Original Article

Evaluation of the Bioactivity of Surface Modified Polyetheretherketone (PEEK) as an Implant Material: An *In Vitro* Study

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Abstract

Purpose: The purpose of this study was to evaluate the bioactivity of polyetheretherketone (PEEK) used as an implant material after surface modification by electron beam deposition of titanium. **Materials and Methods:** Twenty-two samples of PEEK were obtained from a single manufacturer, water jet sectioned, and divided randomly into two groups of eleven each (Group I and Group II). Eleven PEEK samples from Group II were coated with Grade II commercially pure titanium by electron beam deposition technique. One representative sample from each group was evaluated for surface roughness, topography and composition using three dimensional surface profilometer, scanning electron microscope coupled with energy dispersive X-ray (SEM-EDX) analysis. Simulated body fluid (SBF) was prepared and calcium (Ca) content in it was quantitatively analyzed by inductively coupled plasma mass spectrometry (ICP-MS) technique. Ten samples from each group were then immersed in SBF for a period of 21 days and amount of calcium depletion was analyzed to determine the bioactivity of two groups. Surface characteristics and elemental composition of immersed samples were analyzed by SEM-EDX and correlated with results of ICP-MS tests. The data obtained were then subjected to statistical analysis using independent *t*-test. **Results:** Group II samples showed a significant increase in surface roughness compared to Group I ($P < 0.02$). There were significant differences in Ca depletion of Group I and Group II samples when compared to preimmersion Ca content ($P < 0.001$). When compared between two Groups, Group II samples showed higher Ca depletion ($P < 0.001$). **Conclusion:** Within the limitations of this study, it was concluded that PEEK dental implants which were surface modified by electron beam deposition of titanium had enhanced bioactivity when compared to unmodified PEEK. Hence, they can serve as a valuable alternative to conventional dental implant materials.

Keywords: *Bioactivity, e-beam, inductively coupled plasma mass spectrometry, polyetheretherketone, polyetheretherketone implants, simulated body fluid, Titanium*

Introduction

Dental implants increase the quality of life for many patients with tooth loss. Developments in clinical prosthodontics are driven by introduction of new dental materials and technologies.[1] With increase in demands for functional and esthetic prosthetic replacements, research in dental implant materials is continuing at a fast pace since the last decade. For a restorative material to be called as biomaterial, it has to be biocompatible with good mechanical and esthetic properties.

The material of choice for oral endosseous implants is pure titanium. Implants based on titanium and its alloys, such as titanium-aluminum-niobium (Ti-6-A1-7Nb) and titanium-aluminum-vanadium (Ti-6A1-4V), are well evidence based from the late 1960s.[1] The success of implants depends on osseointegration, which is defined as the procedure by which mature bone is deposited directly on implant material without any intervening soft or fibrous tissues.[2]

Commercially pure titanium and its alloys possess good physiochemical characteristics, they promote osseointegration, and have high resistance to fatigue stress.[3] Although it has got many advantages, titanium as an implant biomaterial has some notable disadvantages which hinder their wide medical applications. Most important is their high strength and elastic modulus does not match those of normal human bone tissues. Marginal bone loss around titanium dental implants occurs to a certain level after the 1st year of function.[1,4]

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The long term clinical success of endosseous implants depends mainly on minimizing the amount of marginal bone loss after several years of functional loading.[5] Although the causes of bone loss remains unknown, occlusal overload has been reported to be primary etiological factor.[6] Previous studies have shown that these stiff implants do not adequately strain the bone, which can result in disuse atrophy and bone resorption.[5] This phenomenon is referred to as stress shielding[7] and causes the reduction in volume of the bone around an implant due to the shielding of normal loads by the implant.[8]

Another problem is a potential hypersensitivity to titanium. Although the incidence is low, urticaria, eczema, edema, redness and pruritis of skin, facial erythema, nonkeratinized, edematous, proliferative hyperplastic tissues have all been described in medical literature.[9]

As an alternative to Titanium, ceramic implants are proposed, which were first introduced 40 years ago and were made from aluminum oxide. Due to frequent fracture incidences, this material was substituted by Titanium. Nowadays, ceramic dental implants are made of polyetheretherketone (PEEK) and Zirconia, which seems to be a better suitable alternative to titanium because of its tooth like color, mechanical properties, biocompatibility and low plaque affinity.

PEEK is a synthetic semi-crystalline linear polycyclic aromatic thermoplastic material that was first developed by a group of English scientists in 1978.[10] In the 1980s, PEEK was commercialized for industrial applications, such as aircraft and turbine blades.[11] By the late 1990s, PEEK became an important high-performance thermoplastic candidate for replacing metal implant components, especially in orthopedic and traumatic applications.[3] PEEK was commonly used in vertebral surgery as a material of the interbody fusion cage.[11,12]

The chemical structure of PEEK exhibit stable chemical and physical properties.[11,13] It is wear-resistant and stable at high temperatures amongst polymers.[11] It is resistant to attack by all substances apart from concentrated sulfuric acid.[10,11] It remains stable in sterilization processes. Besides, PEEK exhibits good biocompatibility *in vitro* and *in vivo*, causing neither toxic or mutagenic effects nor clinically significant inflammation.[14] Furthermore, the oral microbial flora attachment to PEEK abutments is comparable to those made of titanium, zirconia and polymethylmethacrylate. More importantly, the mechanical properties of PEEK are close to that of human cortical bone.[13] For example, the elastic modulus of PEEK is approximately 8.3 GPa, which is close to that of human cortical bone (17.7 GPa) and much lower than that of titanium alloy (116 GPa) and cobalt–cromium alloy (210 GPa).[1,15]

The high elastic modulus of titanium and Cobalt-Chromium alloy increases the stress shielding resulting in bone resorption around the implant and causes the failure of implant. However, since the elastic modulus of PEEK is less than that of human cortical bone (8.3GPa), PEEK can be modified easily by incorporation of other materials like Carbon fibres which will increase the elastic modulus up to 18 GPa, which is compatible to that of human cortical bone. The carbon– reinforced PEEK could exhibit lesser stress shielding when compared to Titanium, when it is used as an implant material.[5]

Moreover, tensile properties of PEEK are also analogous to those of bone, enamel and dentin,[4] making it a suitable restorative material as far as the mechanical properties are concerned. These findings suggest that PEEK could substitute titanium as material for dental endosseous implants. PEEK is also widely used in dentistry as an implant healing abutment, removable prosthesis material, obturators, crowns and computer-aided design-computer-aided manufacturing milled fixed partial dentures.[16]

Bhoner *et al*.[17] stated that a bioactive material is "one which has been designed to induce specific biological activity." Bioactivity is the characteristics of an implant material which allows it to form a bond with living tissues.[2,18] Some of the previous studies have shown that PEEK is biologically inert,[13] which has limited its potential applications. Therefore, improving the bioactivity of PEEK is a significant challenge that must be solved to fully realize the potential benefits.[13] Three types of techniques have been advocated to enhance the bioactivity of PEEK. One is by incorporation of bioactive particles during the manufacturing process by either injection molding or compounding, secondly by physical and chemical surface treatments, and thirdly by incorporation of bioactive surface coatings.

The main disadvantage of incorporating bioactive particles is that it may alter the favorable mechanical properties of PEEK. Some physical and chemical treatments have also shown to degrade physical properties of PEEK material as they are biodegradable at 343 degree centigrade.[10]

Surface coatings can improve the interaction with bony tissues and results in better osseointegration of implant materials.[19] These coatings increase the surface roughness and surface wettability thereby increasing the bioactive potential.[20] Some of the most commonly used coating materials are titanium, hydroxy apatite, and bio glass. Titanium is a strong candidate as the coating material for PEEK implants as it has excellent biocompatibility as proved from several studies.[12] Therefore Titanium is coated over PEEK by Electron beam deposition technique.

Various approaches have been suggested to evaluate the bioactivity of implant biomaterials such as *in vitro*, laboratory *in vivo*, clinical trials, and *ex-vivo* analysis. One such *in vitro* test is immersion of implant biomaterials in

solutions like simulated body fluid (SBF), that replicate mineral content of human plasma.[18]

The immersion of implant biomaterial in SBF results in formation of surface apatite on the implant material. The evaluation of surface apatite by means of scanning electron microscopy (SEM) showed that it was similar to bone mineral in its composition and structure.[21] As a result, it was speculated that osteoblasts might preferentially proliferate and differentiate to produce apatite and collagen on its surface. Based on these results, it was proposed that a material which is able to form bone like apatite on its surface in SBF has potential to form apatite *in vivo* and bonds to bone. The *in vitro* testing of bioactivity in SBF has advantages as it represent artificial environment that can be manipulated by researchers in a controlled manner and has also minimized the requirement of animal studies.

Calcium analysis of the SBF solution by induction coupled plasma mass spectrometry (ICP-MS) both prior to and after immersion of samples has been recommended as a method to assess the apatite precipitation, which is considered as indication of bioactivity. This quantitative analysis is correlated with the qualitative analysis done by SEM and energy dispersive X-ray spectroscopy (EDX). The decrease in the quantity of Calcium content of SBF indicates the absorption of Calcium by the implant material, which is related to the bioactivity of the implant material.

In the literature, studies had been reported regarding the bioactivity of the unmodified PEEK implant material. However, studies related to bioactivity of PEEK surface modified by electron beam deposition of titanium is lacking in the literature. Hence, the present study was carried out to determine the bioactive potential of PEEK material after surface modification by electron beam deposition of titanium.

Materials and Methods

Preparation of test samples

Medical grade PEEK (Bredent, GERMANY) was obtained and sectioned using water jet cutting machine (Excel water jet cutting., Chennai, INDIA) to obtain 22 samples with dimensions of 12 mm × 5 mm × 2 mm [Figure 1]. Commercially pure Ti (MIDHANI, Mishra Dhatu Nigam Limited, Hyderabad, India) plates with dimensions of 10 mm X 10 mm X 1 mm were prepared from titanium sheet as a target material to coat over PEEK [Figure 2].

Emery treatment of sectioned samples

The sectioned samples of PEEK and Titanium were subjected individually to emery paper treatment (Sirag Dental Co., Chennai, INDIA). Each PEEK sample was held by an artery forceps (Sirag Dental Co., Chennai, INDIA) and ground using Silicon Carbide Emery Papers of 2000 grit, using a sandpaper mandrel (Sirag Dental Co., Chennai, India) attached to a dental Micromotor (Marathon, Korea).

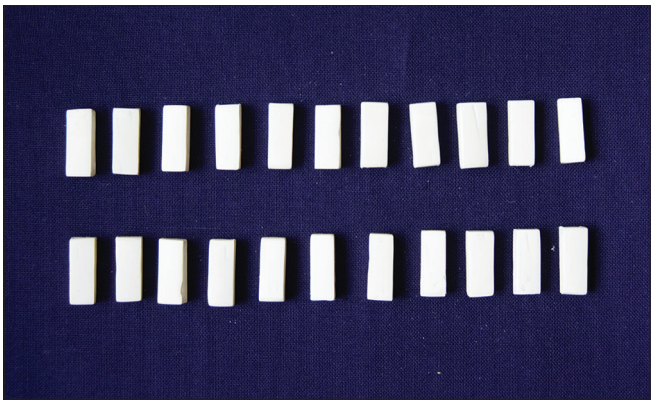

Figure 1: Sectioned peek samples

Commercially Pure Titanium was ground using Silicon Carbide Emery Paper of 220 grit. The samples were then rinsed with distilled water (Merck and Co., Mumbai, India).

Ultrasonic cleaning of samples

The samples of PEEK and Titanium are ultrasonically cleaned by distilled water for 3 min each (Beijing Ultrasonic Co., Beijing, China).

Grouping of samples

The samples were divided into 2 groups, each group comprising of 11 samples of 12 mm × 5 mm × 2 mm. The study groups were designated as Group I and II which are described subsequently.

- Group I samples (n = 10 + 1): Were not subjected to any treatment (unmodified)
- Group II samples (n = 10 + 1): Were subjected to surface treatment with electron beam deposition of commercially pure titanium (surface modified).

Surface treatments of samples

The samples of Groups II are subjected to surface treatments as described under:

Surface treatment of PEEK test samples by electron beam deposition of titanium (Plassys MEB600, FRANCE) [Figures 3 and 4].

Group II samples were coated with a thin film of Ti using an e beam evaporator. The prepared substrate was mounted on a rotating holder in a vacuum chamber and cleaned with an Argon ion beam with a voltage of 90 V and a current of 1.5A for 20 min before coating. Then the Ti film was coated on the PEEK substrate to a film thickness of 1 micro meter at a rate of 0.05 nm/s. The temperature of argon ion beam cleaning and titanium coating processes were approximately 90°C and 120°C, respectively. During the coating process, the substrate holder was rotated at 5 rpm to achieve a uniform thickness. After treatment samples are placed in

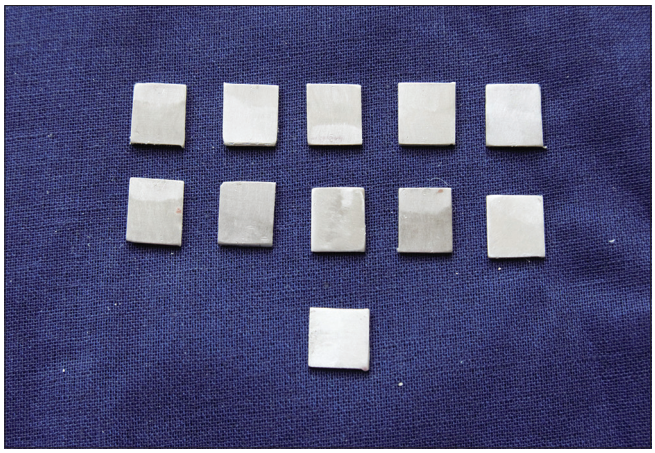

Figure 2: Sectioned titanium samples

Figure 3: Electron beam depositor machine

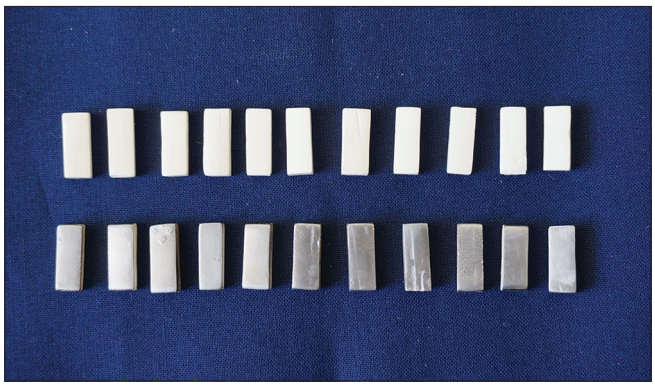

Figure 4: Uncoated and peek coated with titanium samples

desiccator before immersion in SBF. (Tarsons Products Pvt. Ltd., Kolkata, India).

Preimmersion quantitative and qualitative evaluation of surface texture of test samples

One sample from each group was tested for surface characterization using:

a. Three-dimensional (3D) surface profilometry (SP) for quantitative analysis of surface topography. (Taylor-Hobson, United Kingdom)
b. SEM-EDX Spectroscopy for qualitative analysis of surface morphology.(Hitachi S-3400n, Tokyo, Japan).

The photomicrographs of the test surfaces were obtained and the images were studied for the quality of the surface of the samples.

Bioactivity test

Preparation of simulated body fluid[Figure 5]

In the present study, a custom made SBF was used to assess the bioactivity of test specimens.

The custom-made SBF used in this study was prepared as per guidelines given for SBF preparation by Kokubo and Takadama[18] in their study.

In order to prepare 1000 ml of SBF, 700 ml of distilled water was taken in a 1 L plastic beaker (Polylab Industries Pvt. Ltd., Haryana, INDIA) (Polylab Industries Pvt. Ltd., Haryana, India). It was then set on the hotplate with a magnetic stirrer (IKA C-MAG HS., Bangalore, India) and a laboratory thermometer, (IKA ET3-D5., Bangalore, India) immersed in the plastic beaker. The water in the beaker was heated to 36.5°C ± 1.5°C under stirring.

Prescribed quantities of the required analytical reagents were weighed using an electronic weighing machine. The SBF was prepared by dissolving the reagents (Merck and Co., Mumbai, INDIA) in distilled water in the following sequential order [Figure 6]:

- NaCl (8.035 g)
- $NaHCO_3$ (0.355 g)
- KCl (0.225 g)
- $K_2HPO_4 \cdot 3H2O$ (0.311 g)
- $MgCl_2 \cdot 6H_2O$ (0.311 g)
- 1.0M-HCl (39 ml)
- $CaCl_2$ (0.292 g)
- Na_2SO_4 (0.072 g)
- Tris-hydroxymethyl aminomethane, $(HOCH_2)$[3] CNH_2 (6.118 g)
- 1.0M-HCl (0-5 ml).

Initially, the reagents from 1st to 8th in the order mentioned above were dissolved into the solution at 36.5°C ± 1.5°C one by one. The laboratory thermometer was employed to check and control the temperature of the solution. A reagent dissolved only after the preceding one was completely dissolved.

The temperature of the solution was set at 36.5°C ± 1.5°C. Distilled water was added to make the amount of the solution up to 900 ml in total.

The pH meter (Eutech instruments., Singapore) electrode was then inserted into the solution. Just before dissolving the 9th reagent, the pH of the solution was checked with a pH meter to be 2.0 ± 1.0.

With the solution temperature between 35 and 38°C, preferably to 36.5°C ± 1.5°C, the 9th reagent, Tris was

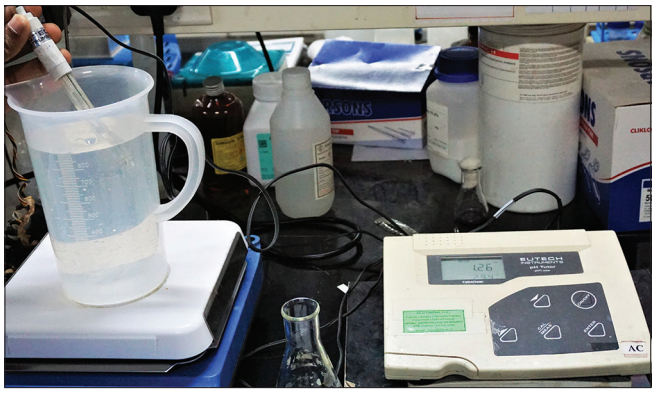

Figure 5: Simulated body fluid preparation procedure

Figure 6: Ingredients for simulated body fluid preparation

dissolved into the solution incrementally taking careful note of the pH change. After adding a small amount of Tris, further addition was discontinued until the reagent already introduced was dissolved completely and the pH had become constant; then more Tris was added to raise pH gradually.

When the pH became 7.30 ± 0.05, the temperature of the solution was maintained at 36.5°C ± 0.5°C, more Tris was added to raise the pH to 7.45.

When the pH had risen to 7.45 ± 0.01, further dissolution of Tris was stopped, and the 10th reagent, 1.0M-HCl was dropped by syringe to lower pH to 7.42 ± 0.01 the remaining Tris was dissolved little by little until the pH had risen to ≤7.45.

The remaining Tris was added into the solution alternately with 1.0M-HCl. This process was repeated until the whole amount of Tris was dissolved keeping the pH within the range of 7.42–7.45. After dissolving the whole amount of Tris, the temperature of solution was adjusted to 36.5°C ± 0.2°C.

The pH of the solution was adjusted (by dropping 1.0M-HCl little by little) at a pH of 7.42 ± 0.01 at 36.5°C ± 0.2°C and then finally the pH was adjusted at 7.40 exactly at 36.5°C.

The pH meter electrode was removed from the solution and rinsed with distilled water and the washings were added into the solution.

The pH adjusted solution was then poured from beaker into 1 L plastic volumetric flask (Polylab Industries Pvt. Ltd., Haryana, India).

The surface of the beaker was rinsed with distilled water and the washings were added into the flask. Further addition of distilled water was done to bring the lower meniscus of the liquid to the marked line and the flask was covered with a lid. The flask containing the prepared SBF was kept in water to cool it down to 20°C.

After the temperature of the solution dropped to 20°C, distilled water was added up to the marked line as required to obtain the recommended SBF for the study.

Preimmersion calcium-content analysis of simulated body fluid

The Ca-content of the prepared SBF solution was verified prior to immersion of the samples using ICP-MS (Agilent Technologies, California, USA) and this value was noted.

Immersion of test samples in simulated body fluid

Twenty-five millilitres of SBF was then poured into each of the 20 polypropylene test tubes (Polylab Industries Pvt. Ltd., Haryana, India), which were labeled to indicate the groups for identification.

One sample from each group were placed 1 per test tube in the SBF only after heating the SBF to 36.5°C ± 1.5°C. The samples were submerged completely in the SBF.

The test tubes were placed in plastic test tube stands (Polylab Industries Pvt. Ltd., Haryana, INDIA) and kept in a bacteriological incubator (Techlab Instruments Co., Chennai, India) at a maintained temperature of 36.5 ± 1.5°C for 21 days [Figure 5].

After soaking at 36.5°C ± 1.5°C in the SBF for 21 days, the samples were taken out from the SBF and washed gently with distilled water.

The samples were dried in a desiccator and stored there until further testing by SEM-EDX SPECTROSCOPY [Figure 7].

The SBF solution in each test tube was preserved for further analysis of postimmersion Ca-content using ICP-MS.

Postimmersion evaluation of simulated body fluid (calcium-simulated body fluid analysis)

The SBF from each test tube of each group was subjected to Ca-SBF analysis by ICP-MS, to assess the Ca-content depletion in the SBF and thereby determine the bioactivity of the specimens. The Ca-content values obtained from each test tube of SBF of all the two groups were recorded.

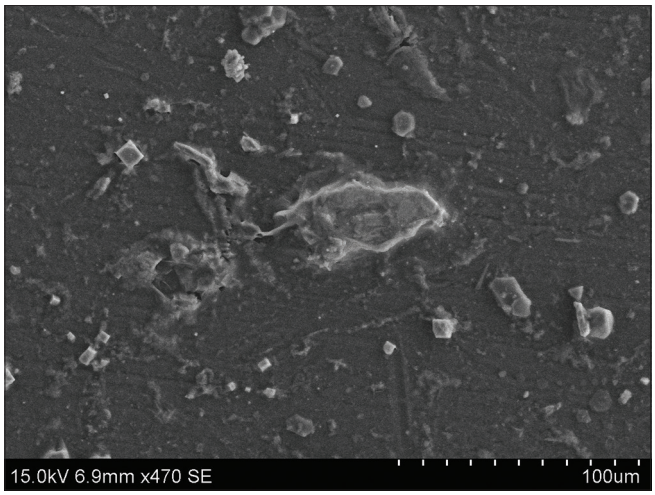

Figure 7: Postimmersion scanning electron microscope image of uncoated sample

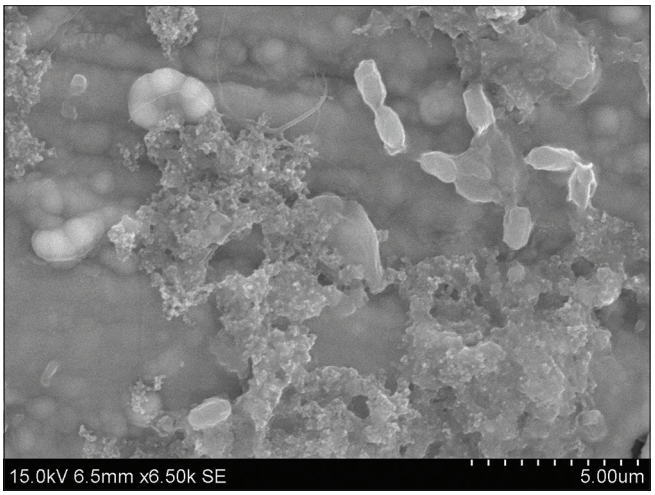

Figure 8: Postimmersion scanning electron microscope image of titanium coated sample

One representative sample [Figure 8] from each group was subjected to postimmersion surface analysis by SEM-EDX under magnification, to qualitatively assess the precipitated phases on the samples surfaces.

Surface elemental analysis or characterization by EDX of the above sample was also done.

Data tabulation and statistical analysis

- The basic data and mean values obtained were tabulated and subjected to statistical analysis
- *t*-test, was done to compare the data obtained for statistical significance with respect to surface roughness and bioactivity of the two groups. The Ca/P ratio was calculated based on the percentage weight of elements obtained by the EDX analysis.

Results

In the present study, the comparative evaluation of mean surface roughness (Ra) between two groups (Group I and Group II) showed higher surface roughness value of Group II than with Group I. Statistical analysis by *t*-test revealed that there is statistically significant difference in surface roughness of samples which are surface modified with titanium by electron beam deposition of titanium [Table 1].

The Calcium content present in the SBF after immersion of Group I and Group II samples is less than the pre immersion Calcium content. 't' Test revealed that this difference is statistically significant [Table 2]. The Calcium content present in the SBF after immersion of Group II samples is lesser than that of Group I samples. *t*-test revealed that statistically significant difference is present between the two groups. The lesser Ca content in SBF after the immersion of Group II sample revealed that Group II (Surface modified) has better bioactive potential compared to Group I (unmodified) [Table 3].

Table 1: Comparative evaluation of mean surface roughness (Ra) between the two groups (Group I and II) by *t*-test

Test groups	*n* (areas measured for Ra value)	Mean	*P*
Group I	4	0.190	0.002**
Group II	4	0.372	

Inference: The surface roughness value is higher with Group II than with Group I and this difference is statistically significant. ***P*=0.002; significant. SBF: Simulated body fluid

Discussion

Improvements in health care and increased life expectancy of the population demand the design of implant biomaterials demonstrating no or minimal deleterious effects on host tissues. Therefore, the development of new biomaterials is of importance for current implantology and also to offer new future possibilities for design solutions and product development.[22]

Despite its good mechanical properties, the adhesion of PEEK implants to bone tissue proceeds slowly because of their relatively low biocompatibility.[3] It is well acknowledged that the quality and quantity of host bone, presence of sufficient primary stability at the time of implant placement and formation of a direct bone-to-implant contact (BIC) are critical parameters that govern the overall success and survival of implants. However, implant surface characteristics (including surface topography, energy, chemistry, and roughness) also play significant role in enhancing osseointegration and BIC.[19,23,24] Studies have reported that increasing surface roughness of implants favors osteoblastic proliferation, collagen synthesis, and expression of integrin in the extracellular matrix, thereby improving the mechanisms associated with osseointegration.[25] In this regard, some studies placed localized organic and inorganic osteogenic coatings on implant surfaces in an attempt to improve implant surface

Table 2: Comparative evaluation of preimmersion Ca-content (reference value) in simulated body fluid with mean postimmersion Ca-content in simulated body fluid of two groups using *t*-test

Preimmersion Ca-content in mg/L (reference value)	Test groups	Mean postimmersion Ca-content in (mg/L)	Mean difference of Ca-content in (mg/L)	*P*
201.24	Group I	128.085	73.154	<0.001**
	Group II	58.671	142.568	<0.001**

Inference: *t*-test was used to calculate statistical significance of the Ca depletion in SBF of Group I and Group II samples. The Ca content present in the SBF after immersion of Group I and Group II samples is less than that of Preimmersion value, which is statistically significant (P<0.001). **P<0.001, **Highly significant. SBF: Simulated body fluid

Table 3: Comparative evaluation of postimmersion Ca-content in simulated body fluid between two groups (Group I and Group II) by *t*-test

Test groups	*n*	Mean	Mean difference	*P*
Group I	10	128.085	69.414	<0.001**
Group II	10	58.671		

Inference: The Ca content present in the SBF after immersion of Group II samples is lesser than that of Group I samples. *T*-test revealed that statistically significant difference is present between the two groups. The lesser Ca content in SBF after the immersion of Group II sample revealed that Group II (Surface modified) has better bioactive potential compared to Group I (unmodified). **P<0.001**; highly significant. SBF: Simulated body fluid

activity and osseopromotive activity.[23] Studies have also reported that implant surface roughness is directly associated with the degree of primary stability achieved and long-term success rate of the implant.[2]

Although PEEK is always physically and chemically stable, it can be modified by some kind of physical or chemical treatments. The commonly used physical treatments are plasma modifications and accelerated neutral atom beam. Similarly chemical treatments such as Sulfonation treatment can chemically modify the surface of PEEK.[10,26]

Composite preparation is another strategy to improve the bioactivity of PEEK, wherein bioactive materials are incorporated into the PEEK matrix during preparation procedure. However the main challenge in composite preparation is to keep intact the excellent mechanical properties of PEEK when impregnating bioactive materials.[10]

Another approach to surface modifications of the PEEK implant is by introduction of bioactive coating materials using various physical and chemical methods, including ionic plasma deposition (IPD), Electron beam deposition, Plasma spray deposition and *in vitro* precipitation. Surface treatment alone or in combination with surface coating can greatly improve the bioactivity of PEEK.[10,27] More recently, a significant amount of research has been conducted to modify PEEK by coating or blending it with Nano sized particles and producing Nano level surface topography. It is reported that incorporating Nano sized particles to PEEK can produce PEEK composites with enhanced mechanical properties, bioactivity and osseointegration.[28,29]

In vitro bioactivity of a test material can be assessed by various methods like by determining apatite formation following its immersion in SBF, alkaline phosphatase activity, human or animal osteoblast cell adherence, proliferation or differentiation, and by experimental animal studies. According to Kokubo and Takadama evaluation of bioactivity using SBF is a reliable method. SBF prepared in laboratory has ion concentrations nearly equal to those of human blood plasma, but not its organic component. They reported that an implant material bonds to living bone with formation of bone like apatite layer on its surface. He reported that this apatite layer can be reproduced in SBF. This means that the *in vivo* bone bioactivity of a material can be predicted by examining apatite formation on its surface in SBF. This method can be used for screening bone bioactive materials before animal testing and the number of animals used and the duration of animal experiments can be remarkably reduced by using this method, which can assist in the efficient development of new types of bioactive materials.[18]

Zhao *et al*.[30] in his study produced a structurally modified PEEK by Sulphonation procedure. These modifications showed 3D porous structure on PEEK substrate and enhanced its osseointegration and bone implant bonding. Jung *et al*.[31] in his study incorporated magnesium into PEEK to prepare PEEK/Mg composite. The results demonstrated active attachment and proliferation of osteoblast cells. Physical methods as described above have shown to alter the physical properties of PEEK in long term. Whereas incorporation of bioactive particles onto PEEK have raised concerns on maintaining the mechanical properties of PEEK intact. Hence surface coatings have been used in literature as an alternative to Physical treatments and composite preparation without damaging the Physical and mechanical properties of PEEK.

In literature various methods have been employed for surface coating of PEEK material. Yao *et al*.[20] employed IPD method for coating to study the change in bioactivity of PEEK. Electron beam deposition technique[3] is used in this study to coat Titanium over PEEK. Studies on this technique have reported that this technique does not damage the PEEK substrate by heat, provides good stability of coating layer and the deposited Titanium is highly crystalline in nature. Other advantages being dense, smooth, uniform and crack-free caoatings.[3]

Cook and Rust-Dawicki[32] coated Ti onto PEEK surface and concluded that the Ti-coated specimens had significantly higher percentages of bone contact than the uncoated specimens at both 4 and 8 weeks.[33] The level of proliferation and differentiation of the osteoblast cells was more than doubled after Ti was coated onto the PEEK surface.[3] Several studies have shown similar results in the literature.[13,29,34] In line with the above mentioned studies, Titanium is employed as a coating material over PEEK in the present study because of its time proven bioactivity and osseointegration.

Albrektsson and Wennerberg[22] stated that the surface properties of any implant biomaterial are reported to play a crucial role in promoting enhanced *in vivo* biological response and is one of the key parameters influencing osseointegration according to and several other researchers.[19,35-37]

Keller *et al*.[36] in his study on characterization of titanium implants determined that surface roughness played a major role in implant osseointegration. Results from several other studies were also in line with these findings.[19,23-25,36] This surface roughness can be determined by various methods like Atomic force microscopy, 3D surface profilometery, SEM analysis, Alpha two step profilometer etc., In the present study, surface roughness were assessed on representative samples of each group to obtain better insights of the unmodified and Titanium coated PEEK surfaces. Surface roughness evaluation was performed by 3D SP to obtain 3D, Nano resolution qualitative and quantitative data. The results showed surface roughness of 0.190 μm for Group I (unmodified) and 0.372 μm for Group II (surface modified by electron beam deposition of titanium). The surface treatment by Electron beam deposition of Titanium resulted in significantly higher surface roughness as compared to the untreated sample ($P < 0.02$).

These results were correlated with the respective 3D images of the sample, which revealed a relatively uniform surface appearance with poorly defined peaks and valleys with an average depth of 0.585 μm for Group I (unmodified) sample. The surface modification by Electron beam deposition (Group-II) revealed a predominantly nonuniform texture with moderate to high and well-defined peaks and valleys with an average depth of 1.319 μm.

These findings indicate that surface modification by Electron beam deposition of Titanium improved the surface roughness of PEEK samples.

SEM-EDX spectroscopy is performed to assess the surface topography at high magnifications and to assess the surface elemental composition, respectively. Zhou *et al*.[38] in his study on PEEK surface modification employed SEM-EDX as a method to observe the surface characteristics of PEEK. As previously mentioned in several studies these interpretations are valuable in understanding study results.[21,38-41] In this study, SEM photomicrographs revealed significant variations in the micro topographies of the unmodified and surface modified samples. Group I (Unmodified) sample exhibited smoother surface morphology with Parallel, shallow grooves, while Group II (Surface modified) sample showed an uniformly roughened surface morphology with distinctive surface nodules of deposited titanium. Presences of few micro cracks were also observed. These observations indicated that surface topography is altered due to deposition of titanium resulting in a more uniformly roughened surface. Respective EDX spectrums revealed the presence of the elements, Carbon, Oxygen, and Chlorine for Group I and Carbon, oxygen and titanium for Group II.

Several studies employing SBFs for analysing the bioactive potential of PEEK material have been documented in literature.[21,38,39,42] Chi *et al*.[43] coated titanium dioxide over PEEK substrate to determine the surface apatite formation over the surface by immersing in SBF for 14–28 days. Similarly Deng *et al*.[44] prepared a PEEK-Hydroxy apatite-Carbon composite and used SBF as one of method to determine the bioactive potential by analysing the surface apatite. Several studies in literature have employed SBF as a reliable source of determining bioactivity of various materials. Hence bioactivity of surface modified PEEK is evaluated in the present study, by employing the SBF. Since SBF is a highly saturated solution the preparation procedure is strictly followed as per the literature.[18]

A decrease in Ca-content of SBF is observed for bioactive materials, which is due to the precipitation of a calcium-rich phase from the fluid on the test surface. Therefore the lower the Ca-content in SBF for a particular test group postimmersion, the higher the Ca-rich phase precipitation and thus, higher the bioactivity for that test surface. Oyane *et al*.[45] in his study on analysis of SBF used ICP atomic emission spectroscope for the determination of dissolved elements such as calcium and phosphorous.[46] Lee *et al*.[47] in his study also employed the same technique to determine Ca ion concentrations.

After preparation, calcium content in SBF was assessed by ICP-MS which is more advanced in accuracy than ICP-atomic emission spectroscopy technique which are quoted in literature.[46] This equipment detects several metals and nonmetals at concentrations in range of 1–10 part per trillion (ppt) and automatically compute the ion concentration, from a 1 ml sample dose.

The preimmersion Ca-content of freshly prepared SBF was found to be 201.24 mg/L and this was used as the reference value for comparison with the postimmersion calcium content for calculating bioactivity.

All test samples (Group I and Group II) were individually immersed in test tubes containing 25 ml of SBF and

incubated at 37°+/-1.5°C for 21 days for standardization of study. In literature several immersion period in SBF ranging from 1 day to 28 days have been reported.[21,39,40,47] In a related pilot study, a 2 weeks immersion protocol was initially tested. However there was no appreciable calcium depletion in SBF or formation of apatite on the samples at the end of this period. Hence in the present study, the immersion of samples was done for a period of 21 days. This period lies in range of 14–28 days as reported in literature.

The SBF after immersion of samples for 21 days is analyzed for its Calcium content by ICP-MS, for both the groups (Group I and Group II).

The Group I (Unmodified) showed a mean postimmersion Ca-content of 128.085 mg/L, Group II (Surface modified) showed a mean postimmersion Ca-content of 58.671 mg/L at the end of 21 days.

The difference between the mean pre- and post-immersion Ca-contents in SBF that is observed is due to the precipitation of calcium-rich apatite phase on the PEEK test surfaces. The lower the postimmersion Calcium content in SBF, the higher the bioactivity for that particular test group. On comparison, the respective mean postimmersion Ca-content in SBF for both the test groups showed statistically significant calcium depletion when compared with the preimmersion Ca-content, indicating highly significant bioactivity for both untreated PEEK as well as the surface modified PEEK ($P < 0.01$).

The results of the present study indicates that the untreated PEEK showed significant bioactivity by virtue of Calcium depletion observed after immersion in SBF for 21 days. However, this result was considerably lesser as compared to that of surface modified group ($P < 0.01$).

Examining the surface characteristics of samples after immersion in SBF for presence of Apatite crystals have been shown in several studies as a qualitative method for assessing bioactivity. This bone like apatite precipitation is considered as an indication for enhanced *in vivo* bioactivity.[21,38,40,41]

The Unmodified and surface modified PEEK samples after immersion for a period of 21 days is qualitatively analyzed by SEM and EDX for its surface characteristics and elemental composition. EDX was also employed to determine Ca/P ratio on the surface of samples. Thus, despite being categorized as a bioinert polymer, there is a definite apatite forming tendency on untreated PEEK at the end of a 3 week immersion period. However, this apatite layer formed on the unmodified sample was found to be a poorly defined, scattered crystals of bone-like apatite, with evidence of uncovered PEEK substrate at certain locations, as evidenced from the postimmersion SEM image. The postimmersion SEM images for Groups II also corroborate this finding of superior bioactivity, in that, isolated clusters of well-formed crystals of apatite structures of varying sizes were observed. In literature Elemental analysis after immersion in SBF have shown to have Ca and P in their surface.

The postimmersion EDX results revealed a higher Ca/P ratio for the surface treated groups as compared to the unmodified group. Group II (Surface modified) exhibited the highest Ca/P ratio of 1.923, followed by Group I (Unmodified) with ratios of 1.037. It has been reported in the literature by authors like Wang *et al*.[13] and Li *et al*.[48] stated that Ca/P ratio of 1.50 indicates apatite formation similar to trabecular bone, whereas, values upwards of 1.60 indicate cortical bone-like apatite formation. All these findings suggest that surface treatment of PEEK serves to significantly enhance its bioactive potential and also results in apatite layer of superior quality as compared to an unmodified surface.

These results indicates that unmodified PEEK surfaces also attracts the calcium present in SBF, but to a lesser extent than surface modified samples. Surface treatments are said to promote bioactivity, since they remove impurities, reduce surface hydrocarbons, increase surface energy, thereby, providing improved surface characteristics such as roughness and wettability, that are critical in promoting cell adhesion and calcium apatite formation. Previous studies evaluating efficacy of surface coating of Titanium by Electron beam deposition method have reported improved Osteoblastic cell adhesion and osseointegration. The results of superior bioactivity of Group II samples obtained after electron beam deposition technique in the present study complement the results obtained from previous cell culture studies.[3]

Conclusion

The results obtained with the present study serves as an encouragement for the use of PEEK as an implant biomaterial. Within the limitations of the present study, it is suggested that the surface modification by electron beam deposition of titanium can be employed to significantly improve the bioactivity of PEEK. Therefore, the same concept can definitely be applied for PEEK implants as well.

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Conflicts of interest

There are no conflicts of interest.

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