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RESEARCH ARTICLE

Differences in *Candida Albicans* hydrophobicity and biofilm formation between thermoplastic nylon and polyether-ether-ketone denture base materials

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ABSTRACT

Polyetheretherketone (PEEK) and thermoplastics nylon are extensively composed as an alternative material for removable denture base. The primary difference between the two materials is associated with water absorption properties. The two materials interact with saliva and microorganisms during application in the oral cavity. Candida is predominantly reported in the cavity. The microorganism is distinguished by its hydrophobicity and ability to form biofilms. The objective of this study was to determine the differences between thermoplastic nylon and poly-ether-ether-ketone as removable denture materials in terms of hydrophobicity and Candida albicans biofilms. It is a laboratory experiment using 20 5 x 2 mm samples for the hydrophobicity test and Candida albicans biofilm. The hydrophobicity test was performed by determining the contact angle with a Goniometer and the WinDrop++ software. The biofilm test samples were divided into 4 groups, encompassing PEG 5% (negative control), PEEK treatment, thermoplastics nylon, and 0.12% chlorhexidine (control positive). The microdilution method was employed to examine biofilm formation. The results of the study were interpreted, and the data were analyzed by employing the t test. The research discovered significant differences in biofilm formation, large contact angle on PEEK and thermoplastics nylon ($p < 0.05$). Biofilm formation on thermoplastics is significantly larger than PEEK ($p < 0.05$). In conclusion, the contact angles of PEEK and thermoplastic nylon are incorporated in the partially wetted. Polyetheretherketone is involved in the non-producers of biofilms while thermoplastic nylon is categorized in of weak biofilm formers.

Keywords: *Candida albicans* biofilm; hydrophobicity; PEEK; thermoplastic nylon

INTRODUCTION

Patients experiencing teeth loss possess limited treatment options encompassing removable partial denture, fixed partial denture and prosthesis teeth implant. The substructure of the base plate and supplementary sustaining dentures in partial dentures promote the recovery of the original tooth function. Modern removable partial dentures are made from a variety of materials, including metal and nonmetal. Acrylic resin, thermoplastics such as polyamide, polyester, polycarbonate, acrylic, and polypropylene are frequently used materials. Dentures made of metal framework (denture frame) are considered to be superior to acrylic due to the properties of a thinner layer that is rigid and solid.[1] However, this type of denture poses risks of allergic reaction allergy in association with metal substance.[2] Thermoplastics provides unique advantages incorporating aesthetic and flexibility,[3] despite water absorbance, rough surface after use and complex manufacturing processes.[4,5] Furthermore, thermoplastic nylon is fluid absorbent which leads to decreased solidity, decolorization and increased surface roughness after one week application, and processing difficulties.[4,5] Surface roughness influences biofilm formation by providing a retention area for food residue and microorganisms. Excessive surface roughness may impede biofilm removal. Surface roughness less than 2 m has been discovered to prevent bacterial colonization.[6]

Research in dentistry has developed reliable inventions to complement the existing material. Polyetheretherketone (PEEK) is a thermoplastic polymer generally utilized for orthopedic care.[7] PEEK contains an excellent elastic modulus,[8] less allergenic and is able to avoid plaque formation.[9,10] Furthermore, PEEK is a non-water-soluble material and non-fluid absorbent.[11] PEEK is a white, radiolucent, and rigid material with eminent thermal stability up to 335.8 °C. PEEK initially adopted in biomedical applications[12] has enhanced to be an alternative component for metal implant, particularly for post-traumatic, orthopedic and spinal implants.[13] Within prosthodontics care, PEEK is an advanced material for removable partial denture, fixed partial denture and maxillofacial.[14] Considering the mechanical properties of PEEK, this material can be utilized as teeth implant, removable partial denture and fixed partial denture.[15]

Contact angle is the boundary between fluid materials with solid surface. Liquid materials when it poured to solid surface will expand to the whole surface. The contact angle is estimated to be zero degrees in this particular circumstance. If the contact angle is between 0° and 30°, the solid material's surface cannot be categorized as hydrophilic. In contrast, a hydrophobic solid surface has a contact angle significantly larger than 150° and may even surpass 180°. During this phenomenon, the liquid rests on the surface without soaking or spreading, indicating that the surface is superhydrophobic. The contact angle determines the ability of a liquid to soak the surface of a solid object. The ability of liquid to soak the surface was reduced as the contact angle increased. Thus, if the contact angle formed is 0 degrees, a liquid can flow completely on the surface.[16] Angle below 30° is considered as water-absorbing or hydrophilic, while 30° – 89° partially wetted, and above 90° categorized into water-repellent or hydrophobic.[17] Several factors affecting the contact angle are the chemical structure, surface smoothness, porous filling, and presence of foreign substances on the surface.[18]

Hydrophobicity is a physics molecular property on the material surface which is repelled by the liquid. The contact angle is estimated to be zero degrees in this particular circumstance. If the contact angle is between 0o and 30°, the solid material's surface cannot be categorized as hydrophilic. In contrast, a hydrophobic solid surface has a contact angle significantly larger than 150° and may even surpass 180°. During this phenomenon, the liquid rests on the surface without soaking or spreading, indicating that the surface is superhydrophobic. The contact angle determines the ability of a liquid to soak the surface of a solid object. The ability of liquid to soak the surface was reduced as the contact angle increased. Thus, if the contact angle formed is 0 degrees, a liquid can flow completely on the surface. The goniometer comprises a CCD camera to record the image of a droplet located onto the surface by employing a microsyringe and a dedicated image processing software to determine the contact angle. Measurements in two different positions were administered for each specimen.[19]

Candida albicans is an organism which easily attaches to water. In a fact that water possesses contact angle smaller than 20 degrees, this substance is unfavorable to attach to solid objects. Therefore, it creates solid objects hydrophobic.[20] Thermoplastic nylon and PEEK, as denture materials, are constantly in contact with salivary fluid and microorganisms. Food residue and plaque tend to adhere to dentures, compromising the oral cavity's health and hygiene by causing stains, calculus, and biofilms. Candida albicans is a fungus that is predominantly discovered in the oral cavity. Food glycoproteins are precipitated by saliva during the mastication process, resulting in the accumulation of a thin layer of pellicle in a short period of time[21] Pellicle adversely promotes microorganism attachment and produces biofilms matrix complex.[21,22] Biofilms generated on teeth surfaces turned into teeth plaque.[23] Plaque also formulates on dentures,[24] however, lack of reported information about complications or biofilm formation on PEEK[12] surfaces.

Candida albicans could proliferate and cause infection when individual immune system and the microorganism altered due to pH changes, antibiotics discharge and immunosuppression. Symptoms affected by Candida varied from mucosa dermal layer infection as thrush, diaper rash, vaginal infections, to Candidemia.[25] Biofilms are layers constructed by microbe colonies that are immobile, mucus slimy, and difficult to detach from substrate surfaces. Deposition, microbial bond formation (planktonic) on films, growth, bacteria colonization, and biofilm formation are all stages in the development of biofilms.[26] The process of biofilm formation begins with microorganisms attached to the pellicle forming colonies.[27] Once the colony was established, microorganisms generated matrix complex. Matrix serves protection, provides nutrition, adhesion, and stabilization during the biofilm formation process.[28,29] *Candida albicans* biofilms are developed through four processes. First, yeast cells attach to solid surfaces (mucosa, skin, and medical implants) through seeding, which requires 60-90 minutes. Second, *Candida albicans* cells proliferate and yeast cells begin to produce filament. Third, biofilm maturation is characterized by the discovery of cell morphology, such as hyphae, pseudohyphae, and yeast, which produce extracellular matrix for thick and solid biofilm. The maturation process can last up to 24 hours and may be observed under a microscope. The fourth and final process is dispersion, which occurs when yeast cells break free from biofilms to grow in a new location.[30]

Formation of *Candida albicans* biofilm in oral cavity occurs through three phases, comprising of adhesion (0-12 hours), initiation (12-42 hours), maturation (42-72 hours) and dissemination above 72 hours, SEM and crystal violet employed to observe biofilm formation.[31] A biofilm detection test can be conducted with Microtiter Plate Assay method.[32] The objective of this study was to investigate the difference between the two materials on hydrophobicity and *Candida albicans* biofilm.

MATERIALS AND METHODS

This experimental laboratory research has been approved by the ethical board with number 00697/KKEP/FKG-UGM/EC/2021. A total of 20 pieces 5x2 mm made of thermoplastic nylon were incorporated in the sampling. Thermoplastic nylon and PEEK were administered with injection molding method. Both materials were examined for hydrophobicity and biofilm. The hydrophobicity test was conducted by calculating contact angle. Sample surfaces were pulverized by employing sandpaper 4 grit 5000 and washed by ultrasonic cleaning. A beam arranged to produce a surface plate and locate the sample on top of the glass slide. The camera focus was adjusted to capture detailed images within a close distance. Distilled water of 6 µm was dropped upright on the glass slide continuously for 5 minutes. At 5 minutes, drops of distilled water were photographed. The images were observed and processed by administering the ImageJ application. The t-test was utilized to statistically evaluate the measurement results on both samples before and after contact with *Candida albicans*.

The biofilm formation test was conducted by employing the microdilution method: both samples of *Candida albicans* suspension were administered with BHI and 2% sucrose into polystyrene U microplates. The total volume of each well was 90 µL. In the positive control group, 0.12% chlorhexidine was utilized while negative control employed PEG 5%. Microplates were incubated for 24-hours at 370 °C, rinsed with water 3 times. 1% crystal violet solution in 125 µL was added for each well, and further incubated at room temperature for 15 minutes. The microplate was rinsed with water 3x. Addition of 200 µL 96% ethanol to each well applying a 50-200 µL micropipette was conducted. Incubation period of 15 minutes in a room temperature were performed. Each 150 µL of solution was transferred to a 24 well flat bottom polystyrene microplate. The biofilm was observed by Biorad Benchmark® microplate reader with a wavelength of 595 nm. In this method, the presence of biofilms was identified by optical dentistry (OD) score. Control negative employed as blank value to identify exists biofilm formation. If the OD value of microbes is higher than blank OD value, the biofilms formation is in progress. Calculation of the ODcut off completed with the following approach: ODcut= Mean control OD Negative + (3x Standard deviation (SD) negative control). Microbial OD= Mean ODmicrobial – ODcut. The values of the ODcut off and ODmicrobial are interpreted with this approach: If ODmicrobial ≤ ODcut. It can be interpreted that there is no biofilm formation (non-biofilm producer). ODcut < ODmicrobial ≤ 2x ODcut, indicated as weak biofilm formation (weak film former). If 2x ODcut < ODmicrobial ≤ 4x ODcut categorized as moderate biofilm formation (moderate-biofilm former), and 4xODcut<Microbial OD, strong biofilms (strong-biofilm formers).[32] A t-test statistical analysis was employed to calculate the mean difference between two materials in the result of this study. Furthermore, SEM observations were performed on thermoplastic nylon and PEEK before and after the *Candida albicans* test.

RESULTS

Candida albicans biofilm formation and contact angle on thermoplastic nylon and PEEK as denture bases were evaluated. The hydrophobicity method was conducted to determine the contact angle of two materials, thermoplastic and PEEK, and was analyzed by employing two methods: the paired t test and the independent test. Paired t tests were administered to calculate data on nylon thermoplastics and PEEK before and after contact with *Candida albicans*. Data from the reduction results before and after contact with *Candida albicans* were utilized in an independent test to determine the effect of the difference in contact angles between thermoplastic nylon and PEEK. The results of calculating the mean and standard deviation before and after contact with *Candida albicans* for both materials are presented in Table 1.

Calculations by employing the paired t test demonstrated that the contact angle on both materials was revealed before being contacted with *Candida albican*s was greater than after being contacted, the contact angle had decreased. The correlation before and after contact with *Candida albicans* on thermoplastic nylon material (0.219 > 0.05) and on PEEK (0.419 > 0.05) unveiled no relationship between the influencing variables. Before and after being contacted with *Candida albicans* on each material, both demonstrated sig 0.00 < 0.05. It illustrates that there was difference. Thus, before and after contact with *Candida albican* on nylon thermoplastic materials, a significant difference was revealed. These results are also perceived in PEEK.

To determine the effect of *Candida albicans* on the contact angle, it was examined by independent t test. The data was acquired from reduction between before and after contact with *Candida albicans*. The calculations were performed by independent test analysis. Moreover, the results were in the forms of mean and standard deviation of Thermoplastic nylon 22.413 ± 5.311 and PEEK 9.385 ± 4.118. Both materials possess normally distributed data (0.274 and 0.562 > 0.05) and homogeneous (0.615 > 0.05). This data produces a significant t-value of sig 0.00 < 0.05, which indicates that there was difference in the mean result of thermoplastic nylon and PEEK. The contact angle on thermoplastic nylon is greater than that of PEEK. *Candida albicans* biofilm formation on the thermoplastic nylon and PEEK material were calculated by statistics analysis, mean and standards deviation as provided in Table 2.

Table 1. Mean and standard deviation of contact angle of thermoplastic nylon and PEEK before and after *Candida albicans* exposure (°)

Intervention	Mean	Standard deviation (±)	t measured	t table
A pre-test	105.335	5.899	12.836	2.262
A post-test	83.685	2.393		
B pre-test	86.700	4.139	7.206	2.262
B post-test	77.315	2.311		

Description:
A: Thermoplastic nylon
B: Poly-ether-ether-ketone (PEEK)

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Table 2. Mean of biofilm formation on thermoplastic nylon and PEEK

Materials	Mean ± SD
Control – (Blank)	0.412 ± 0.015
Control + (positive)	0.218 ± 0.465
Thermoplastics nylon	0.967 ± 0.367
PEEK	0.543 ± 0.137

It was also revealed that the mean and standard deviation for thermoplastics nylon were greater than PEEK, by 0.967 ± 0.367 > 0.543 ± 0.137. The Levene normality and homogeneity test resulted 0.144 > 0.05, which confirmed homogeneous data. The Shapiro-Wilk normality test on thermoplastics nylon was 0.807 while on PEEK 0.882, implied normally distributed data. T-test demonstrated t count 2.649 > t table 2.015, indicated as significant difference between thermoplastic nylon and PEEK. Thermoplastic nylon encountered greater biofilm formation in comparison with PEEK.

Based on the formula, the calculation results for OD cut off = mean negative OD + (3 x SD negative) = 0.457, while microbial OD on thermoplastic nylon= mean thermoplastic nylon OD – cut off OD = 0.51. Meanwhile, OD PEEK = 0.086. The results of the interpretation of the strength of film formation[32] : microbial OD ≤ ODcut in this study produced thermoplastics nylon with a value of 0.51 > 0.457, implying that nylon thermoplastic materials are incorporated in the category of producing biofilms

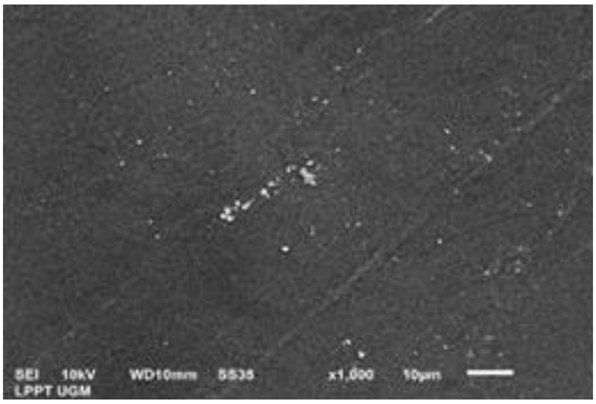

Figure 1. Thermoplastics nylon SEM prior to biofilm test

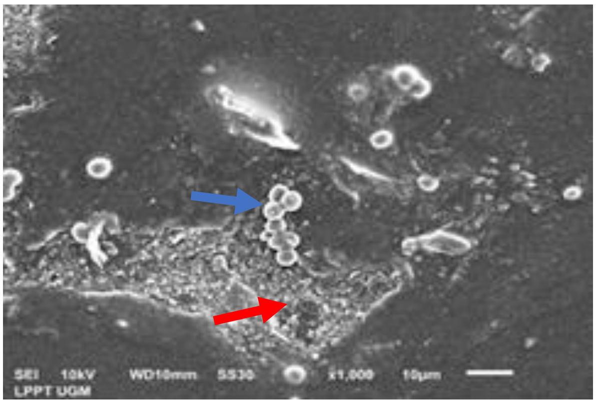

Figure 2. Thermoplastics nylon SEM upon the biofilm test

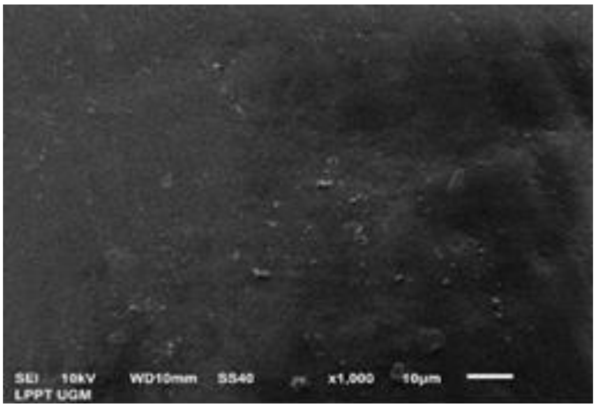

Figure 3. PEEK SEM prior the biofilm test

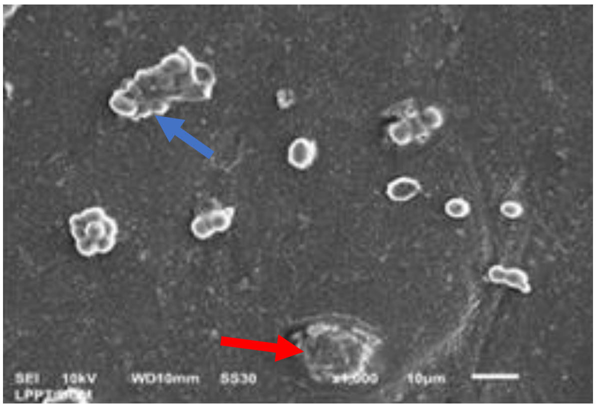

Figure 4. PEEK SEM after the biofilm test

Description
: Candida albicans biofilms
: Matrix

(biofilm formers), while in PEEK, the value is 0.086 < 0.457, indicating PEEK materials did not generate biofilms (non-biofilm formers), in accordance with the nature of PEEK which is not easily soluble in water and possesses low absorption capacity for liquids13. If Biofilm production in thermoplastic nylon material based on the guide with ODcut < ODmicrobes < 2x ODcut returned value 0.457 < 0.51 < 0.914, it will represent weak biofilm producer of the thermoplastic nylon material.

SEM observation implied that thermoplastic nylon before exposure with *Candida albicans* and with after *Candida albicans* exposure (Figure 1 and 2). Figures 3 and 4 demonstrated *Candida albicans* exposure to PEEK material. It is indicated that after *Candida albicans* exposure, biofilm observed in mature phase with complex matrix was generated in 24-hours.

DISCUSSION

The research of contact angle unveiled significant differences in the mean contact angle on thermoplastic nylon and PEEK before and after contact with microorganisms. After being exposed to candida, the contact angle on thermoplastic and PEEK decreased. Thus, before being exposed to Candida albicans, the material's surface has a large contact angle or is hydrophobic. Candida albicans attaches randomly to the surface of the material when presented to it. It will have an effect on the roughness of the material's surface. As it is difficult for water to spread on a rough surface, the contact angle becomes small. PEEK is smaller than thermoplastic nylon when compared to the average value of the contact angle both before and after contact with Candida albicans. PEEK materials incorporate non-allergenic, plaque preventing properties,[9] non-water soluble and lower resorption to liquids,[11] the number of biofilms was less compared to thermoplastic nylon.

The contact angle is associated with wettability. The limit for wettability is hydrophilic when the contact angle is 0°-30°, while at a contact angle > 150° is hydrophobic. The resulting contact angle between thermoplastic nylon and PEEK before and after contact with *Candida albicans* is between 77° - 105°, the result is > 30° but < 150°, indicating that the wettability of the two materials is between hydrophilic and hydrophobic before and after contact with *Candida albicans*. It corresponds to the 30° - 89° category, which is partially wetted.[17] The two materials tend to make it easier for Candida albicans to stick to the surface of the material in this partially wetted condition, but due to the nature of the PEEK material, it is a material that does not cause allergies and plaque does not stick easily.[9] Furthermore, it owns the property of not being easily soluble in water and owns a low absorption capacity of liquids,[11] hence, in PEEK, the number of biofilms is smaller than thermoplastic nylon.

The surface properties of medical devices are significant in determining the presence of *Candida albicans* biofilms, as the surface properties of medical devices, the material contact angle, and the hydrophobicity index were revealed to be positively associated with initial adhesion and *Candida albicans* biofilm formation.[33,34.]

The hydrophobicity of matter is an essential factor in microbial adhesion and/or biofilm formation. In determining the effect of the contact angle on *Candida albicans*, it was revealed that thermoplastics discovered a mean of 22.431° and a PEEK of 9.385°. The greater the contact angle of thermoplastic nylon over PEEK, as well as its rugged nature, the more it influences biofilm formation by providing a retention area for food debris and microorganisms. Surface roughness can also escalate surface roughness, complicating the removal of biofilm. Bacterial colonization will occur if the surface roughness exceeds $\leq$ 2 μm.[6]

In the research results, it was discovered that the thermoplastic nylon possessed a value of 0.51 > 0.457, implying OD microbes > OD blank. According to the interpretation, it is incorporated in the category *Candida albicans* producing biofilm, while in PEEK the value is 0.086 < 0.457, indicating that PEEK material does not produce biofilm.[30] Biofilm formation can be perceived from the amount of the two materials which are different and the formation of biofilm on nylon thermoplastic which is more than that of PEEK

Surface support for biofilm formation depends on the properties of the biomaterial, the properties of thermoplastic nylon which are greater in their absorption of water, and the property of easily forming roughness which makes biofilms easy to adhere to and develop, in which PEEK material contains smooth properties and low affinity for plaque formation.[9] It is consistent with the formation of biofilms affected by environmental nutrition, pH, temperature as well as the chemical and physical properties of the surface of the object. As this study was conducted in vitro, it did not accurately reflect the full range of oral conditions. It was one of the study's limitations. Mechanical stresses and strains experienced in the mouth differ from those experienced in the laboratory, in which specimens are treated to each condition individually. Future research is highly recommended to be conducted in a manner that more closely resembles the conditions that exist in vivo in order to produce more significant results.

CONCLUSION

The contact angles of Polyetheretherketone and thermoplastic nylon are incorporated in the partially wetted. Polyetheretherketone is involved in the non-producers of biofilms, while thermoplastic nylon is incorporated in of weak biofilm formers.

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