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BIO-FILM FORMATION ON CAD-CAM VERSUS PRESSED PEEK FOR OBTURATOR PROSTHESIS

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ABSTRACT

OBJECTIVE: This research aims to assess biofilm formation and microbial colonization of the normal oral and respiratory flora on the commercially available forms of polyetheretherketone (modified PEEK); the CAD-CAM and the pressed PEEK in comparison to heat polymerized polymethyl methacrylate (PMMA) and correlate them to average surface roughness.

METHODS: Oral, nasal, and nasopharyngeal swabs were taken from a healthy volunteer. The swabs were collected carefully to avoid touching non-involved surfaces. Specimens were sent to the laboratory once collected.

Thirty-six circular discs were processed forming three groups: Group I: twelve (PEEK) discs were prepared with CAD-CAM technique, Group II: twelve PEEK discs with Pressed technique, Group III: twelve heat polymerized PMMA. finishing and polishing were accomplished mimicking the clinical situation. Microbiological procedures were performed including microbiological sampling, isolation, purification, identification, and biofilm formation. Average Surface roughness correlation to biofilm formation test was performed.

RESULTS: No statistically significant positive correlation was found between biofilm formation and average surface roughness in all groups. CAD-CAM PEEK has lower biofilm formation than pressed PEEK, despite the rougher surface. There was no statistically significant difference between CAD-CAM PEEK and PMMA regarding biofilm formation.

CONCLUSIONS: Surface roughness isn't the sole parameter for biofilm formation. Both PEEK processing techniques were positive for biofilm formation, Though CAD-CAM PEEK showed a rougher surface, it showed less biofilm formation than pressed PEEK. Therefore, it can be used in maxillofacial removable prosthodontics.

KEYWORDS: Biofilm, Digital, CAD-CAM, Surface roughness, PEEK, Maxillofacial prosthesis.

RUNNING TITLE: Bio-film formation on PEEK for obturator prosthesis.

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INTRODUCTION

A maxillary obturator prosthesis is a device that parts the oral cavity from the nasal cavities. The prosthesis allows the patient to accomplish several functions of deglutition, mastication, and speech and it is also comfortable and esthetic. (1, 2)

An artificial palate is also included in the obturator prosthesis. (3) It is naturally thick and bulbous. It is often made using the hollow bulb technique and acrylic resin.(4) PMMA has been declared as the gold standard as it has proven successful through years of clinical service, but it has been reported that PMMA has a great potential for harbouring microorganisms owing to its porosity (23 pores at 0.01 µm) hence the need for a new candidate that will overcome this drawback.(5) also, the weight of the prosthesis may pose a significant issue. That's why modified polyetheretherketone (PEEK) (BioHpp) is a good candidate. It offers superior mechanical performance, high-temperature resistance, and chemical stability. The bulb section of an obturator prosthesis would be lighter in weight since it has a low specific gravity (1.31 g/cm3) like PMMA, ease of polishing, and machinability. (6)

The critical disadvantage of PMMA is the fact that microorganisms thrive in the best conditions possible on its surface. Biofilm formation caused by microbial contamination may result in local or systemic infection. (7) Studies of biofilm formation and bacterial colonization with these different forms are limited and the available literature lacks enough information on PEEK's microbiological properties to determine its biocompatibility with the upper respiratory tract flora (oral-nasal-nasopharyngeal) owing to their complex nature.

Unfortunately, it is difficult to prevent microbial adherence to dental materials' surfaces, so searching

for an alternative material with enhanced properties has become a necessity.

PEEK may be constructed in various techniques since it has a low fusing temperature of 343°C. The material can be compressed using a specialized vacuum-pressing mechanism. It could also be milled using CAD/CAM technology. (8)

In this work, biofilm formation on pre-pressed CAD-CAM blanks was tested and compared with pressed PEEK pellets, and PMMA, and correlated them to their average surface roughness (Ra).

MATERIALS AND METHODS

Ethical Approval

All procedures performed in the study involving human participants were following the ethical standards of the institutional research committee (Medical Research Ethics Committee of Faculty of Dentistry Alexandria University, Egypt) and with the 2008 Helsinki declaration and its later amendments or comparable ethical standards.

Statement of Informed Consent: An informed written consent was obtained from the patient before inclusion in the study.

This study is a comparative experimental study, in which a swab was taken from a healthy volunteer. The specimens were delivered to the laboratory as soon as possible after collection. to assess the biofilm formation on the different available commercial forms of BioHPP (Bredent GmbH & Co. KG, Senden, Germany) was assessed in comparison to PMMA and determine the correlation of biofilm to average surface roughness.

For this study, thirty-six circular specimens were prepared to form 3 groups, group I (study) includes twelve CAD-CAM PEEK specimens, group II (study) includes twelve pressed PEEK specimens, and group III (control) includes twelve conventional PMMA specimens. Each specimen was a circular disc of 8mm diameter and 3 mm thickness. Each specimen was microbiologically evaluated for biofilm formation, bacterial colonization (Aerobic and Anaerobic), and fungal growth.

2.1. Specimen construction:

2.1.1 CAD-CAM technique (Group I).

Twelve PEEK specimens were prepared from breCAM circular blank. On a computer software (Dental Wings DWOS. CAD-CAM designing and milling software) and milling machine (SHERA eco-mill 5x. Germany), a circular study disc of 8 mm diameter and 3 mm thickness was virtually designed and used to standardize the dimensions of all specimens for all three groups. (fig. 1,2). (6)

2.1.2. Conventional press technique (Group II)

Twelve circular PEEK discs of the same dimensions were created using a conventional press method utilizing BioHPP pellets in its special pressing device.

Figure 1: Twelve milled BioHPP circular discs.

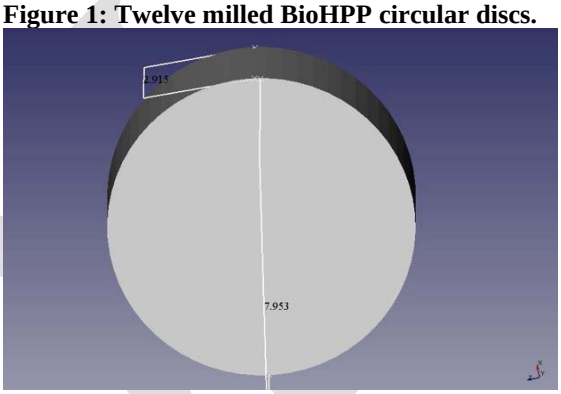


Figure 2: Circular disc design.

Processing technique

First: A circular wax blank of 14 mm thickness (Katana wax disc, Kuraray Noritake Dental Inc., 300 Higashiyama, Mioshi-Cho, Japan) was used to produce twelve circular wax discs of 8 mm diameter and 3 mm thickness (fig. 3). Next: Spruing was done then the muffle formers were filled with the investment material. Once the material melted, a disposable press plunger was attached to the muffle, which was placed into the pressing unit (BioHPP®, Bredent GmbH & Co. KG, Senden, Germany) and the pressing table was closed manually. Finishing and polishing were performed following Bredent protocol.Finally, twelve circular PEEK discs of 8 mm diameter and 3 mm thickness, produced via the pressing method were created.

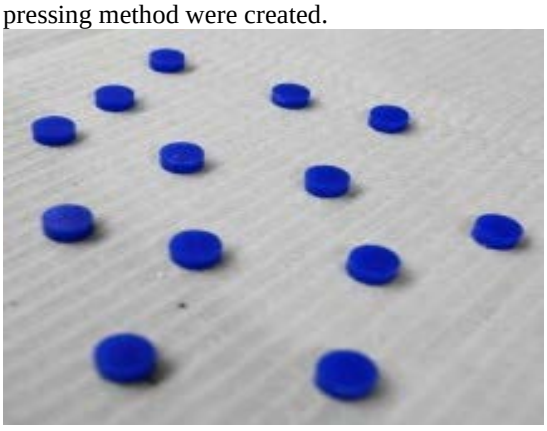


Fig. (3): Milled circular wax discs from KATANA wax blanks.

2.1.3 Heat-cured PMMA (Group III).

Twelve heat cured PMMA circular discs (8mm diameter, 3mm thickness) were processed by the conventional processing technique to be the control group for the study. (9)

2.2. Surface roughness calculation (10, 11).

Average surface roughness (Ra) was determined for all groups by using a surface roughness tester. Traversing length Lt (distance) 1.75 mm, with a 2 μm diamond stylus tip, and a measuring force of 0.7 mN.

2.3 Specimens (discs) preparation.

All the study discs PEEK and PMMA were sterilized by gas plasma sterilizer; low-temperature, hydrogen peroxide plasma sterilizer (HUMANMEDITEK– Gas plasma sterilizer. Toronto, Ontario M3B 3P9 Canada).

2.4. Microbiological Preparations and Methodology

2.4.1. Microbiological sampling (12)

Oral, nasal, and nasopharyngeal samples were collected with a sterile cotton swab. Samples were taken from a healthy volunteer; they were collected carefully to avoid touching non-involved surfaces or mucosa.

2.4.2. Isolation of microorganisms

The isolated microbial flora was cultured on (blood agar, chocolate blood agar, MacConkey's agar, Sabouraud dextrose, and mitis salivarius agar) blood agar in restricted anaerobic conditions as well. Then, to detect the mature biofilm, the plates were incubated aerobically and anaerobically for 72 hours at 37°C.

2.4.3. Specimen purification and identification

The isolated bacterial strains were purified by sub-cultures on blood agar to obtain a young and fresh culture. The sub-cultures were identified to the species level by the use of Matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS) identification device (Ultra Flex Extreme, Bruker Corporation, Massachusetts, USA). MALDI utilizes a soft ionization mechanism, a saturated low-mass organic solution called the matrix (a UV-absorbing MALDI solution) (Bruker Matrix; α-Cyano-4-hydroxycinnamic acid. Billerica, Massachusetts, US) was used (fig.4).

The mass analyzer, a part of the mass spectrometer, examined the ionized proteins in the sample to disclose distinctive details about the sample's composition in the context of mass-to-charge ratios. When spectra are obtained, a comparison to a database of established reference spectra enables the identification of microorganisms. .(9) After MALDI-TOF MS identification, thirteen oral, nasal, and nasopharyngeal species were identified at the species level, based on which the following steps proceeded.

Fig. (4): Biological material smeared over MALDI Target plate.

2.4.4. MALDI identified microbial flora.

From the oral cavity: *Streptococcus cristatus, Streptococcus pseudo pneumoniae, and Streptococcus pneumoniae* were identified. From the nasal cavity *Meyerozyma guillermandii, Moraxella catarrhalis, Staphyococcus aureus and Staphylococcus epidermidis*. From the nasopharynx: *Streptococcus gordoni, Streptococcus salivarius, Candida albicans, Scopulariopsis brevicaulis, Lactobacillus paracasei and staphylococcus aureus* were identified.

2.4.5. Biofilm formation on PEEK and PMMA Discs

After identification, another fresh subculture was done. From the 13 isolates, bacteria were harvested from each plate and inoculated onto a sterile flask containing 500 ml nutrient broth (Thermo Fisher Scientific, Oxoid Products and Remel, 100-1926 Merivale Rd, NEPEAN, Ontario K2G 1E8), with 1% glucose, turbidity was adjusted to 10^8 (0.5 McFarland turbidity standard).

The flask was incubated for 24 hours, at 37°C, and then the culture was diluted 1/100. A rack with 36 sterile test tubes was prepared and divided into three groups of sterile test tubes (group I: twelve for the CAD-CAM PEEK, group II: twelve for the Pressed PEEK, group III: twelve for PMMA).

From the diluted broth, 10 ml was delivered to every corresponding tube. Thirty-six sterile PEEK and PMMA discs were aseptically delivered.

All tubes with discs were incubated at 37°C for 72 hours aerobically to help biofilm establishment. After incubation, the discs were removed from the test tubes and transferred into another dry sterile test tube before washing.

The discs were washed with sterile saline 4 times with gentle shaking every time, to get rid of the floating surplus germs. Biofilm formed on discs was fixed with 1% methanol for 15 minutes, then it was stained with 0.1% crystal violet (5ml) on each disc for another 15 minutes in sterile cups (13, 14)

Excess crystal violet stain was eliminated by washing the discs in sterile distilled water four times (avoiding direct application on the specimen), and then it was allowed to dry overnight at room temperature. Two ml at 30% acetic acid was added to each disc to elute the crystal violet stain from the biofilm formed on the surfaces (12) in sterile labelled test tubes.

2.4.6. Quantitative evaluation of biofilm formation (colourimetric staining assays).

The stain eluted from every disc was subjected to an optical density ELISA (enzyme-linked immunosorbent assay) reader (OD) of 570. (15) To examine the stain absorbance. The amount of dye solubilized by the solvent (acetic acid), was directly proportional to the amount of biofilm formation (14) (fig.5), diagrammatic representation of the workflow (fig.6).

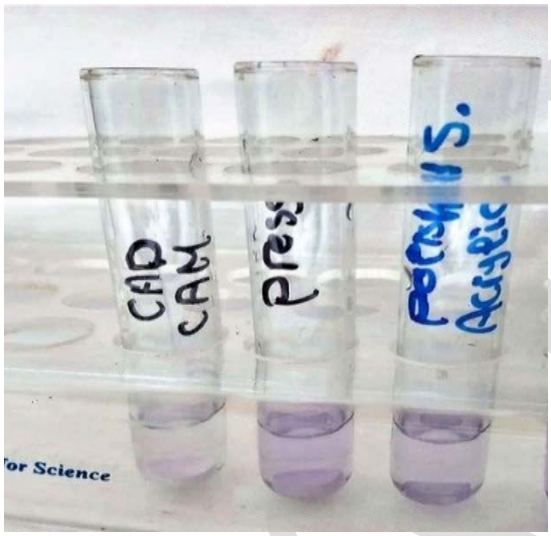


Fig. (5): Different acetic acid elusion colours owing to different stain uptake, corresponding to different amounts of biofilm formation.

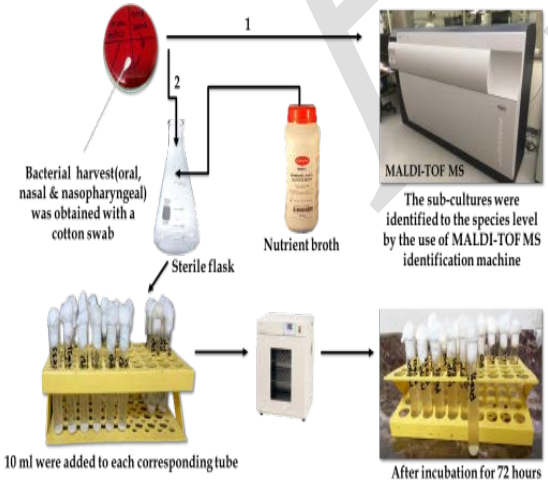


Fig. (6): Diagram of Biofilm formation steps.

Statistical analysis

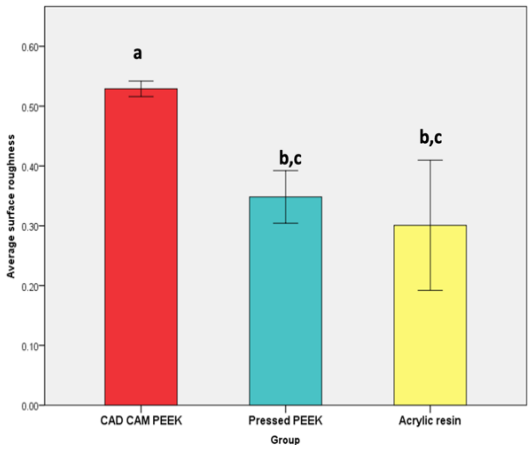
To identify an effect size of 1.082, a sample size of 12 discs per group (for a total of 3 groups) is required. (minimal difference in mean microbial colonization and biofilm formation) (16, 17) of the primary outcome (18, 19), as statistically significant with 82% power and at a significance level of 95% (alpha error = 0.05). The sample size per group does not need to be increased to control attrition bias. The sample size was calculated using GPower version 3.1.9.2. (20)

Data were collected and entered into the computer using SPSS (Statistical Package for Social Science) program for statistical analysis (ver 21).(21) Data were entered as numerical or categorical, as appropriate. The Shapiro-Wilk test of normality revealed no significance in the distribution of the variables, so parametric statistics were adopted. (22). Data were described using minimum, maximum, mean, and standard deviation.

Comparisons were carried out between more than two independent normally distributed subgroups using a one-way Analysis Of VAriance (ANOVA) test. (23) When F ratio of ANOVA was significant Levene test of homogeneity of variances was done, and if significant Brown-Forsythe Robust test was adopted. Post-hoc multiple comparisons were done using the Games-Howell method. (24) Bar chart of mean and 95% CI. An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%. Pearson's correlation was performed between average surface roughness and optical density results among the three studied groups (CAD CAM PEEK, Pressed PEEK and PMMA).

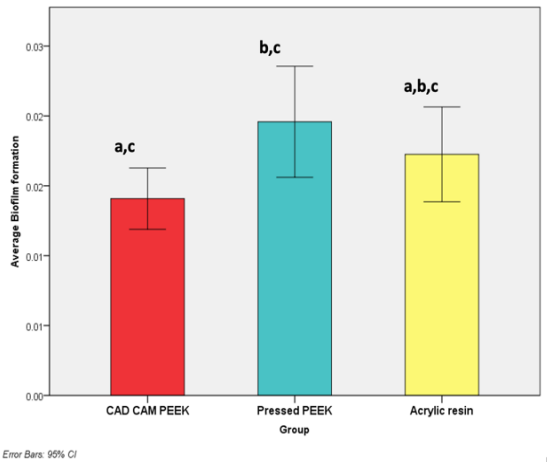
RESULTS

The average surface roughness of CAD-CAM PEEK (Group I) ranged from 0.51600 to 0.54800 with a mean value of 0.52900 ± 0.01233. While in the Pressed PEEK (Group II), it ranged from 0.30100 to 0.38900 with a mean value of 0.34833±0.04191, and in acrylic resin (Group III), it ranged from 0.18000 to 0.45000 with a mean value of 0.30083±0.10381. There was a statistically significant difference among the three study groups (F=20.566, p=0.001*). Pair-wise comparison using the Games-Howell multiple comparison method indicated that the CAD-CAM PEEK group had the highest average surface roughness when compared with the two other groups, further, the CAD-CAM PEEK group had higher average surface roughness when compared with the acrylic resin group. Finally, the Pressed PEEK group had lower average surface roughness when compared with the CAD-CAM PEEK group; indicating a smoother surface.) (Graph. 1).



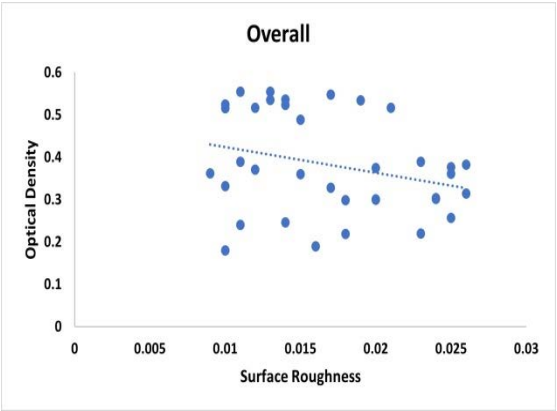
Graph. (1): Bar chart of mean and 95% CI of the average surface roughness in the three studied groups. Different superscript letters indicate significant difference using Games-Howell multiple comparison method.

Optical density (OD) results representing biofilm formation in CAD-CAM PEEK (Group I) ranged from 0.0100 to 0.0210 with a mean value of 0.0141 ± 0.0034, while in Pressed PEEK (Group II), it ranged from 0.0090 to 0.0260 with a mean value of 0.0196 ± 0.0063. While, in the acrylic resin (Group III), OD values ranged from 0.0100 to 0.0260 with a mean value of 0.0172 ± 0.0053. Pair-wise comparison using the Games-Howell multiple comparison methods proved that there was a statistically significant difference among the three study groups (F=3.444, p=0.044*). The pressed PEEK group had statistically significantly higher biofilm formation OD when compared with CAD-CAM PEEK (p=0.041*) other pair-wise comparisons were statistically insignificant. (Graph.2).



Graph. (2): Bar chart of mean and 95% CI of the biofilm formation in the three studied groups.
Different superscript letters indicate significant difference using the Games-Howell multiple comparison method.

There was no statistically significant positive correlation between average surface roughness and biofilm formation in the whole samples (n=36), for CAD CAM r =-0.084, p=0.795, for pressed PEEK r=-0.084, p=0.795, for PMMA r =0.216, p =0.500 (Graph.3).



Graph. (3): Pearson's correlation between average surface roughness and optical density scatter plot.

DISCUSSION

In the current study, surface roughness and bacterial colonization of oral and respiratory flora were assessed on the two commercially available forms of PEEK (BioHPP)® (CAD-CAM form and Pressed PEEK) that were successfully used in maxillofacial prosthodontics, in comparison to heat-cured PMMA (Acrostone) the gold standard for maxillofacial prosthodontics. and to determine whether surface roughness is a crucial factor in bacterial colonization on well-established materials.

The gas plasma sterilization method was chosen for all specimens to avoid inducing any chemical or mechanical changes to the material as suggested by previous studies that used other sterilization techniques. (25, 26)

From an ecological perspective a bacterial mixture containing oral, nasal, and nasopharyngeal microorganisms; aerobes, anaerobes, and fungi was performed in one study (test) to get a diversified perspective of all the microorganisms related to the maxillofacial prosthesis.

Choosing quantitative evaluation of biofilm formation (colourimetric staining assays) Using ELISA microwell auto reader was supported by considering that it is the gold-standard method for biofilm detection as recommended by various microbiological investigations. (13, 14)

Our results revealed that the Pressed PEEK group had lower Average surface roughness when compared with the CAD-CAM PEEK group, which is in agreement with Rochford et al., (27) who stated that machining (CAD-CAM) of PEEK results in a relatively rough surface with non-uniform features, while injection molding (Pressing) a topography that is largely smooth, with few plateaus and ridges (a reflection of the mould), when using PEEK.(6).

Despite the rougher surface, CAD-CAM PEEK showed less biofilm formation than the pressed PEEK. That observation was supported by Yu P. et al., (28) who concluded that increasing hydrophobicity and surface roughness have increased S. mutans' adhesion forces and early attachment (2 and 4 hours) was affected, but not the subsequent growth of the mature biofilm (6 to 24 hours); it only affects early attachment, not the entire biofilm formation process. They believed that neither roughness nor nano roughness had an impact on bacterial attachment; rather, wettability had a significant impact and was substantially linked with adhesion, indicating that bacterial adherence could not rely solely on roughness.

In agreement with our results, Ammar et al., (29) showed that the existence of valleys on the rough surface did not play a considerable role in estimating biofilm formation under conditions favourable for bacterial adhesion i.e., high ionic strength and hydrophobic substrate and that it only affects the initial bacterial adhesion rather than the mature biofilm, Some researchers noted that surface roughness has a much more significant effect on biofilm detachment than initial adhesion, where it only plays a minor influence. Flint et al. declared that there was little correlation between adhesion and surface roughness. (29-31)

From another perspective, Stawarczyk et al., (8) claimed that the reliability and stability of PEEK restorations were improved by commercial pre-pressing of blanks (CAD-CAM PEEK). As porosities decreased. (32-34) .

This may explain why CAD-CAM specimens showed less biofilm formation than pressed PEEK pellets as our test measured the optical density of the eluted stain that is directly proportional to the quantity of biofilm uptake by the specimen that is necessarily relevant to the material's porosity as biofilm didn't only nurture on the surface but rather permeated through the whole specimen.

Our results are in disagreement with the published study by Kawai et al., which found a positive correlation between surface roughness and the amount of plaque accumulation. However, they found that roughness values ranging from 0.12 to 0.53 µm did not result in a significant increase in bacterial adhesion which was the case in our study. Also, Dutra et al.,(35) stated that topographical irregularities of restorative surfaces played a limited effect on in vitro bacterial retention, while a higher impact was observed in vivo studies.

Maryam Gharechahi et al., (36, 37) showed that the roughening of the surface increases the available area for bacterial adhesion. this might be attributed to the fact that they measured (Ra) on different materials; PMMA, metallic biomaterials, glass ionomer cement, and ceramic with other biofilm measuring parameters rather than ELISA in our study; they measured only surface biofilm rather than bacterial colonization through-out the whole specimen. Also, our study tested the effect of a bacterial and a fungal mixture rather than a single bacterial strain. (38-40)

The multifactorial process of biofilm production depends on several variables, including Lifshitzevan der Waals forces, that develop as a result of the electrical charges of the bacterium and the biomaterial surface. Last but not least, forces created between two highly polar molecules containing hydrogen are defined by Lewis acid-base interactions. Hydrophobicity and hydrophilicity of the biomaterial and the microorganism are crucial factors as well. (27)

CONCLUSION

Within the limitations of this study, it could be concluded that: Surface roughness is not the sole parameter for determining biofilm formation. CAD-CAM PEEK showed advantages over pressed PEEK in biofilm formation. Thus, is a promising alternative to PMMA in intra-oral maxillofacial prosthetics given its superior physical and mechanical properties. However, clinical studies are required to verify these preliminary results.

Compliance with Ethical Standards

Conflict of interest: None.

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