Biomimetic Evaluation of Polyetheretherketone as a Promising Implant Material: *In vitro* study

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Abstract: Introduction: Dental implants are currently one of the main pillars of restorative dentistry. Titanium and its alloys were and still are the gold standard for dental implant materials. However, Titanium is not a perfect material thus the search for a more ideal material is ongoing. Zirconia and Polyetheretherketone are two viable alternatives to titanium as dental implant materials. These two materials show different mechanical and biological behavior *in vitro* and *in vivo*. **Objectives:** Evaluation of mechanical performance and bioactivity of Polyetheretherketone as an implant material following controlled biomimetic simulations. Zirconia was used as a comparable implant material. **Materials and Methods:** Microbars of Polyetheretherketone and yttrium-stabilized tetragonal polycrystalline zirconia were prepared using precision cutter under water cooling and used for biomimetic assessment of the following properties: Flexural strength and elastic modulus for unaged and aged microbars using cyclic loading, Vickers Microhardness and *In vitro* Bioactivity using simulated body fluid. **Results:** Biomimetic mechanical evaluation displayed significance decrease of flexural strength and elastic modulus values for both unaged and aged Polyetheretherketone compared to zirconia. No significance was detected for Polyetheretherketone after aging. While, zirconia showed significant decrease of flexural strength and elastic modulus values after aging. There was also a significant decrease in microhardness values of Polyetheretherketone corresponding to zirconia. *In vitro* Bioactivity showed the higher affinity of zirconia to bone formation compared to Polyetheretherketone. **Conclusions:** Polyetheretherketone could be recommended as a promising alternative to titanium and zirconia as a dental implant material.

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1. Introduction

Missing teeth with their supporting oral tissues are considered as one of the major concerns in modern dentistry. Traditional management included replacement using fixed or removable partial dentures, which despite their wide use had several drawbacks(1). Dental implants offer a reliable alternative, that improved quality of life for most patients with tooth loss(2). The material chosen for endosseous implants has been and still is commercially pure titanium, introduced in 1969 by *Branemark et al.*(3). Although the use of titanium (Ti) and Ti alloys as the main material for dental implants is the gold standard, a range of problems were attributed to their use(4). Titanium hypersensitivity was a potential problem(5). Furthermore, its dark grayish color with lack of light transmission which occasionally appears through thin gingival biotype compromising the esthetic outcome of the treatment. This risk is greatly emphasized when replacing teeth in the esthetic zone and in patients with high smile line(6). Ceramic implants were proposed as an alternative to overcome Ti drawbacks as well as responding to the increasing demand of completely metal-free dental reconstructions. Aluminum oxide implants were the first ceramic implants introduced 40 years ago(7). However a frequent fracture incidence was reported impeding their wide use(8). Currently, zirconia is the material of choice for ceramic dental implants, offering adequate mechanical and physical properties compared to Ti implants(9). Yttrium-stabilized tetragonal polycrystalline zirconia (Y-TZP) dental implants were shown to promote osseointegration, produce an excellent soft tissue response, low plaque affinity, and can have a natural tooth-like color(10). It was reported that, minimizing marginal bone loss after years of functional bone loading could be considered as one of the main parameters in assessing long-term clinical success of dental implants(11). Another crucial issue for a dental implant to succeed is stress shielding that could be primarily attributed to the great difference in elastic moduli between implants and their surrounding bone, eventually leading to peri-implant bone loss(12). The elastic modulus of Ti and zirconia were found to be 110Gpa and 210 Gpa, respectively, which are 5–14 times greater than that of compact bone (15 Gpa)(13). In contrast, Polyetheretherketone (PEEK) could be a promising polymeric implant material due to its exceptional elastic modulus of 3.6 Gpa(14), which also could be modified by adding carbon fibers to achieve a modulus of 18 Gpa similar to that of cortical bone(15). PEEK is a polyaromatic semicrystalline linear polymer with good biocompatibility, excellent mechanical properties, and is found to be translucent in radiographs(16). Moreover, PEEK is a high-performance thermoplastic polymer which over the past few decades has been extensively used in orthopedic applications and spinal implants since it was proposed as a suitable biomaterial in the 1980s(17). The PEEK-Optima is a composite mixture of Polyetheretherketone and inert materials. The addition of filler materials such as glass and carbon fibers resulted in a significant increase in the PEEK mechanical properties making it more suitable for more demanding applications such as hip replacement and dental implants(18). The superior mechanical and biological properties of PEEK-Optima convincingly proposed it as a dental implant material. Moreover, it possesses many other advantages including; its natural beige color, reduced magnetic resonance imaging artifacts, and radiolucency on X-rays(19). Biomimetic approach for dental implants is a significant topic of study in material science and a smart elegance in dentistry(20). Mechanical aging with cyclic loading to mimec the biological performance of dental implants coresponding to their mechanics is an excellent method to predict the success of different dental implant materials(21). The bioactive surface properties of implant materials were reported by different studies as a key for successful dental implantation with improved osseointegration(22,23). The bioactivity of dental implants could be *in vitro* evaluated througth investigation of the calcium phosphate phases formed as bone minerals on the implant surface after soaking into a biomemitic simulated body fluid (SBF)(24). From this viewpoint, the current study aimed to evaluate the mechanical performance and bioactivity of PEEK as a promising implant material following *in vitro*, controlled biomimetic simulations analogous to those occurring in the oral physiological environment. Y-TZP was used as a comparable commanding implant material.

2. Materials and methods

2.1. Materials

Two implant materials of different classes were used during the present study; CAD CAM polymeric discs of Polyetheretherketone (PEEK) (PEEK OPTIMA Juvora Ltd, Lanchire, UK), and partially sintered, CAD CAM ceramic discs of Yttrium-stabilized tetragonal polycrystalline zirconia (Y-TZP) (Ceramill Zolid Amman Girbach AG, Koblach Austria).

2.2. Methods

2.2.1. Specimens Preparation

One hundred and forty-eight (148) microbars specimens were prepared by cutting using microtome precision cutter (Micracut 151 Metcon Instruments Inc.) under water cooling. Specimens were separated according to the used implant materials into two groups; Polyetheretherketone (PEEK) group, and Yttrium-stabilized tetragonal polycrystalline zirconia (Y-TZP) group, (74 each).

2.2.1.1. Preparation of PEEK Specimens

PEEK discs were sawed into 74 microbars of dimensions (5*25*1.5 mm) according to ASTM C1161(25). Then, the prepared PEEK microbars were divided into 40 microbars for flexural strength test, 20 for microhardness test(26), and 14 for *in vitro* biomimetic-bioactivity test(24).

2.2.1.2. Preparation of Y-TZP Specimens

Partially sintered Y-TZP discs were cut to prepare 40 microbars of dimensions (1.5 *20*1 mm) according to ASTM D790 for flexural strength test(27). The other Y-TZP specimens were prepared to produce 34 microbars of dimensions (5*25*1.5 mm), that were divided into 20 microbars for microhardness test(28), and 14 specimens for *in vitro* biomimetic-bioactivity test(24). All prepared Y-TZP microbars were then completely sintered using high temperature furnace according to manufactures instructions; (Heat Rate 600 (°C/h), Holding Temperature and Time 900; 0.5 h; further with 200 °C/h, Final Temperature 1450 (°C), Holding Time 2 (h), Cooling Rate 600 (°C/h)) (29).

2.2.2. Biomimetic Mechanical Aging(21)

Twenty flexural strength specimens of both groups (PEEK or Y-TZP) were randomly separated and subjected to biomimetic mechanical aging in a cyclic loading chewing simulator that applied loading cycles of 1 stroke per second with a uniform load of 1 Kg each, and 240 thousand cycles were performed for each specimen to mimic one year of clinical service.

2.2.3. *In Vitro* Tests

2.2.3.1. Flexural Strength Test(25,27)

Four studied subgroups were subjected to the flexural strength test; unaged PEEK, aged PEEK, unaged Y-TZP, and aged Y-TZP subgroups, (n=20). Three-point bending test was performed to calculate the flexural strength through stress-strain curve. Specimens were submitted to the flexural strength test in a universal testing machine at a cross head speed of 0.5 mm/min until fracture (Comten 700 series, Comten Industries, Inc.). The maximum fracture load of each specimen was recorded in Newton, and the flexural strength (FS) was calculated in MPa. Additionally, elastic modulus (E) was calculated in GPa.

2.2.3.2. Vickers Microhardness Test(28,30)

Twenty microbars of each studied group either PEEK or Y-TZP were highly finished and polished to assess microhardness using Vickers microhardness tester (Instron Wolpert HMV-2000) with a diamond indenter in the form of a right pyramid with a square base and an angle of 136 degrees between opposite faces, that was adjusted to apply a load of 500 g for PEEK and 1 kg for Y-TZP specimens.

2.2.3.3. Biomimetic-Bioactivity Test(24)

Simulated body fluid (SBF) was freshly prepared according to Kokubo protocol to act as a biomimetic environment into which each specimen was soaked at 37°C for 4 weeks to assess its bioactive properties. Each 14 microbars of PEEK and Y-TZP groups were equally divided into 2 subgroups; control subgroup that was soaked in distilled water and the second one was soaked in SBF, (n=7). Specimens of both subgroups were kept at 37°C for 4 weeks. Afterward, *in vitro* bioactivity assessment was performed to analyze apatite minerals precipitated on the surfaces of studied specimens. X-ray diffraction analysis (XRD) (PANalytical (Holand), X Pert PRO) was conducted to investigate the phase crystallography of calcium phosphate minerals precipitants, also scanning electron microscopy (SEM) (JEOL JSM-5300- JSM, Tokyo, Japan) was operated at 25 KV after gold sputter-coating to inspect surface morphology.

2.2.4. Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The distributions of quantitative variables were tested for normality, Student t-test was used to compare two groups for normally distributed quantitative variables while ANOVA was used for comparing between more than two groups and followed by Post Hoc test (Tukey) for pair wise comparison. Significance of the obtained results was judged at the 5% level.

3. Results

Comparison among the four studied subgroups (unaged PEEK, aged PEEK, unaged Y-TZP, and aged Y-TZP) according to flexural strength values in MPa and elastic modulus values in GPa was shown in table 1. Flexural strength test for PEEK specimens displayed mean value of 27.5±2.1 for unaged subgroup, and 26.7±4.2 for biomimetic mechanically aged one. Therefore, no statistically significant difference was detected between the flexural strength mean values of PEEK subgroups. On the other hand, the flexural strength mean value of unaged Y-TZP subgroup was 135.1±22.9 that was significantly decreased to 91.8±11.0 for cyclic loading aged Y-TZP one. It is worth affirming that, PEEK specimens recorded a significantly lower flexural strength mean values compared to Y-TZP for both unaged and mechanically aged subgroups ($P<0.001$) ($F=334.66$). Similarly, statistically significant outcomes were recorded for elastic modulus mean values of both PEEK and Y-TZP subgroups ($P<0.001$) ($F=537.41$), Figure 1. Vickers microhardness results were 32 ± 3 for PEEK and 1261 ± 87 for Y-TZP group, accordingly PEEK recorded an evident significant lower microhardness values than Y-TZP ($P<0.001$) ($F=63.031$), Figure 2. Regarding to the *in vitro* bioactivity results, control PEEK specimens soaked in distilled water revealed only the distinctive x-ray diffraction peaks of Polyetheretherketone represented as (110), (111), (200) and (211). While, other diffraction peaks of different calcium phosphate phases included; $Ca_2P_6O_{17}$ – CaP_2O_6 – $Ca_2P_2O_7$ – $Ca_3H_2P_4O_{14}$ – $Ca(H_2PO_4)_2H_2O$ – $Ca_3(PO_3)_610H_2O$ – $Ca_3(PO_4)_2$ and $Ca(PO_3)_2$ were obviously detected in addition to the previously mentioned PEEK diffraction pattern upon XRD investigation of the PEEK surfaces soaked in SBF for 4 weeks, Figure 3. Likewise, only the characteristic diffraction peaks of tetragonal yttrium zirconium oxide crystal phase represented as (101), (002), (110), (112), (200), (103) and (211) were perceived for Y-TZP specimens soaked in distilled water. While, Y-TZP surfaces soaked in SBF for 4 weeks reflected the diffraction peaks of tetragonal yttrium zirconium oxide crystal phase in addition to the characteristic XRD peaks of hydroxyapatite represented as (211), (112) and (300). Also, different calcium phosphate phases were noticed included; $CaHPO_4$ - $Ca(H_2PO_4)_2$ and $Ca_2P_2O_7$, Figure 4. Scanning electron microscope (SEM) images of both PEEK and Y-TZP surfaces soaked in distilled water showed the morphological features of these milling surfaces reflecting their homogenous irregularities. On the other hand, a respected difference was observed for either PEEK or Y-TZP surfaces soaked in SBF represented by few aggregations of calcium phosphate minerals found to be scattered on the PEEK surfaces soaked in SBF compared to control surfaces, (Figure 5: A-C). Also, apparent accumulations of apatite minerals were moderately cover Y-TZP surfaces soaked in SBF corresponding to those soaked in distilled water, (Figure 6: A-C). It was obvious that, the quantity of calcium phosphate minerals deposits on PEEK surfaces was less than that observed on Y-TZP surfaces.

Table (1): Comparison between the different PEEK and Y-TZP studied subgroups according to flexural strength values in MPa and elastic modulus values in GPa.

	PEEK group		Y-TZP group		F	p
	Unaged subgroup	Aged subgroup	Unaged subgroup	Aged subgroup		
Flexural strength values in MPa						
Median	27.9[c]	27.1[c]	136.1[a]	97.6[b]		
(Min. – Max.)	(24.2–30.3)	(20.2–32.7)	(107.3–168.3)	(71.0–100.7)	334.66*	<0.001*
Mean ± SD.	27.5±2.1	26.7±4.2	135.1±22.9	91.8±11.0		
Elastic modulus values in GPa						
Median	6.0[c]	6.3[c]	217.8[a]	161.9[b]		
(Min. – Max.)	(4.5–7.3)	(5.4–6.8)	(17.9–269.9)	(118.6–166.9)	537.41*	<0.001*
Mean ± SD.	6.0±0.9	6.2±0.5	216.2±36.6	150.8±18.0		

Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant)
*: Statistically significant at $p \leq 0.05$

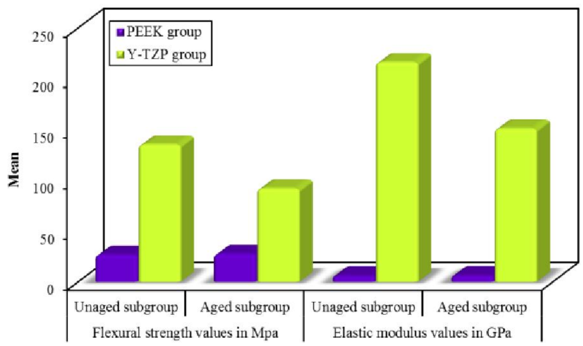

Figure (1): Biomimetic mechanical evaluation diagram of PEEK and Y-TZP studied subgroups regarding to flexural strength values in MPa and elastic modulus values in GPa.

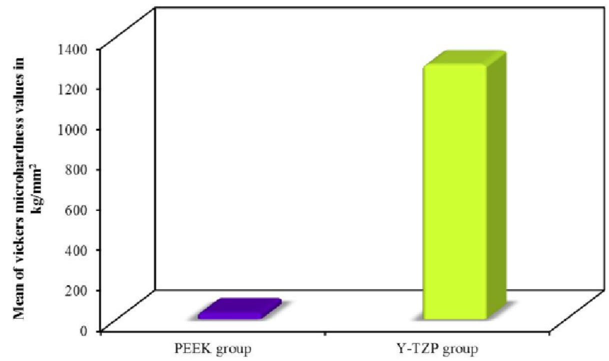

Figure (2): Comparison between Vickers microhardness mean values of PEEK and Y-TZP tested groups.

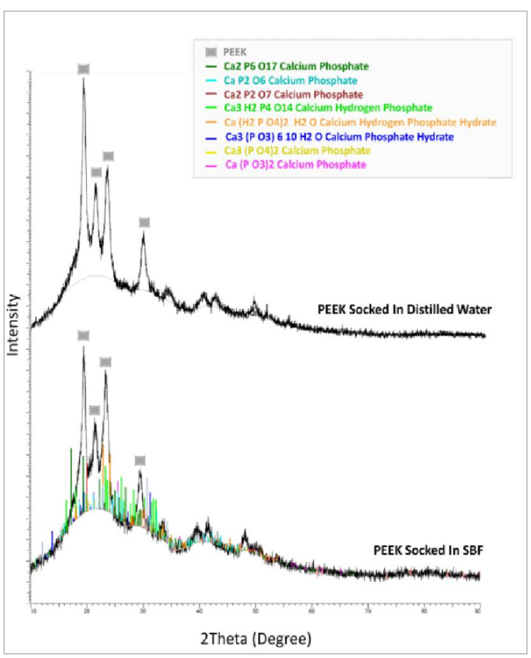

Figure (3): XRD spectrums of Polyetheretherketone (PEEK) microbars socked for 4 weeks either in distilled water or simulated body fluid.

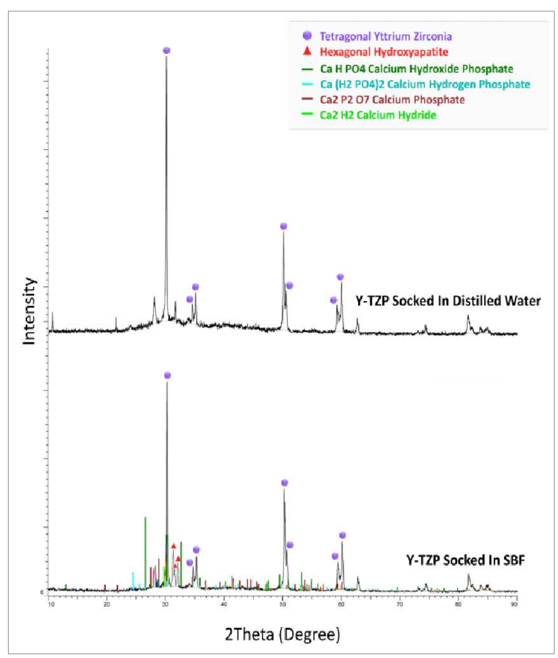

Figure (4): XRD spectrums of yttrium-stabilized tetragonal polycrystalline zirconia (Y-TZP) microbars socked for 4 weeks either in distilled water or simulated body fluid.

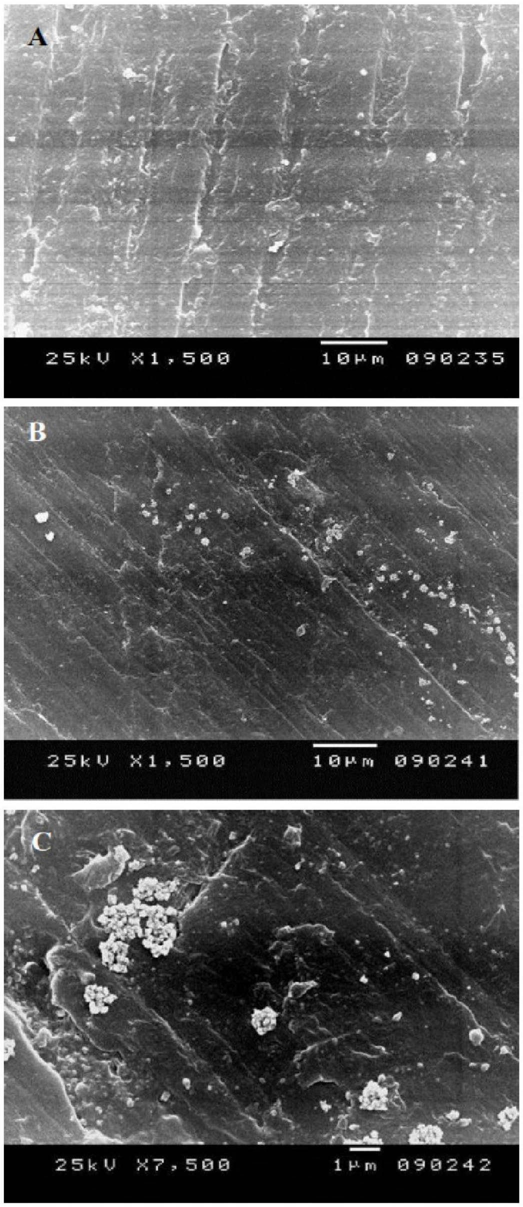

Figure 5.(A-C): SEM images demonstrating the 4-weeks *in vitro* biomimetic-bioactive assessment of Polyetheretherketone (PEEK) surfaces. A: 1500x, showed homogenous irregular topography of PEEK surfaces socked in distilled water. B: 1500x, displayed few minerals aggregations randomly dispersed on PEEK surfaces socked in simulated body fluid. C: 7500x, a higher magnification screening the formation of calcium phosphate bone minerals crystals on PEEK surfaces after 4 weeks in simulated body fluid.

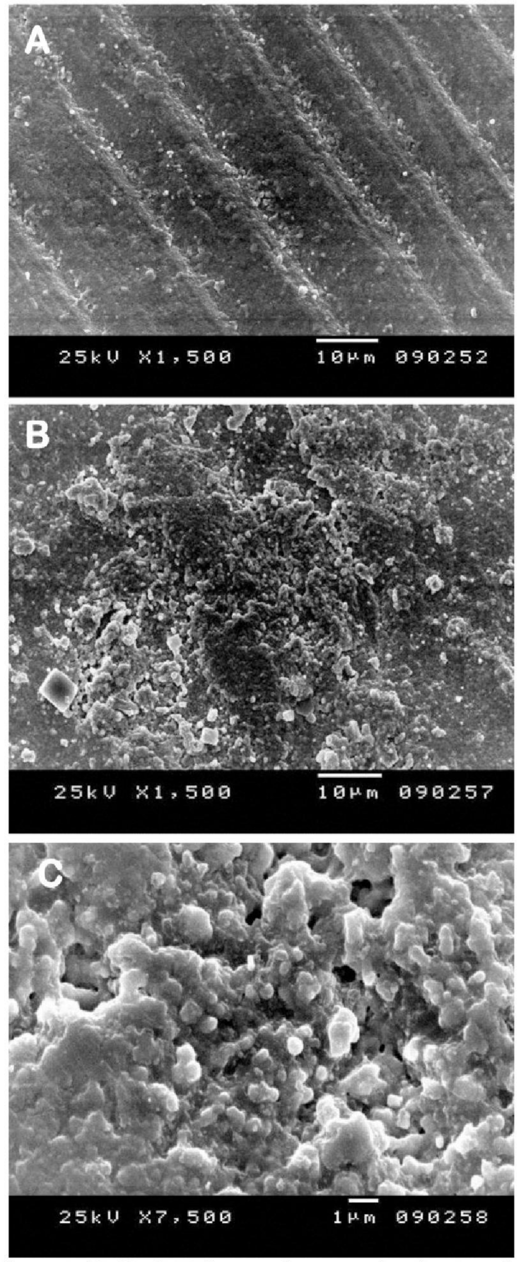

Figure 6. (A-C): SEM images demonstrating the 4-weeks *in vitro* biomimetic-bioactive assessment of yttrium-stabilized tetragonal polycrystalline zirconia (Y-TZP) surfaces. A: 1500x, showed the rough morphological pattern of milled Y-TZP surfaces socked in distilled water. B: 1500x, reflected accumulations of apatite minerals partially extended along Y-TZP surfaces socked in simulated body fluid. C: 7500x, a higher magnification showing the augmented formation of hydroxyapatite bone minerals on Y-TZP surfaces compared to PEEK after 4 weeks in simulated body fluid.

4. Discussion

Endosseous dental implant is a surgical device inserted surgically into the jaw bone to support a prosthodontic or orthodontic appliance(31). For decades Ti and its alloys have been the universal choice of implant materials(32). However, Ti appeared to have a number of drawbacks hence multiple researches were focused on studying alternatives to Ti over the past few years(33). Yttrium-stabilized tetragonal polycrystalline zirconia (Y-TZP) is a ceramic alternative for dental implants owing to its excellent mechanical and physical properties besides its biocompatibility and acceptable osseointegration features(10). However, its modulus of elasticity is much higher than that of bone which might lead to bone loss due to stress shielding(13). Accordingly, polymeric implant materials have been developed as another prospect. Polyetheretherketone (PEEK) is currently tested as a dental implant material, this is could be due to its natural beige color and its excellent modulus of elasticity which is close to that of bone(19). However, the potential of PEEK to replace titanium as a material for dental implants is still debatable(34). Dental implant is a complex treatment which requires meticulous mechanical and biological considerations to achieve optimal osseointegration along with attainment long-term clinical success. Furthermore, the main goal of biomimetic dentistry is to provide dental treatments mimic natural teeth as much as possible(20). Corresponding to this perception, the main purpose of the present study was focusing on biomimetic mechanical and biological evaluation of PEEK when used as an implant material in comparison to Y-TZP. Flexural strength and modulus of elasticity are mechanical properties of potential importance for ideal dental implant materials. One of the main issues concerning the choice of dental implant material is its modulus of elasticity as when a large difference between elastic moduli of both the implant material and its surrounding bone is recorded, stress shielding might occur leading to increased rate of peri-implant bone loss(19). Another main concern is the influence of regular performance and long-term aging on the mechanical properties of dental implant materials. Cyclic loading is considered as one of the essential aging methods that mimic mechanical loading on dental implants during normal masticatory function(21). By this means, it was designated in this study to apply cyclic loading as a mechanical aging imitating one-year performance under masticatory forces to allow biomimetic mechanical evaluation of both PEEK and Y-TZP as implant materials through assessment of their flexural strength and elastic moduli values. The current results demonstrated no statistically significant difference in the flexural strength and elastic modulus mean values between unaged and mechanically aged PEEK subgroups indicating the optimal performance of PEEK as an implant material under mechanical masticatory stress. Comparable outcomes were found by *Schwitalla et al.*(35) who tested the elastic modulus of different PEEK composites, and displayed 4.09 ± 0.80 Gpa as an elastic modulus mean value for PEEK Optima. Similarly, Another recent study by *Selvam et al.*(36) recorded the flexural strength of PEEK as 29.56 N/mm2 and its modulus of elasticity as 3.7 Gpa. Furthermore, *Schambron et al.*(37) conducted an experiment to evaluate the effect of aging and cyclic loading on the flexural strength of carbon fiber reinforced PEEK (CF/PEEK), and also showed no significant change in the flexural strength of (CF/PEEK) after cyclic loading. Similar findings were concluded recently in 2017 by *Dworak et al.*(38). Conversely, the mechanically aged Y-TZP subgroup showed significant decrease in both flexural strength and elastic modulus mean values compared to unaged one indicating the prevailing influence of cyclic loading on Y-TZP performance as a dental implant material after one year. Similar findings were recorded by other different studies of the same interest (21,39,40). The surface hardness is the resistance of a material to surface abrasion(41). Consequently, surface microhardness assessment could be considered as a crucial property when testing and comparing different dental implant materials. This is mainly owing to the critical demand of different dental implants for variable surface treatments to achieve adequate osseointegration and gain long-term success(42). The Vickers microhardness results of this study revealed statistically significant lower mean values for PEEK compared to Y-TZP indicating that, the surface hardness of PEEK as an implant material was clearly lower. This low surface hardness value of PEEK might be due to its polymeric plastic properties which are completely different from those of Y-TZP ceramics that recorded significantly high surface hardness values. These Vickers microhardness values of PEEK were in agreement with *Goyal et al.*(30) and *Wang et al.*(43). As well, Y-TZP microhardness results agreed with different previous studies assessed surface hardness properties of Y-TZP(28,39,41,44,45). Comparison of PEEK to Y-TZP showed statistically significant decrease in both flexural strength and elastic modulus mean values either for unaged or cyclic loading aged subgroups. Parallel significance was recorded in Vickers microhardness values of PEEK relative to Y-TZP. This could be attributed to the semicrystalline linear microstructure and flexible properties of PEEK as a polymeric material(46) in contrast to the polycrystallinity, highly rigid and brittle nature of Y-TZP ceramic materials(47). Based on the biomimetic mechanical evaluation findings, it could be stated that, PEEK showed promising mechanical properties supporting its performance as a dental implant material. This was mainly attributed to its contiguous rigidity to bone consenting reduced stress shielding and peri-implant bone loss with subsequent successful osseointegration. Also, PEEK kept its flexure values and exhibited minimal fatigue when subjected to cyclic loading with absence of crack propagation hypothesized a good prognosis of its performance as dental implant materials. Moreover, surface modifications of PEEK might be simple owing to its low surface hardness, and so different techniques of implant surface treatment could be useful to enhance the biological responses of PEEK. Concerning to the bioactivity of implant materials, *Kokubo et al.*(24) concluded that, the ability of a material to form apatite minerals on its surface after soaking in simulated body fluid (SBF) is directly related to its ability of apatite formation when implanted in the living body, and bonds across this surface apatite layer to living bone. Hence, *in vitro* bioactivity test using SBF could be considered a reliable method to evaluate the bone bonding ability of any implant material. In this present work, XRD patterns of PEEK surfaces after soaking in SBF for 4 weeks revealed the formation of different calcium phosphate crystal phases signifying the ability of PEEK to precipitate derivatives of bone minerals on its surface when biomimetically used as an implant material. These outcomes were confirmed with the scanning electron microscope (SEM) images that showed random growths of minerals distributed in a limited pattern on the PEEK surfaces. Similar results were obtained by *REN et al.*(48). On the contrary, previous studies found no deposits occurring on the surface of PEEK after immersion in SBF(49–52). These negative results might be due to the compositional difference of PEEK-Optima used in this study. Diffraction patterns of Y-TZP surfaces after socking in SBF for 4 weeks showed the hydroxyapatite characteristic peaks, in addition to other calcium phosphate phases. This result established the bioactive response of zirconia to form apatite minerals deposits appeared as frequent accumulations on its surfaces as showed by SEM morphological analysis. *Bozzini et al.*(53) in addition to other recent studies similarly found moderate amounts of crystalline precipitates on the zirconia surfaces after soaking in SBF for variable time intervals(54,55). Conversely, *Liang et al.*(56) immersed Y-TZP specimens in SBF for 7 days and found no precipitates on the surface when examined by SEM and XRD. This could be attributed to the shorter soaking period in SBF. Upon comparing the PEEK biomimetic-bioactivity results to those of Y-TZP, it was clearly observed that, even though fewer amount of different bone minerals were deposited on PEEK surfaces with lack of hydroxyapatite detection indicating the higher affinity of Y-TZP to form bone apatite minerals when implanted in living bone, the deposited amount of bone minerals on zirconia surfaces is still considered inadequate for adequate osseointegration. Accordingly, it could be deduced that, different mechanical and physicochemical surface treatments could be the crucial to increase the bone deposition abilities of both PEEK and Y-TZP and thereby achieve proper osseointegration. To the best of our knowledge no direct comparison was done between PEEK and zirconia.

Conclusions

Polyetheretherketone (PEEK-Optima) used in this study demonstrated exceptional mechanical performance when assessed as an implant material exhibited its flexural strength and elastic modulus properties significantly unchanged under biomimetic one-year cyclic loading. Additionally, the recorded significant low microhardness values simplified its surface modification to improve its biological performance when implanted into the living bone. However, *in vitro* bioactivity evaluation using biomimetic simulated body fluid concluded the reduced ability of PEEK-Optima to precipitate different crystal phases of bone minerals on its surface when compared to zirconia. Consequently, PEEK could be recommended as a brilliant alternative optimally serving as a dental implant material when compared to highly rigid and hard zirconia ceramics.

Recommendations

Further studies should be conducted to evaluate the influence of different surface modifications techniques on topographical, physicochemical and bioactive surface properties of Polyetheretherketone implants to enhance their biological activity and osseointegration.

Availability of data and materials

All data presented in the manuscript are available for publication.

Competing interests

Youssef M. Kassem and Dawlat Mostafa declare that they have no competing interests.

Funding

No funding conflicts are related to the study.

Authors' contributions

Author Youssef M. Kassem prepared the specimens and conducted the *in vitro* biomimetic mechanical aging. Author Dawlat Mostafa performed the *in vitro* tests and interpreted the outcomes. Both authors shared in the preparation of the manuscript. Also, both authors read and approved the final manuscript.

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