3a**). In contrast, the total volumes on PVC (A and B) and PE (A) were significantly higher than the control.

Figure 3c displays the adhesion results for saliva coated surfaces. For PVC and PTFE, surfaces after treatment B (fine) showed a total cell volume that was significantly higher than the control, but also significantly higher than their respective A-treated surface. Cell adhesion on PTFE (A) was also significantly lower than on all other surfaces. In contrast, compared to the control, cell volume was significantly increased on both PE surfaces. When comparing saliva coated with uncoated surfaces, the strongest difference could be observed for PTFE (B): Whereas the surfaces showed significantly decreased cell volume without saliva, it was the opposite after saliva coating. The application of artificial saliva could also affect the adhesion of *C. albicans* to PTFE B as shown in **Figure 3e** and **Figure 3f**.

The ratio of viable to dead cells is depicted in **Figure 3b** and **Figure 3d**. Despite some small, even though statistically significant differences, the values remained largely constant. At least 90% of the cells were viable.

Figure 3: Mean and standard deviation (SD) of the total cell volume (μm^3 per $184.52 \mu\text{m}^2$, a, c) and the percentage of live/dead cells (b, d) of *C. albicans* adhesion on polymeric materials with different treatment and without (a, b) and with (c, d) saliva coating. (n = 36).

*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001

Representative CLSM images of *C. albicans* adhesion on different polymeric materials without (e) and with (f) saliva coating (magnification 63'; white bar: 20 µm for each image; Imaris x64). Green fluorescence indicates vital cells, whereas dead cells are shown in red. The acquired images were visualized in 3D.

3.3 Biofilm Formation of *C. albicans* on the Polymeric Materials with and without Saliva Coating

Finally, the different polymeric materials were analysed for *C. albicans* biofilm formation followed by fluorescence staining and microscopic evaluation. **Figure 4a** illustrates the total biofilm volume on surfaces without saliva coating. All values were significantly lower than the control (well-plate bottom), and also differences between the three surfaces could be observed. On PVC (A and B), the biofilm volume remained significantly lower than on the other materials. Conversely, biofilm formation was significantly higher on PE (A and B) than on PVC and PTFE. Especially on PE (A), more cells were able to form hyphae (**Figure 4e**). Effects of the different surface treatments could not be observed for biofilm volume.

Figure 4c illustrates the biofilm volume results for saliva-coated surfaces and revealed notable contrasts to uncoated surfaces. The data showed that saliva coating had a significant effect on biofilm formation, and resulted in a significant reduction in biofilm volume on all surfaces. When comparing biofilm volume amongst polymeric surfaces, with the exception of PTFE (A), the lowest biofilm volume was observed for PVC (A), while the highest volume was observed for PE (A) (**Figure 4f**).

The images in **Figure 4e** and **Figure 4f** also indicate that the application of artificial saliva reduced the formation of hyphae by *C. albicans* especially on the control surfaces.

The ratio of living and dead cells in biofilms on the different polymeric materials is displayed in **Figure 4b** and **Figure 4d**. Although there were some significant differences between the materials, the percentage of living cells exceeded the percentage of dead cells by more than 90%.

Figure 4: Mean and standard deviation (SD) of the total cell volume (μm^3 per $184.52 \mu\text{m}^2$, a, c) and the percentage of live/dead cells (b, d) of *C. albicans* biofilm formation on polymeric materials with different treatments and without (a, b) and with (c, d) saliva coating (n = 36). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$

Representative CLSM images of biofilm formation (control, PVC, PTFE, PE) without (e) and with (f) saliva; magnification $63\times$; white bar: $20 \mu\text{m}$ for each image; Imaris x64, where the acquired images were visualized in 3D. Green fluorescence indicates vital cells, whereas dead cells are shown in red.

4 Discussion

Colonization of polymeric prosthesis materials by the facultatively pathogenic yeast *C. albicans* in the oral cavity can be the onset of severe and chronic tissue infections. To contribute to the elucidation of the underlying microbiologic processes, this study investigated the adhesion and biofilm formation of *C. albicans* on three general polymeric materials (PVC, PTFE, and PE), and focussed on the effects of surface roughness, SFE, and artificial saliva coating. The results demonstrated that *C. albicans* cell adhesion and biofilm formation mainly depended on the polymeric material and its intrinsic SFE, with

lower adhesion on materials with lower SFE. Roughness and saliva coating had a minor effect, which was material-specific and equally prominent for cell adhesion. In contrast, for biofilm formation, no effect of surface roughness could be observed, whereas saliva coating reduced biofilm formation on all materials.

In this baseline study, a single *Candida* strain was selected to ensure reproducibility and comparability while minimizing variability. Using a well-characterized reference strain allows for fundamental insights into adhesion and biofilm formation while avoiding strain-specific complexities. This approach simplifies data interpretation, conserves resources, and is well-suited for preliminary investigations. In the future, the results of this study should be verified with different, preferably clinical *Candida* strains. Likewise, artificial saliva was used for coating, as it allows for a higher reproducibility compared to (pooled) saliva from volunteers. Preliminary experiments from our group have already indicated that the artificial saliva used produces comparable results to natural saliva regarding bacterial adhesion [33]. Saliva plays a critical role in microbial colonization because the surface energy of the pellicle influences microbial adhesion by the binding of specific proteins (adhesins) on microorganisms to receptors in the pellicle [34–36]. The pellicle contains glycoproteins and molecules that bind microorganisms, which could explain the variable levels of adhesion to surfaces [17, 34].

As substrate materials, the polymers PVC, PTFE and PE were selected for this study due to their distinct surface properties. PVC is a polar polymer with a moderate SFE and relatively high wettability. In contrast, PTFE has a very low SFE and extreme hydrophobicity, which can make microbial adhesion more difficult [37]. PE is a non-polar polymer with a moderate SFE. All materials were analysed with two different surface roughness. As commonly referenced in microbial adhesion studies, the $R_a = 0.2 \mu\text{m}$ roughness threshold [7, 14] was used as one of two reference surface treatments in the current study. In addition, an extremely smooth surface with a roughness of $R_a = 0.04 \mu\text{m}$ was produced to assess whether minimal surface roughness can reduce *C. albicans* adhesion [7]. The compliance with these theoretical parameters was verified by line roughness measurement. Profilometric surface analysis is a standard measurement approach, even though having distinct limitations, including potential surface damage resulting from the diamond tip and its dependency on the resolution on tip size. Nevertheless, this method features the advantage that the data can be directly compared with further research.

After cell seeding, live/dead staining and CLSM were used to examine the adhesion and biofilm formation of *C. albicans*. This technique uses specific dyes to differentiate between live and dead cells based on their membrane integrity and facilitates the assessment of cell viability [38, 39]. In addition, CLSM produces high-contrast 3D images that can be used to study the native morphology of *C. albicans* cells and biofilm [40, 41].

4.1 Adhesion without Saliva

The adhesion of *C. albicans* showed significant dependencies on the polymeric material, SFE, and roughness, which supports our research hypotheses. The most decisive influence could be observed for the polymeric material and SFE. The adhesion of *C. albicans* on PTFE was generally lower than on PVC and PE, which aligns also with the material's SFE. It is a general observation that low SFE corresponds to reduced wettability and, as a result, less microbial adhesion, which is consistent with the hydrophobic nature of such surfaces. The hydrophilicity of a material directly influences cell adhesion by modifying cell-surface interactions, as these are governed by cell adhesion molecules anchored within the cell membrane [42]. PTFE is known as a repellent, self-cleaning material, thereby enhancing oral hygiene [37], which in turn reduces the likelihood of cell adhesion. Therefore, the reduced adhesion of *C. albicans* to PTFE surfaces is most probably a result of their surface properties, including hydrophobicity and low SFE. This is also in line with other studies that have shown a greater tendency for adhesion of *Candida* species to PVC catheters used for central venous access *in vitro* [43, 44] than to PTFE catheters [45], highlighting the material-dependency of microbial adhesion.

In contrast to material type and SFE, roughness had a varying effect depending on the polymeric material. For PTFE and PE, significantly more *C. albicans* cells were detected on the rougher surface. A rougher surface offers *C. albicans* a larger surface area and, thus, better adhesion possibilities [46, 47]. For bacterial adhesion on PTFE, several studies have demonstrated that lower surface roughness could potentially reduce bacterial colonization, although this effect remains strongly material-dependent. PTFE's smooth surface, despite its small pores, provides limited area for cell attachment, and thereby contributes to its effectiveness in minimizing bacterial adhesion in a dental context [37]. In contrast for PVC, reduced roughness did not lead to reduced cell adhesion. Probably, this observation can be attributed to a substantially higher SFE on the rough surface, resulting in greater wettability, which promotes adhesion. These results underscore the fundamental role of material composition and SFE as key contributors to *C. albicans* adhesion.

4.2 Adhesion with Saliva

In the presence of artificial saliva, *C. albicans* adhesion varied depending on both material and surface roughness. The rough surface of PVC produced decreased adhesion, while finer-polished PTFE exhibited increased adhesion in the presence of artificial saliva. When taking SFE results into account, artificial saliva influenced adhesion particularly among specimens with similar SFEs, such as PVC (A) and PTFE (B) (**Supplement 1**) – probably due to matching physicochemical characteristics. This observation indicates that artificial saliva exhibited only a minor effect on *C. albicans* adhesion in comparison to the influence of SFE. Additionally, judging from the current data, SFE rather than roughness may influence adhesion in the presence of an artificial salivary pellicle, although the overall effects of saliva on adhesion remain limited in comparison to the effect observed by the different materials and surface treatments without artificial saliva.

4.3 Biofilm without Saliva

As the previous adhesion results only allowed yeast-surface interactions for a limited period of time and without supportive nutrients, clinically relevant biofilm formation over 24 hours in full medium was analyzed, too. The biofilm results again confirm the hypothesis of this study that surface treatments and material properties significantly influence biofilm formation and cell morphology of *C. albicans*. Compared to the well-plate control, polymeric materials exhibited significant reduction in hyphae formation. However, independently of roughness values, variations in biofilm volumes highlighted SFE as a key factor for biofilm formation. Materials with lower SFE exhibited significantly reduced biofilm volumes compared to materials with higher SFE, even though this effect was not as pronounced as for cell adhesion. This is most probably again due to limited wettability that hindered cell attachment and spread. In contrast to the adhesion experiments, PVC showed lower cell density than PTFE after 24 h of incubation. This phenomenon is perhaps due to elevated temperatures or the presence of CO₂ during biofilm formation conditions [48]. This finding is additionally supported by data published by Estivill et al., who reported less *C. albicans* biofilm formation on PVC than on PTFE, independently of the surface parameters [49].

In conclusion, the analysis of bare polymeric surfaces supports the observation that SFE is the primary determinant of *C. albicans* biofilm formation, while surface roughness had only a minor impact.

4.4 Biofilm with Saliva

When comparing the results of biofilm formation with and without saliva pre-treatment, the application of artificial saliva significantly reduced biofilm volume across all materials, with the control group showing a reduction of over 50% (**Supplement 2**). This decrease may be attributed to salivary proteins that coat the surfaces, blocking cell adhesion sites and thereby inhibiting biofilm formation [50], particularly on PTFE. Similar findings by Martínez-Hernández et al. confirm that saliva surface treatment reduces biofilm formation [51]. Interestingly, this effect could only partly be seen for the previously performed adhesion experiments. Possibly, different adhesion sub-processes occur in a timely manner and saliva effects it at a later stage. Rough surfaces continued to exhibit lower biofilm volumes than finely polished surfaces, which is consistent with the observations made in the absence of saliva. Furthermore, PE specimens with different roughness levels showed similar biofilm formation, and this is probably due to comparable SFE values.

In summary, artificial saliva effectively reduced biofilm formation on all experimental surfaces. Artificial saliva had a stronger influence on the biofilm formation than roughness and SFE. While roughness had a minor role, SFE remained crucial – lower SFE led to decreased biofilm formation. If exposed to artificial saliva, SFE appeared to be a more relevant factor in biofilm reduction than roughness. Furthermore, saliva exposure reduced hyphal formation, in particular in the control and PE. PE consistently showed

higher biofilm volumes than other materials, indicating that its surface properties may more readily support adhesion, hyphal development, and robust biofilm formation.

4.5 Limitations of the Study

Even though the above data nicely describe the different and hierarchical effects of SFE, roughness, and saliva treatment on *C. albicans*, they have to be interpreted within the limitations of the study, some of which are due to its *in vitro* design. A major limitation is the use of a single *Candida* strain, although it is the most common yeast in oral infections and a primary cause of denture stomatitis. While this ensures reproducibility, it may not capture strain-specific differences in adhesion and biofilm formation. Future studies should include multiple strains and clinical isolates for a more comprehensive analysis. Furthermore, limited incubation periods may have affected morphological characteristics and hyphal formation. Shear stress during incubation and experimentation could also have impacted cell adherence. Due to the *in vitro* design, some relevant *in vivo* factors, such as host immune response and the natural oral microbiome with its multiple interacting microbial species, could not be adequately simulated. Future studies should therefore also consider multispecies biofilm models, for example via analysis of *in situ* grown biofilms.

5 Conclusion

This study highlights the significant and hierarchical effects of SFE, roughness, and saliva coating on the adhesion and biofilm formation of the clinically relevant yeast *C. albicans* on a variety of standard polymeric materials. For both, initial adhesion and biofilm formation, the strongest effect could be observed due to the material's SFE. Materials with lower SFE showed reduced adhesion and biofilm formation. Variation in roughness affected only *Candida* adhesion by influencing the SFE and providing different available surface areas for the cells. Saliva treatment in general led to a reduced adhesion and especially biofilm formation, the latter probably due to the increased incubation time. Overall, the study suggests that reducing polymer surface's SFE by material choice and pre-treatment may be an effective strategy to minimize *C. albicans* adhesion and biofilm formation, and potentially to reduce the risk of denture-associated candidiasis.

Declarations

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Author contributions

L.B. and S.T. contributed to experimental design, data acquisition, and the analysis of the results, as well as wrote the original manuscript. L.B. was responsible for the material-specific part and S.T. for the

microbial part. S.H. and N.K. acquired funding, supervised the study, contributed to design of the experiment, and revised the manuscript. M.S. and K.D.-N. supervised the study and contributed to the revision of the written work. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interests

The authors declare no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

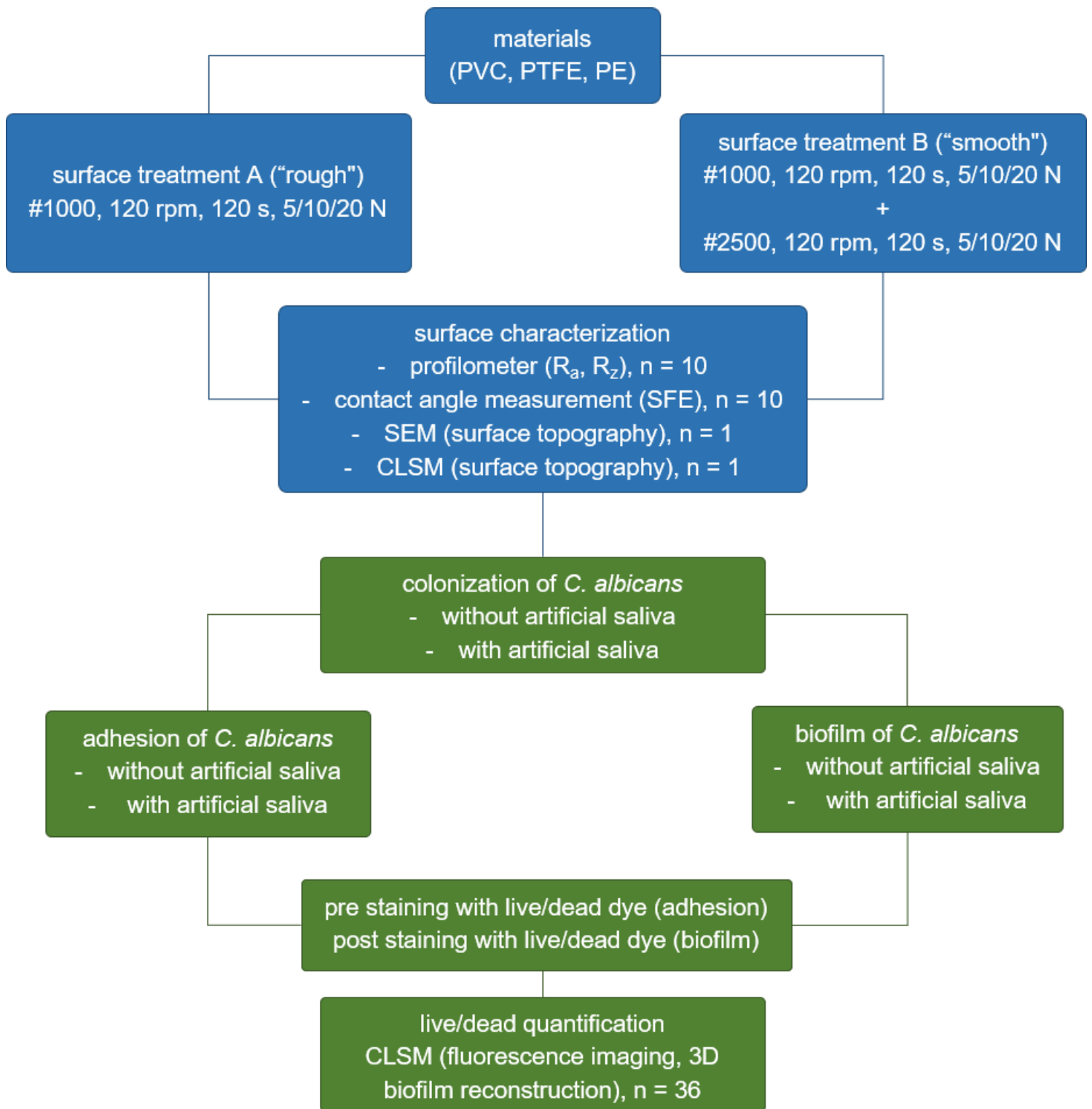


Figure 1

Study design.

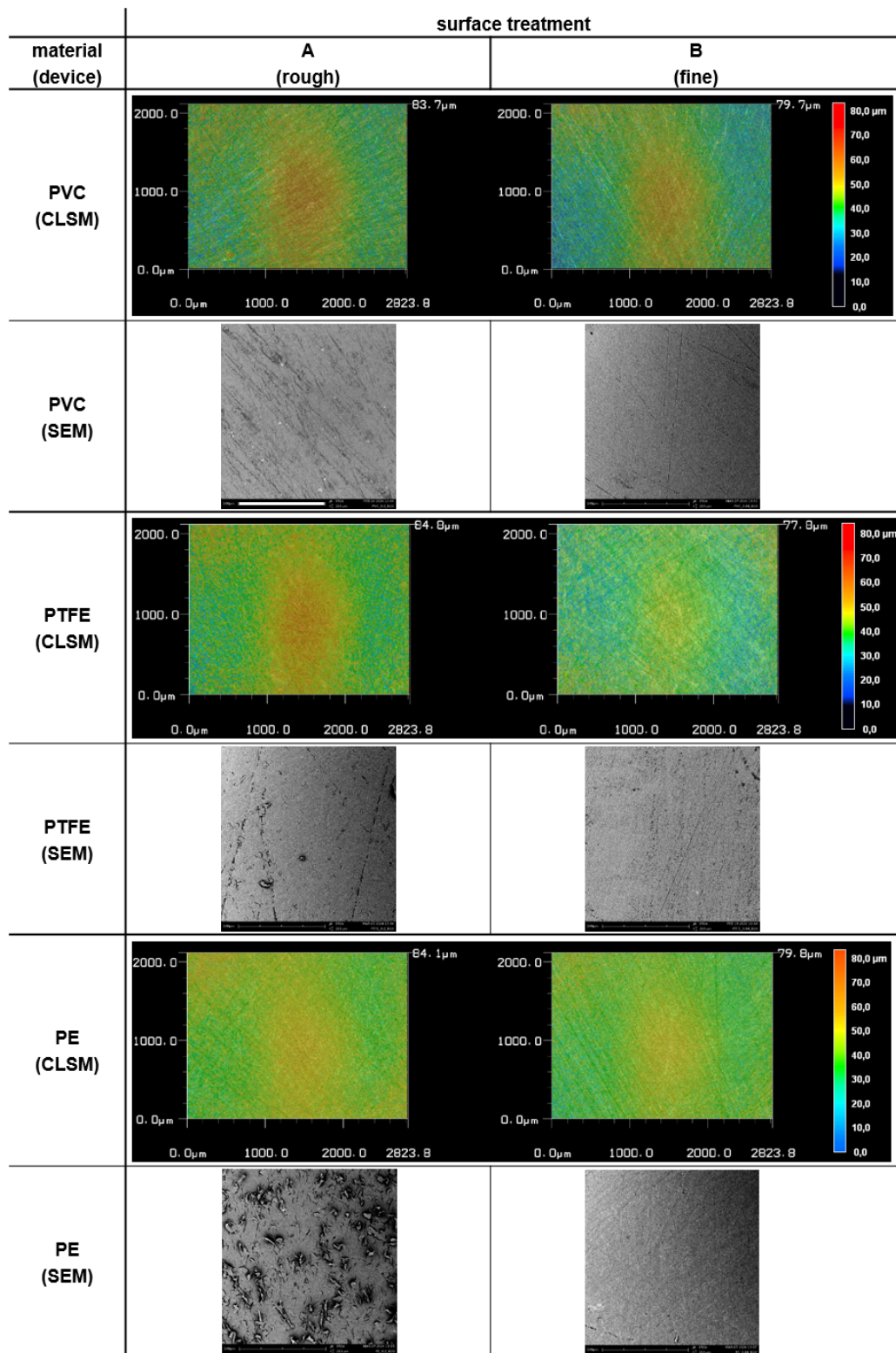


Figure 2

Exemplary CLSM and SEM images of the three polymeric material surfaces after the different treatments (CLSM: magnification 5'; SEM: magnification 850'; white bar: 140 μm for each image).

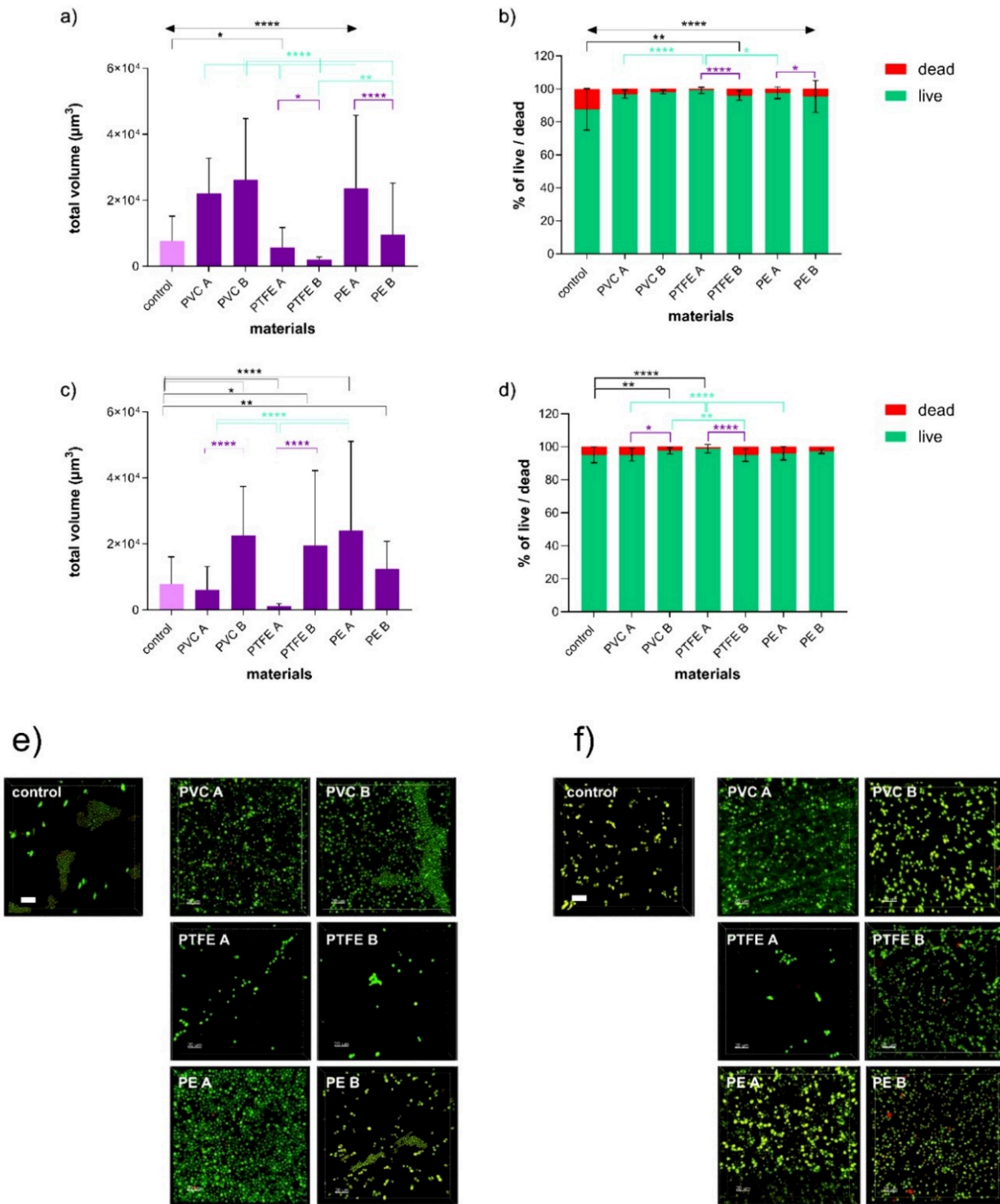


Figure 3

Mean and standard deviation (SD) of the total cell volume (μm^3 per $184.52 \mu\text{m}^2$, a, c) and the percentage of live/dead cells (b, d) of *C. albicans* adhesion on polymeric materials with different treatment and without (a, b) and with (c, d) saliva coating. (n = 36).

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$

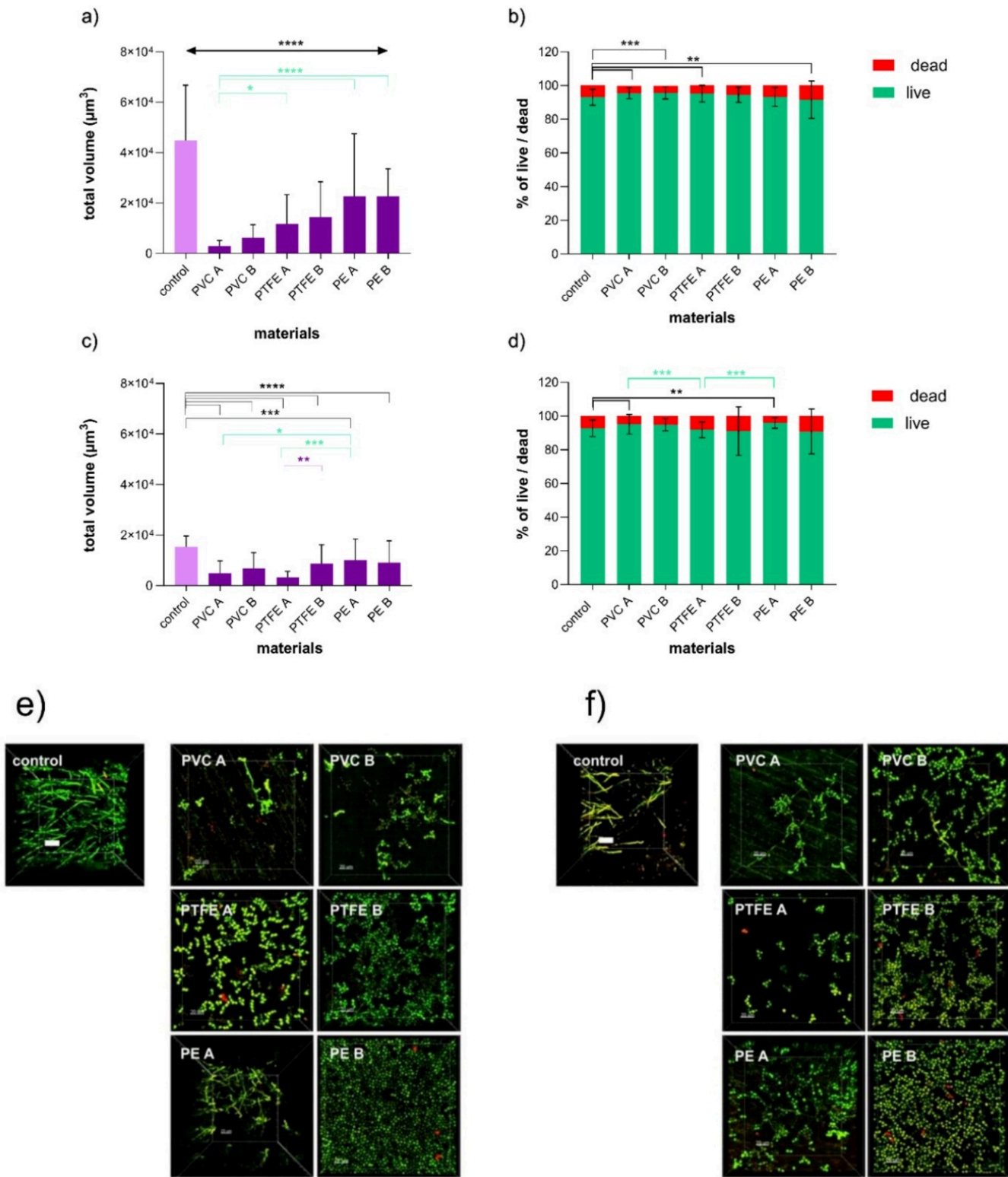


Figure 4

Mean and standard deviation (SD) of the total cell volume (μm^3 per $184.52 \mu\text{m}^2$, a, c) and the percentage of live/dead cells (b, d) of *C. albicans* biofilm formation on polymeric materials with different treatments and without (a, b) and with (c, d) saliva coating (n = 36). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$