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Evaluation of The Effect of Different Polyetheretherketone Materials on Biofilm Formation: An *in vitro* Study

Farklı PEEK Materyallerinin Biyofilm Formasyonuna Etkisinin Değerlendirilmesi: Bir *İn Vitro* Çalışma

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ABSTRACT

Objective: The aim of this *in vitro* study was to investigate microorganism adhesion and biofilm formation between pure and ceramic-reinforced polyetheretherketone (PEEK) materials.

Methods: A total of 72 rectangular (8 x 8 x 4 mm) samples were prepared from pure-PEEK without filler and PEEK (Ceramic-reinforced PEEK - bio high-performance polymer) containing 20% nano-ceramic filler. A profilometer contact surface measurement device was used to assess the surface roughness of the samples. PEEK groups (36 pure PEEK, 36 Ceramic-reinforced PEEK) were divided into 4 sub-groups of 9 according to the microorganism strains. *Staphylococcus aureus* [American Type Culture Collection (ATCC 29213)], *Acinetobacter baumannii* (ATCC 19606), *Enterococcus faecalis* (ATCC 29212), and *Candida albicans* (ATCC 10231) standard strains were used for microbiological analysis. Blocks were added to 24-well microplates containing suspensions of microorganisms and were incubated at 37 °C for 72 hours. Microplates were read at a wavelength of 490 nm using crystal violet.

Results: No significant difference was determined between the PEEK groups in terms of surface roughness. No significant

ÖZ

Amaç: Bu *in vitro* çalışmanın amacı iki farklı tip polietereterketon (PEEK) materyali (saf ve seramik ile güçlendirilmiş) üzerinde mikroorganizma tutulumu ve biyofilm formasyonunun değerlendirilmesidir.

Yöntemler: Çalışmada doldurucu içermeyen saf PEEK (Juvora) ve %20 nano-seramik doldurucu içeren PEEK (Seramik PEEK - yüksek performanslı polimer) materyallerinden 8 x 8 x 4 mm boyutlarında dikdörtgen şeklinde toplam 72 örnek hazırlandı. Numunelerin yüzey pürüzlülüğünü değerlendirmek için profilometre temas yüzeyi ölçüm cihazı kullanıldı. Her iki PEEK grubu mikroorganizma suşları dikkate alınarak 4 alt gruba ayrıldı (n=9). Mikrobiyolojik analizde *Staphylococcus aureus* [American Type Culture Collection (ATCC 29213)], *Acinetobacter baumannii* (ATCC 19606), *Enterococcus faecalis* (ATCC 29212), *Candida albicans* (ATCC 10231) standart suşları kullanıldı. Her mikroorganizma için eşit sayıda blok kullanıldı (9 saf PEEK ve 9 Seramik PEEK). Çalışma blokları mikroorganizma süspansiyonlarını içeren 24 kuyucuklu mikroplaklara eklendi ve 37 °C'de 72 saat inkübe edildi. Mikroplaklar kristal viyole kullanılarak 490 nm dalga boyunda okundu.

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ABSTRACT

differences in biofilm formation of *S. aureus*, *A. baummanii*, *E. faecalis*, and *C. albicans* strains were found between the PEEK groups (p>0.05). In the pure-PEEK, the highest adhesion was recorded in *S. aureus* (p<0.001), and the lowest adhesion in *C. albicans* (p<0.001). In the ceramic-reinforced PEEK group, *S. aureus* and *A. baummanii* adhesions were observed more than *E. faecalis* and *C. Albicans* (p<0.001).

Conclusion: The results of this investigation demonstrated no significant differences in the biofilm formation of different strains between PEEK materials. This was a preliminary study to define the biological characteristics of ceramic-reinforced PEEK. There is a need for further comparative and clinical studies on this subject.

Keywords: PEEK, ceramic-reinforced PEEK, BioHPP, biofilm formation

ÖZ

Bulgular: Yüzey pürüzlülüğü açısından PEEK grupları arasında anlamlı fark saptanmadı. Her iki PEEK materyali arasında *S. aureus, A. baummanii, E. faecalis* ve *C. albicans* suşları adezyonu açısından anlamlı farklılık bulunmadı (p>0,05). Saf PEEK bloklarında en yüksek tutulum *S. aureus* mikroorganizmasında görülürken (p<0,001), en düşük tutulum *C. albicans*'ta saptandı (p<0,001). Seramik PEEK grubunda ise *S. aureus* ve *A. baummanii* adezyonları *E. faecalis* ve *C. albicans*'tan fazla bulundu (p<0,001).
Sonuç: Bu araştırmanın sonuçları, PEEK malzemeleri arasında farklı suşların biyofilm oluşumunda önemli bir fark olmadığını gösterdi. Bu, seramikle güçlendirilmiş PEEK'in biyolojik özelliklerini tanımlamaya yönelik bir ön çalışmaydı. Bu konuda daha fazla karşılaştırmalı ve klinik çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: PEEK, seramikle güçlendirilmiş PEEK, BioHPP, biyofilm formasyonu

Introduction

Microbial dental plaque is defined as a type of highly structurally and functionally organized biofilm consisting of bacterial and non-bacterial microbes aggregations surrounded by an extracellular matrix that includes substance from serum, saliva, and blood (1). Periodontitis/peri-implantitis are pathological conditions associated with biofilm in the tissues surrounding teeth/dental implants. Although certain risk factors such as smoking, inadequate oral hygiene, and poorly controlled diabetes mellitus have been identified in the deterioration of periodontal and peri-implant health, prosthetic factors such as improper prosthetic design, unstable occlusion, the violation of supracrestal adherent tissue, uncleansable bridges, over-contoured crowns, and surface properties of prosthetic materials also play an important role in the development of periodontal and peri-implant diseases (2-7). In addition, the amount and composition of biofilm formation may be influenced by the implant and prosthetic material's chemical and physical properties such as surface roughness and surface energy (8,9).

Polyetheretherketone (PEEK) has a partially crystalline polymer structure and exhibits high-temperature resistance (over 300 °C), high mechanical strength, and good chemical resistance (10). In addition, the elastic modulus of PEEK material is close to the elastic modulus of human bone and thus, it has been reported that the longevity of PEEK material in the human body is excellent (11). PEEK has been preferred for use as an alternative to metallic implants in the field of orthopedics and traumatology because of its favorable biomechanic properties and high performance (12). It has also been reported that PEEK is resistant to chemicals in aging environments (13).

Many applicable methods such as direct surface modification, nano-level surface modification, and/or various filler additives can be produced to increase the bioactivity and osteoconductive properties of PEEK (14,15). Recently, modified PEEK materials have been used as alternative materials to titanium, zirconium, and metal alloys in various fields of dentistry, such as infrastructure material in fixed partial dentures, components in removable prostheses, temporary abutments, healing caps, and even implant materials (15,16). A modified PEEK, bio high-performance polymer (BioHPP), has been introduced as a novel material with higher biocompatibility. This modified and ceramic-reinforced PEEK is obtained by adding 20% ceramic fillers (aluminum oxide and zirconium oxide) to the PEEK material. The ceramic microparticles are approximately 0.3-0.5 microns in size, (17,18) and the addition of ceramic microparticles has been shown to improve the polishability and stability of PEEK material. The ceramic-reinforced PEEK material can be used to produce higher-quality prosthetic restorations (13,17). Moreover, the addition of filler has been reported to improve hydrophilic properties compared to pure PEEK material (19).

Prosthetic implant dentistry is an area that is constantly open to new modifications and materials such as ceramics, polymers, and modified PEEK/PEEK. At the same time, it would be beneficial for clinicians to know about improved dental materials that inhibit bacterial adherence and delay biofilm formation. Although there has been a limited number of studies to date, the research has focused on comparing PEEK and titanium surfaces with biofilm formation (20-22). This *in vitro* study was designed to compare microorganism adherence and biofilm formation of *Staphylococcus aureus, Acinetobacter baumannii, Enterococcus faecalis*, and *Candida albicans* on pure PEEK and ceramic-reinforced PEEK.

Methods

The sample size was calculated with a software program (G*Power 3.0.1). The minimum total number of specimens was determined as 72 with 0.403 effect size (f), 0.80 power (1-β err probe), and 0.05 significance level (α err probe). Considering the result of the power analysis, a total of 72 specimens were prepared in the current study, as 9 in each group.

The surfaces of pure PEEK (Juvora; Juvora Ltd. Thornton Cleveleys, Lancashire, England) and ceramic-reinforced PEEK

(BioHPP; Bredent GmbH, Senden, Germany) materials were prepared using the same processes. Nine pieces of each pathogen and a total of 72 (36 pure PEEK; 36 ceramic-reinforced PEEK) 8 x 8 x 4 mm rectangular samples were obtained from the manufacturer's prefabricated blocks with computer-aided design and computer-aided manufacturing and a precision cutting tool (Micracut 151; Metkon Instruments Inc. Bursa, Türkiye) at 400 rpm/min under water cooling. The samples were polished with 180,400,600,800,1200,1800 silicon carbide papers (ScanDia Hans P. Tempelmann) respectively under water spray at 25 N pressure in an automatic polishing device (Tegranim-20, Struers, Ballerup, Denmark) for 15 sec. All the samples were then rinsed in an ultrasonic machine (UT-206; Sharp, Osaka, Japan) with distilled water for 5 min, and the residue was cleaned from them. The samples were sterilized by autoclaving at 134 °C and 3 bar with a 60-minute program.

Surface Roughness

The surface roughness of the samples was determined with a profilometer contact surface measurement device (Sutronic S-series, Taylor/Hobson, Lester, England) using a standard diamond tip (tip angle: 90°, tip radius 2 μm) and a cut-off level of 0.25. A total of 5 measurements (3 vertical and 2 horizontal) were made from all samples. The mean roughness value (R_a) was calculated by averaging the values obtained from each sample.

Microbial Cultures

All the test microorganisms used in this study were the American Type Culture Collection (ATCC) strains including; *S. aureus* (ATCC 29213), *A. baummanii* (ATCC 19606), *E. faecalis* (ATCC 29212), and *C. albicans* (ATCC 10231).

The microorganisms were first inoculated into 5 mL nutrient broth and were incubated at 37 °C for 24 h. Microorganisms from these cultures were transferred onto a solid medium and incubated overnight. Tryptone Soy Agar (Oxoid, UK) was used for *S. aureus, E. faecalis,* and *A. baummanii*, and Sabouraud Dextrose Agar (Biolife, Italia) was used for *C. albicans*. After growth, selected colonies were transferred into a liquid medium and incubated for 4-6 h to achieve log phase growth. Tryptone Soy Broth (TSB) (Oxoid, UK) for bacterial strains and Sabouraud Dextrose Broth (BD, Difco, USA) for *C. albicans*, were the selected media for this purpose. The optical density of each culture at 490 nm (OD 490) was adjusted to 1-3x10^8 colony forming units (CFU)/mL.

Biofilm Formation Assay

Biofilm formation was measured with a modification of the method used by Peng et al. (23). One mL of a microbial suspension of *S. aureus, E. faecalis, A. baummanii,* and *C. albicans* (10^6 CFU/mL) was inoculated in all the wells of a 24-well plate. The testing samples (pure PEEK and ceramic-reinforced PEEK) were added to the wells and incubated for 72 hours at 37 °C. At the end of the incubation, the broth medium was removed and the wells were washed twice with phosphate-buffered saline (PBS) and air-dried for 1 h. After adding crystal violet (0.1% w/v) to each well, the plate was allowed to remain at room temperature for 15 min. After 15 minutes, the stain was aspirated and the plate was rinsed four times with PBS. In the last step, 1 mL of 33% acetic acid was added to the wells. Absorbance was determined at 490 nm wavelength (OD 490) on a microplate reader (Biotek, ELx800 Absorbance Microplate Reader, USA). TSB and SDB without microbial suspension were used as negative controls.

Statistical Analysis

Data were analyzed using IBM SPSS V21 software (SPSS IBM, Chicago, IL, USA). According to the Kolmogorov-Smirnov test, the data showed normal distribution (p>0.05). The comparison of the two groups was analyzed with the Independent Samples t-test. Intra-group analyses were performed with a two-way analysis of variance (ANOVA) followed by Post hoc Bonferroni adjustment. The level of statistical significance was determined as 0.05. The correlation between surface roughness and biofilm formations were analyzed with pearson correlations.

Results

The surface roughness was mean 0.56±0.88 R_a for pure PEEK, and 0.52±0.83 R_a for ceramic-reinforced PEEK, with no significant difference determined between the groups (p=0.054) (Table 1). There were no significant correlations between surface roughness of peek materials and biofilm formation (Juvora; r=-0.190, p=0.266, BioHPP r=-0.018, p=0.916) (Table 2, Figure 1). The results obtained from the microbial analysis of *S. aureus, E. faecalis, A. baummanii,* and *C. albicans* are shown in Table 2. No significant difference was found in terms of the biofilm formation of the PEEK groups for each pathogen (p>0.05). In each group evaluation, the highest biofilm formation was found in *S. aureus*, followed by *A. baummanii*, *E. faecalis,* and *C. albicans* in the pure PEEK group (p<0.001). The highest biofilm accumulation in ceramic-reinforced PEEK was recorded in *S. aureus* and *A. baummanii*. The biofilm formations of *E. faecalis* and *C. albicans* were significantly lower than those of *S. aureus* and *A. baummanii* (p<0.001) (Table 3).

Discussion

Microbial dental plaque is considered a primary etiological factor for the development of periodontal disease and should be controlled as the first step in periodontal treatment. The attachment of microorganisms to surfaces occurs through complex chemical and physical mechanisms. The surface and chemical properties such as roughness, hydrophilicity, nano topological structure, and modifications with antibacterial coatings of a prosthodontic or implant material can be effective in inhibiting biofilm covering (24,25). Therefore, it is important to know the properties of prosthodontic biomaterials that

Table 1. Comparison of PEEK materials according to surface roughness

Juvora (mean R_a ± SD)	BioHPP (mean R_a ± SD)	p-value
0.56±0.88	0.52±0.83	0.054

Independent samples t-test (Significant level <0.05). SD: Standard deviation, PEEK: Polyetheretherketone, BioHPP: Bio high-performance polymer

Table 2. Correlation analyses between surface roughness of PEEK materials and biofilm formations

Juvora		Biofilm formation	Surface roughness
Biofilm formation	**Pearson correlation**	1	-0.190
	Sig. (2-tailed)		0.266
	n	36	36
Surface roughness	**Pearson correlation**	-0.190	1
	Sig. (2-tailed)	0.266	
	n	36	36
BioHPP		**Biofilm formation**	**Surface roughness**
Biofilm formation	**Pearson correlation**	1	-0.018
	Sig. (2-tailed)		0.916
	n	36	36
Surface roughness	**Pearson correlation**	-0.018	1
	Sig. (2-tailed)	0.916	
	n	36	36

Juvora; r=-0.190, p=0.266, BioHPP r=-0.018, p=0.916. PEEK: Polyetheretherketone, BioHPP: Bio high-performance polymer

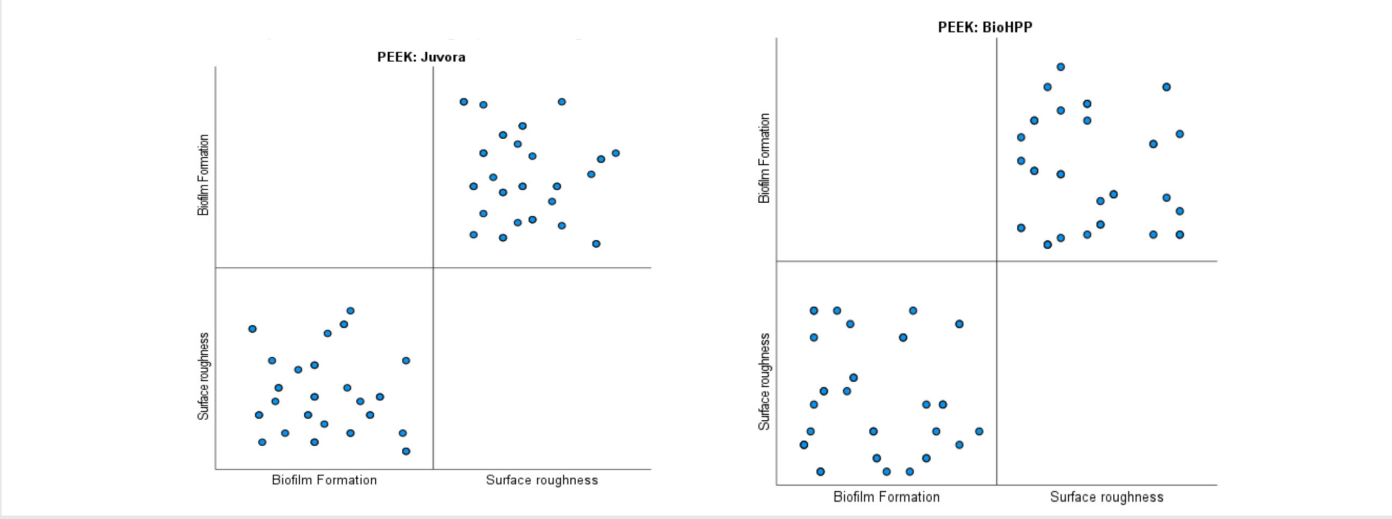

Figure 1. Scatterplot Matrix Graphics for correlations between surface roughness of PEEK materials and biofilm formation
PEEK: Polyetheretherketone, BioHPP: Bio high-performance polymer

Table 3. Comparison of PEEK materials according to biofilm formation

Pathogen	PEEK		P[a] values
	Juvora	BioHPP	
S. aureus	0.888±0.0062[aA]	0.776±0.0105[aA]	0.14
A. baumannii	0.736±0.0065[aB]	0.822±0.0117[aA]	0.071
E. faecalis	0.660±0.0045[aC]	0.576±0.0079[aB]	0.14
C. albicans	0.523±0.0037[aD]	0.480±0.0040[aB]	0.30
P[b] values	p<0.001	p<0.001	

[a]Independent samples t-test; bold p-values indicate statistical significance (p<0.05) values. SD: Standard deviation, PEEK: Polyetheretherketone, BioHPP: Bio high-performance polymer
[b]ANOVA test results (p<0.05).
The same lowercase letters (a, b) indicate that there is no significant difference in horizontal comparisons of independent peek groups (p<0.05)
The same capital letters (A, B, C, D) indicate that there is no significant difference in vertical comparisons based on Holm-Bonferonni adjustment for multiple testing (Bonferroni adjustment value α=0.05/6=0.008)).

enhance biofilm formation. A search of the literature revealed a few studies that analyzed biofilm formation on PEEK surfaces. This investigation aimed to evaluate the biofilm formation of different strains on pure PEEK and ceramic-reinforced BioHHP materials. The second aim of this study was to contribute to the literature by defining the properties of BioHPP. The hypothesis of the study was that there was a difference in biofilm formation between PEEK and ceramic-reinforced PEEK materials.

Previous studies have noted the importance of good mechanical properties of BioHPP such as good marginal quality, fracture resistance, retention, high polishing, low absorption properties, wear resistance, and aesthetics (26-29). Jin et al. (26) suggested that BioHPP, compared to titanium, could be used as an alternative material to be veneered with composite resin due to higher shear bond strength compared to composite resin. Porojan et al. (17) found that BioHPP was less affected by aging than pure PEEK due to the nanosurface topography and nanoroughness. BioHPP is reinforced with ceramic molecules; aluminum oxide, and zirconium oxide (18). Zirconia is known to attract low plaque accumulation (30,31). Therefore, assessing biofilm formation on ceramic-reinforced and pure PEEK materials would be of interest. In the current study, the differences in biofilm formation between pure PEEK and BioHPP were not significant. Wiessner et al. (32) conducted an *in vivo* study to examine the formation of biofilm on various materials, including titanium, zirconia, PEEK, and PEEK-BioHPP. They employed fluorescence microscopy and image analysis software to quantify the biofilm formation. Their findings indicated that zirconia exhibited the least biofilm formation, followed by titanium, PEEK, and PEEK-BioHPP. Their observations align with the outcomes of our study, as no difference in biofilm formation was observed between PEEK and modified PEEK materials.

Higher surface roughness can influence bacterial attachment, increasing the surface area, and causing unsmooth and uncleanable surfaces (9). A key strength of the present study was that no difference was determined in the surface roughness between the PEEK groups. In a recent study, Barkarmo et al. (22) found that while there were no significant differences in biofilm adhesion of streptococci strains between smooth PEEK and titanium surfaces, blasted PEEK material had significantly more biofilm formation. Hahnel et al. (33) reported in a laboratory study that biofilm formation of *Streptococcus gordonii*, *Streptococcus mutans, Actinomyces naeslundii*, and *C. albicans* on the surface of PEEK from zirconia and titanium abutment materials was equal or less. In addition, the surface roughness of PEEK surfaces was also found to be lower than that of zirconium and titanium abutment surfaces. In addition, one study found biofilm formation on ceramic-reinforced PEEK, BioHPP. Similarly, in this study, the adhesion of *S. aureus* and *C. albicans* on dental polymers was mainly affected by surface roughness (34). These results might be explained by the fact that the surface roughnesses were different between blast, rough, polished, and smooth surfaces and increased surface roughness had an impact on the biofilm adhesion.

In the current study, tests were made of four different biofilm-producing strains of microorganisms, namely S. *aureus, E. faecalis, A. baummanii,* and *C. albicans,* on pure PEEK and ceramic-reinforced PEEK material obtained by adding 20% ceramic (aluminum oxide and zirconium oxide). *S. aureus* is an important pathogen of implant-associated osteomyelitis infections (35,36). *Candida spp*. have been associated with denture stomatitis (37). *E. faecalis* acts as an important agent in oral infections and *A. baummanii* is a multidrug-resistant bacteria of clinical importance (38,39). The present study showed considerably high *S. aureus* accumulation and biofilm formation on both biomaterials when compared with the other pathogens. In contrast to *S. aureus, E. faecalis* and *C. albicans* strains formed fewer accumulations. Although not statistically significant, *A. baummanii* formed more biofilm on the ceramic-reinforced BioHHP group than on pure PEEK surfaces. These results led to a review of the previous studies that investigated the antimicrobial properties and biofilm-forming characteristics of aluminum oxide and zirconium oxide, which were added to PEEK material.

Compared to the nanoparticles of oxides of metals such as silver, iron, zinc, and copper, there have been few investigations of the antimicrobial efficacy of aluminum oxide nanoparticles. According to the results of a recent review, studies about the inhibitory effect of aluminum oxide nanoparticles have reported that aluminum oxide nanoparticles cause a decrease in the growth rate of *S. aureus* and *C. albicans*, as well as show an inhibitory effect at moderate concentrations and a bactericidal effect at high concentrations for *A. baumanii.* Compared to other metal oxide nanoparticles, aluminum oxide nanoparticles have the lowest microbial inhibitory effect (40).

Zirconia has also been used introduced to the field of dentistry due to mechanical stability and it can be used as pure zirconia or alumina-toughened zirconia (41). To date, limited scientific data regarding bacterial accumulation around zirconia dental implants and abutments are available and the results are conflicting. Studies investigating zirconia implants have shown less oral biofilm in vivo and conversely, more biofilm *in vitro* when compared with titanium implants. As these data can not allow a clear preference for the use of zirconia, more studies have been carried out to provide further information (42). Zeller et al. (43) investigated biofilm formation on discs of metal alloys, zirconia, and PEEK in vivo and detected higher biofilm mass formation, and the zirconia and PEEK levels were similar. Roehling et al. (44) compared biofilm formation on zirconia and titanium implant surfaces and detected a statistically significant reduction in biofilm formation. Abualsaud et al. (45) evaluated the antimicrobial effects of zirconium dioxide nanoparticles reinforcement of poly(methyl) methacrylate on surface roughness and *C. albicans* biofilm and an insignificant reduction of *C. albicans* biofilm formation was observed.

Study Limitations

The results of the current study, indicating no difference between the two tested biomaterials by means of microbial

attachment, can be interpreted through comparisons with the reports of some former studies concerning low antimicrobial efficiencies and the biofilm formation inhibitory effects of zirconia and aluminum oxide. However, as zirconia and aluminum oxides are mixed with PEEK to obtain ceramic-reinforced BioHHP material, interpretation is not straightforward. These conflicting results can be associated with the nature of the machined or modified PEEK surfaces. Moreover, biofilm involvement can be affected by many clinical factors such as the plaque removal efficiency of the patient the location of restorations, and improper finishing/polishing of restorations. Therefore, conducting our study under *in vitro* conditions is one of the limitations of our study. The certain limitation of this study was the surfaces of PEEK were standardized with silicon carbide papers and the effect of different surface modifications on biofilm formation on the surface of the PEEK materials was not evaluated. The effect of different surface modifications on biofilm formation on PEEK surfaces should be evaluated in further investigations. Another limitation was that the study evaluated only two specific PEEK materials, whereas there were various PEEK materials in the dentistry field. This study was a preliminary study to define the biological characteristics of ceramic-reinforced PEEK. Biofilm retention of PEEK materials could be evaluated with further clinical studies, and comparative evaluation of the effects of various PEEK materials on biofilm formation would be effective in obtaining comprehensive findings.

Conclusion

The study results showed no difference in terms of biofilm formation between pure and ceramic-reinforced PEEK materials. It can be suggested that the association of biofilm formation on modified PEEK materials be investigated in future clinical studies.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Authorship Contributions

Surgical and Medical Practices: S.K.Y., K.A., B.K., S.S., A.D.K., Concept: S.K.Y., K.A., B.K., S.S., A.D.K., Design: S.K.Y., K.A., S.S., Data Collection or Processing: S.K.Y., K.A., S.S., Analysis or Interpretation: S.K.Y., K.A., K.D., Literature Search: S.K.Y., K.A., A.D.K., K.D., Writing: S.K.Y., K.A., A.D.K., K.D.

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