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Effect of microbial adhesion on different esthetic implant materials over titanium- A newer approach towards dentistry

Pooja Singh[1], Subhabrata Maiti[2*], Deepak Nallaswamy[3], Smiline Girija AS[4]

[1]Post graduate student, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077,TamilNadu ,India.

[2]Assistant Professor, Department of Prosthodontics, Saveetha Dental college and hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077,TamilNadu ,India.

[3]Professor, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077,TamilNadu ,India.

[4]Professor, Department of microbiology, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077,TamilNadu ,India.

*__Corresponding author:__ Subhabrata Maiti, Assistant Professor, Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu, India, Email: drsubhoprostho@gmail.com

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ABSTRACT

Background: Biofilm development complications are common in implantology, accounting for one-quarter of all infections each year.To prevent these bacterial biofilms from forming, it is critical to investigate and measure bacteria adhesion to various surfaces.The current in vitro investigation aims at comparing PEEK and PEKK biofilm formation abilities for the use of these materials as alternatives to dental titanium and zirconia as conventional implant materials.

Materials and methods: Alloy material—titanium grade 5 (Ti-6Al-4V), ceramic material—yttria-stabilized tetragonal zirconia polycrystal (Y-TZP), polymer material—PEEK, and polymer material—PEKK (n=48) were investigated. The testing samples were inoculated with the prepared broth suspension, and were incubated at 37 °C for 48hrs.After incubation, the colonies were counted using a digital colony counter and the results were recorded as colony forming units/ml (cfu/ml). Samples were also tested for surface topography and contact angle before microbial study. The data was analyzed with paired t-test and one-way ANOVA with post hoc Tukey's honest significant difference (HSD) tests to compare replicate mean values between different dental material groups in each condition.

Results: The biofilm formation of the Ti-6Al-4V, regardless of the culture times or types of bacteria (S. mutans or E fecalis), was significantly higher than those of the other testing materials along with the value of surface roughness ($p<0.05$).

Conclusion: When compared to titanium alloys (Ti-6Al-4V) and zirconia-based ceramic materials(Y-TZP), PEKK and PEEK were much less conducive to biofilm formation, PEKK showing less adhesion than PEEK and hence can be used as alternative implant materials in aspect of biofilm formation.

Keywords: *Dental implants, PEEK, PEKK, biofilm, peri-implantitis*

INTRODUCTION

Dental implants have been used over several years, with evident benefits for patient care, and are today regarded as the best technique for tooth replacement [1].

The great majority of oral implants used nowadays are made of titanium.[2] However, advances in implant materials demonstrate that high-performance ceramics are increasingly capable of replacing titanium, allowing a single material to be used for a full restoration.[3] Ceramics' advantages include chemical stability, biocompatibility, great mechanical strength, and corrosion resistance.They are also non-allergenic. With the advancement of ceramic as a material for use in implantology, zirconium oxide, particularly the so-called tetragonal zirconia polycrystal (TZP), has gained popularity. It has a high fracture strength and fracture toughness that is twice as great as that of aluminum oxide.[4] Polymers, as one of the most important materials in dentistry, have great physical, mechanical, and biocompatibility qualities. Polymers are used to make a variety of removable appliances, restorations, and denture base materials[5]. Polyetherketoneketone (PEKK) is a novel polymeric material that has piqued the interest of researchers due to its remarkable characteristics and potential applications [6]. The PEKK is a high-performance thermoplastic polymer that is free of methacrylates[7]. Bonner initially invented PEKK in 1962, and it has subsequently been employed for a variety of industrial and military purposes [8]. PEKK has recently gained popularity as a biomaterial with features suited for dentistry and medicinal applications[9].The PEKK has numerous uses in restorative, prosthetic, and implant dentistry. PEKK is a promising material for cranial and orthopedic implants. Because of their superior mechanical strength and the presence of the second ketone group, which allows for more surface modification of their surface, they have a wide range of biomedical applications.

The two most well-known members of the polyaryletherketone (PAEK) family are PEKK and polyetheretherketone (PEEK). The PAEK family of thermoplastic polymers has been used in engineering since the 1980s and has great mechanical and chemical resistance[10]. The PAEK family of thermoplastic composites exhibits ultra-high performance (superior mechanical and chemical resistance) in relation to their processing parameters. PEEK originated as a semi-crystalline material in the late 1990s and demonstrated good biological, mechanical, and physical capabilities for biomedical applications[10,11].

Numerous oral bacteria can colonize the surface of dental implants, causing an infection known as peri-implantitis. Peri-implantitis is a severe concern in today's dental community, with infection rates as high as 28%. The attachment and development of a colonized bacterial biofilm onto the subgingival implant surface causes peri-implantitis. Biofilm development complications are common in implantology, accounting for one-quarter of all infections each year. Despite considerable advances in the research of biomaterials, device-related infections continue to be a major concern. To prevent these bacterial biofilms from forming, it is critical to investigate and measure bacteria adhesion to various surfaces. Preventing pathogenic bacteria's early adhesion and biofilm formation would be a big step towards preventing bacterial infection of implants. Our progress towards ideal implant surface designs is hampered by a lack of quantitative, high throughput adhesion approaches. Furthermore, biocompatibility analyses during implant design focus solely on the implant-host reaction, ignoring the influence of bacteria-implant-host response. Understanding the mechanisms that lead to strong biofilm surface adherence at implant interfaces might help guide the design of surfaces that prevent harmful biofilms and enhance osseointegration.

Only a few research, to the best of our knowledge, have examined biofilm formation and removal assays on the surface of PEEK and PEKK. A direct comparison between cell adhesion to implant and biofilm adhesion to implant could aid in the bioassessments of implants by quantifying the tradeoffs among different surface parameters. A bioadhesion assessment that compares the adhesion of both bacteria and host cells onto implant surfaces is needed. Our team has extensive knowledge and research experience that has translated into high quality publications[12–26]. More research is needed to determine whether PEEK and PEKK materials have a lower biofilm forming ability and are suitable for implants or abutments. The current in vitro investigation aims at comparing PEEK and PEKK biofilm formation abilities for the use of these materials as alternatives to dental titanium and zirconia as conventional implant materials where Null hypothesis stated that there is no difference of biofilm formation among all implant materials.

MATERIALS AND METHODS

Sample size calculation

The sample size (n=48) was calculated with G-Power software version 3.0.10 with a power of 95 percent and a High-intensity alpha error of 0.05.

Sample Preparation

Alloy material—titanium grade 5 (Ti-6Al-4V), ceramic material—yttria-stabilized tetragonal zirconia polycrystal (Y-TZP), polymer material—PEEK, and polymer material—PEKK (n=48) were investigated for biofilm formation. All testing samples had a diameter of 10 mm and a thickness of 2 mm. The design was made in 3D cad software and manufacturing was done using 3D printing technology; thermal resin 3D printer (FUNMAT PRO 410, INTAMSYS, China) for PEEK and PEKK , Titanium was manufactured using DMLS (Direct Metal Laser Sintering) metal printing (EOS, Munich, Germany), and Zr was milled using milling machine (iMES iCORE Coritec 350i). (Table1) Ultrasonic cleaning (RUC-101, REXMED Industries Co., Ltd., Kaohsiung, Taiwan) and air drying was done for samples before testing. Prior to the experiment, all samples were submerged in deionized water at 37 °C for 24 hours.

TABLE 1: Dental materials used in the study and their composition

Materials	Abbr.	Main Composition	Manufacturer
Alloy material			
EOS	Ti-6Al-4V	Ti, Al, V, others	EOS, Munich, Germany
Ceramic material			
Nobilium	Y-TZP	ZrO_2, Y_2O_3, others	Huge Dental, China
Polymer material			
Intamsys PEEK	PEEK	Poly-ether-ether-ketone	Intamsys, Shanghai, China
Intamsys PEKK	PEKK	Poly-ether-ketone-ketone	Intamsys, Shanghai, China

Test for contact angle

The hydrophilicity of the testing samples (n = 48) was determined using a contact angle analyzer (FTA-125, First Ten Angstroms, Portsmouth, VA, USA). A droplet of distilled water (~10 µL) was extruded vertically from a 31G needle onto the testing samples at room temperature, and a charge-coupled device (CCD) was triggered to allow continuous image recordings. A non-spherical fitting approach measured the contact angle of each water drop on the picture. (Figure 1)

Test for surface topography

We were able to gauge the roughness of PEEK,PEEK, Ti, Zr surfaces (n=48) that had undergone plasma activation using AFM measurements. The measurements were conducted using silicon cantilevers with a Si3N4 coating, a tip radius of 20 nm, a spring constant of 40 N/m, and a resonance frequency of 325 kHz (NSC15/A1BS, Mikromasch, CA, USA) on a DimensionTM 3100 instrument (Veeco, Mannheim, Germany) in Tapping Mode® in ambient air under dry conditions. More information about the nanostructures created by the plasma may be provided by tips with a radius of less than 20 nm. 2 x 2 m2 and 1 x 1 m2 of the scan area were chosen, respectively. The software Nanoscope 6.13R1 was used for the data processing and roughness evaluation (Veeco Instruments Inc., Santa Barbara, CA, USA).(Figure 1)

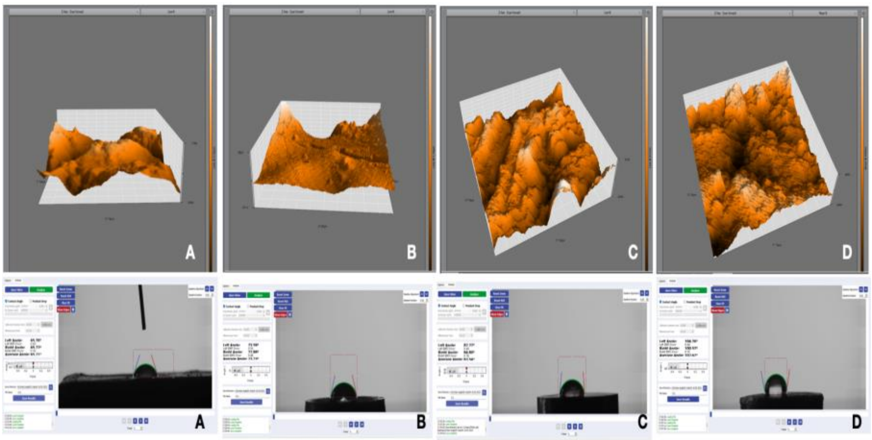

FIGURE 1: Contact angle and Surface topography of groups (A=Ti, B=Zr, C=PEKK, D=PEEK)

Microbial Cultures

In the investigation, Streptococcus mutans and Enterococcus fecalis were employed. S. mutans was grown in tryptic soy broth, while E.fecalis was grown in brain heart infusion broth. The bacteria were inoculated into 3 mL nutritional broth slant through loop transfer from frozen tubes and were kept at 37 °C for 24 hours with continual shaking at 200 revolutions per minute (rpm). Bacteria from these cultures were incubated overnight in an appropriate solid medium. To achieve log phase growth, selected colonies were transferred to a suitable liquid medium and incubated for 4-6 hours to achieve 0.5 McFarland standard turbidity.

The testing samples (Ti-6Al-4V, Y-TZP, PEKK, and PEEK) were inoculated with the prepared broth suspension, and were incubated at 37 °C for 48hrs. After incubation, the discs were washed with sterile distilled water. Using a sterile swab, the discs were touched and were made as lawn culture onto sterile brain heart infusion agar and the plates were incubated at 37 °C for 24hrs. After incubation, the colonies were counted using a digital colony counter and the results were recorded as colony forming units/ml (cfu/ml).(Figure 2)

Statistical Analysis

One-way ANOVA with post hoc Tukey Test was performed. significant difference (HSD) tests to compare replicate mean values between different dental material groups in each condition. All statistical analyses were performed with SPSS (version 19, IBM SPSS Statistics, IBM Corporation, New York, NY, USA) ($\alpha = 0.05$).

RESULTS

The biofilm formation of the Ti-6Al-4V, regardless of the culture times (24 h, 48 h, or 72 h) or types of bacteria (S. mutans or E fecalis), was significantly higher than those of the other testing materials ($p < 0.001$). It is imperative to note that compared with the Ti-6Al-4V, neither the Y-TZP nor the PEEK nor PEKK were conducive to biofilm formation.(Table 2&3)

There was a significant difference of contact angle and surface roughness among groups($p<0.05$). Ti-6Al-4V was showing maximum roughness (169± 28.6) based on Ra value followed by Y-TZP (122.75± 23.63), PEKK (100.3 ± 4.5) and PEEK (85.28 ± 1.79), where the contact angle was inversely proportional indicating maximum wettability in Ti-6Al-4V followed by Y-TZP, PEKK and PEEK (Figure 3)

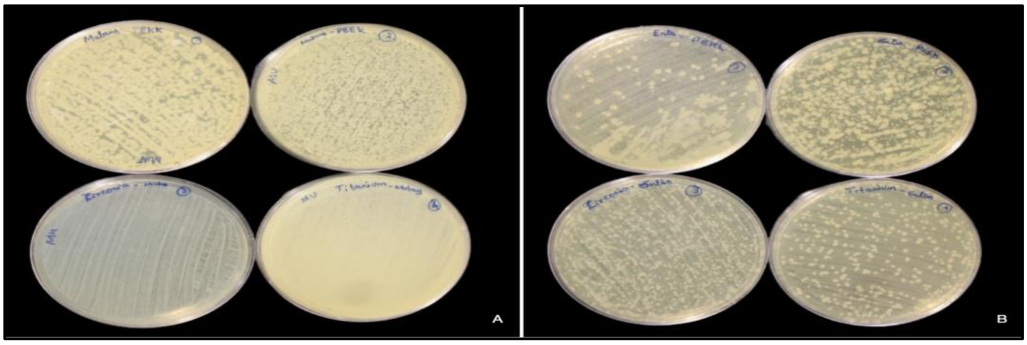

FIGURE 2 A: Colonies for S.mutans b: Colonies for E. fecalis

TABLE 2: One Way ANOVA to compare groups (Ti, Zr, PEEK and PEKK) based on bacterial adhesion.

One Way ANOVA								
Bacteria	Groups	Mean ± SD(×10^7)	SE	95% CI		df	F-value	P-value
				Lower	Upper			
S.mutans	Ti	1176 ± 105.26	47.07	1045.3	1306.69	3	206.9	0.00*
	Zr	470 ± 15.83	7.08	450.73	490.06			
	PEEK	424 ± 47.22	21.11	365.36	482.63			
	PEKK	390 ± 7.56	3.38	380.8	399.59			
E. faecalis	Ti	1622.8 ± 41.89	18.73	1570.78	1674.81	3	2931.18	0.00*
	Zr	974 ± 19.49	8.71	449.79	998.2			
	PEEK	282 ± 17.88	8	259.78	304.21			
	PEKK	248.4 ± 21.27	9.51	221.97	274.82			
*P-value significant at 0.05, P-value was derived from One Way ANOVA								

TABLE 3: Pairwise comparison between groups (Ti, Zr, PEEK and PEKK) based on bacterial adhesion.

Pairwise comparison between groups						
Bacteria	Groups	Mean difference	SE	95%CI		P-value
				Lower	Upper	
S.mutans	Ti v/s Zr	705.6	36.9	600.02	811.17	0.00*
	Ti v/s PEKK	785.8	36.9	680.22	891.37	0.00*
	Ti v/s PEEK	752	36.9	646.47	817.57	0.00*
	PEEK v/s PEKK	33.8	36.9	-71.77	139.37	0.797

E. faecalis	Ti v/s Zr	648.8	17.05	600.01	697.58	0.00*
	Ti v/s PEKK	1374.4	17.05	1325.61	1423.28	0.00*
	Ti v/s PEEK	1340.4	17.05	1292.01	1389.58	0.00*
	PEEK v/s PEKK	33.6	17.05	-15.18	82.38	0.24
*P-value significant at 0.05, P-value was derived from Post Hoc Tukey test						

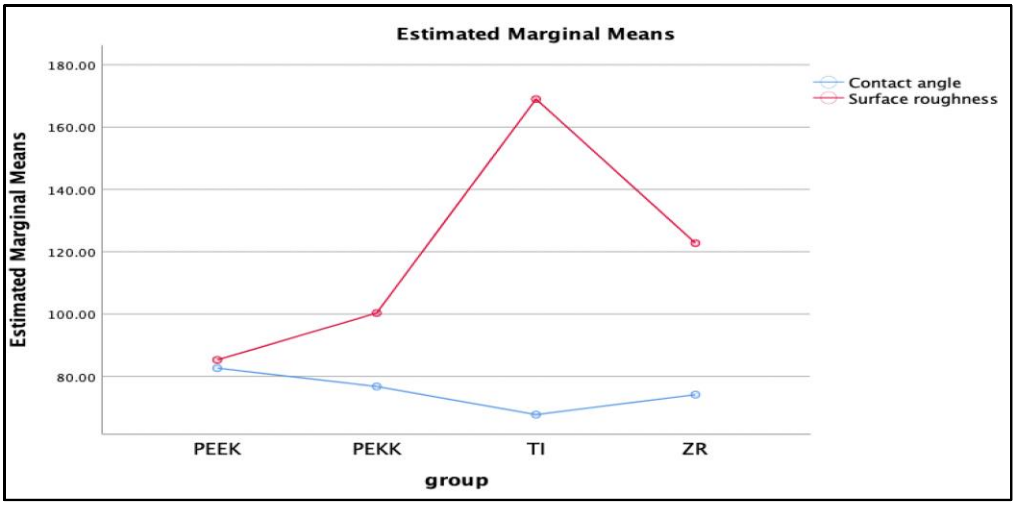

FIGURE 3: comparison of groups (Ti, Zr, PEEK and PEKK) based on contact angle and surface roughness.

DISCUSSION

PEKK and PEEK are poly-aryl-ether-ketone polymers (PAEKs) that are semicrystalline, linear, high-performance thermoplastic polymers with aromatic molecular chain backbones interconnected by ketone and ether functional groups [27]. PEKK and PEEK have excellent mechanical qualities, chemical resistance, and an elastic modulus that is similar to natural bone[10]. Furthermore, these materials may absorb occlusion force and decrease occlusion stress, making them suitable for implants or abutments. PEEK and PEKK were used as target testing materials in this work, with titanium alloys (Ti-6Al-4V) and zirconia-based ceramic (Y-TZP) serving as controls.

Surface roughness and surface free energy may influence the ability of biofilms to form: (a) hydrophobicity and hydrophilicity, and (b) surface roughness. Hydrophilicity was found to be more important than surface roughness in biofilm formation by De-la-Pinta et al. [10,28].The current study confirmed previous findings that revealed hydrophilicity (Pearson r value > 0.9) was more connected to biofilm formation ability than surface roughness (Pearson r value 0.5). Regardless of the kind of bacteria, hydrophobic materials displayed poorer biofilm forming potential when subjected to the same growth conditions. These data confirm the notion that PEEK and PEKK have a lesser biofilm forming potential than Ti-6Al-4V. Bacteria are known to adhere and become sheltered in rougher surfaces of materials [10,28,29], implying that anti-adhesive action against bacteria would occur on the Y-TZP surface.For PEKK , even it has more rough surface than PEEK , due to micro porosity and the material property made the surface antibiofilm formation by nature , showed less biofilm than PEEK.

Gram-positive S. mutans colonize dental plaques as both a primary and secondary coloniser [30]. The microbial species detected around peri-implantitis sites are highly similar to those reported near periodontal disease sites, with the

Gram positive E fecalis being the most common [31]. The PEEK and PEKK results in this investigation also showed that S. mutans was more effective than E fecalis.

The advantages of PEEK and PEKK, such as hydrophobicity and chemical inertness, may prove to be disadvantages for their use in dental implantology. These factors would have a deleterious impact on cell adhesion, bone attachment, and osseointegration. Human osteoblast-like (MG-63) cells are extensively used in osteoblastic models for studying bone cell survival, adhesion, and proliferation on load-bearing biomaterial surfaces[31,32]. Because of their great mechanical strength, chemical stability, biocompatibility, and high osseointegration ability, Ti-6Al-4V and its derivatives are gold standard materials for implants or abutments .According to Martins et al., the bone response to Y-TZP implants is equivalent to that seen around Ti-6Al-4V implants [33].

Based on the results of this study's verification and the data presented in the literature [33,34], it can be concluded that PEEK and PEKK have anti-adhesive bacterial capabilities. Microbiologically, they are appropriate materials for dental implants or abutments.Hence the study rejected the null hypothesis.Further in vivo studies proving the efficacy of the same have to be done.Out of the polymers, PEKK showed less biofilm adhesion than PEEK as compared with Ti-6Al-4V and Y-TZP .Titanium was showing maximum rough surface and polymers was showing minimum, hence this indicates the need for surface modification for osseointegration.

CONCLUSION

When compared to titanium alloys (Ti-6Al-4V) and zirconia-based ceramic materials(Y-TZP), PEKK and PEEK were much less conducive to biofilm formation. Out of the polymers, PEKK showed less biofilm adhesion than PEEK as compared with Ti-6Al-4V and Y-TZP .A thorough examination of the results of this in vitro investigation reveals that PEEK and PEKK are potential alternative materials for dental implants or abutments in the aspect of biofilm formation. More research and in-vivo experiments are needed to corroborate the findings.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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