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Sulfonated polyether ketone ketone (SPEKK) implant as an alternative to titanium implant- in vivo study on Wistar Albino rat mandible

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Abstract

Background Titanium is commonly used for dental implants due to its high biocompatibility and durability. However, concerns about metal ion release and aesthetic limitations in certain tissue biotypes have driven the search for alternative materials. Polyetherketoneketone (PEKK), a high-performance polymer, has emerged as a promising option due to its biomimetic properties. Surface modifications, such as treatment with sulfuric acid (H2SO4), may further enhance its osteogenic potential.

Aim The study aimed to evaluate the osteogenic efficacy of H2SO4-modified PEKK implants in comparison to titanium implants.

Methodology Three groups were assessed: Titanium, unmodified PEKK and H2SO4-modified PEKK(SPEKK). Surface characteristics were analyzed using scanning electron microscopy (SEM). Wettability was checked through contact angle evaluation. Cell viability was evaluated through MTT assays. Implants were placed in rat mandibles, and bone formation was analyzed after 6 weeks using nano-CT and histological assessments. Toxicity was as Statistical comparisons were performed using one-way ANOVA and Tukey's post-hoc test.

Results Acid-modified PEKK implants exhibited the highest bone formation (280.09 ± 12.03) significantly outperforming Titanium (265.12 ± 11.08) and unmodified PEKK (266.52 ± 7.28) ($p < 0.05$).

Conclusions H2SO4-modified PEKK (SPEKK) implants demonstrated superior osteogenic properties compared to titanium, suggesting that these modified polymers could be viable alternatives for aesthetic implants.

Keywords PEKK, Sulfonation, Surface modification, Osseointegration, Animal study

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Introduction

The field of dental implantology has a long history, having progressed from prehistoric tooth replacement attempts to more sophisticated contemporary methods [1]. Dental implants have a long history, having been utilized to replace lost teeth in ancient cultures using materials like seashells and carved stones [2]. Millions of dental implants are now inserted annually throughout the world, giving patients long-lasting and useful replacements for lost teeth [3–5]. Despite its widespread use, titanium dental implants have a number of drawbacks [6]. Their vulnerability to corrosion is a significant issue, especially when it comes to the oral cavity. Titanium particles are released by corrosion into the surrounding tissues, which may cause inflammatory reactions and damage to the DNA of oral epithelial cells [7]. Moreover, inflammatory markers including interleukin IL–1β, IL–6, and Tumor necrosis factor TNF-α can be activated by titanium particles released during implant procedures or maintenance, such as ultrasonic scaling, which can cause tissue irritation or bone loss [8]. Titanium allergies are uncommon—affecting only 0.6% of the population—but for certain people, they can lead to problems [9, 10]. An additional disadvantage of titanium implants is their susceptibility to weakening over time, particularly in the presence of corrosion, thus jeopardizing their long-term stability and strength. Titanium's high Young's Modulus (110–120 GPa) makes it stiffer than bone (10–30 GPa), potentially causing stress shielding and bone resorption. On the other hand, PEEK, with its lower Young's Modulus (3–4 GPa) similar to that of bone, facilitates better load transfer, minimizes stress shielding, and improves long-term implant stability, positioning it as a promising alternative to titanium for dental implants.

Polyetherketoneketone (PEKK) is an emerging polymeric material that has gained significant attention from researchers due to its exceptional properties, making it suitable for a wide range of applications [11, 12]. PEKK is a high-performance thermoplastic that is free of methacrylate, offering durability and versatility. A strong osseointegration property is essential for the long-term success of permanent orthopaedic and dental implants, as it directly influences the implant's stability over time. Poor osseointegration can result in the formation of a layer of fibrous connective tissue (fibro-integration) at the interface between the implant and the host bone. This tissue layer can cause clinical instability, particularly under load-bearing conditions, which may lead to implant loosening and eventual failure. Polyetherketoneketone (PEKK), a non-degradable and bio-inert polymer, has shown limitations in osseointegration [13]. Some studies have reported the formation of fibrous encapsulation around PEKK implants, which weakens the implant's ability to bond with bone and reduces its osseointegration properties [14]. This poses a potential drawback for the use of PEKK in applications where strong bone integration is critical, such as in orthopaedic and dental implants. Extensive research has been undertaken to improve the osseointegration capabilities of PEKK [15, 16]. Various surface modification techniques, such as surface roughening, plasma treatments, and chemical alterations, have been applied to enhance cell adhesion and bone integration [17, 18]. These modifications aim to overcome PEKK's inherent limitations in osseointegration, making it more effective for use in orthopedic and dental implants.

In order to boost the longevity and success of dental implants in human subjects, researchers are attempting to improve the process of converting laboratory discoveries into clinical applications, which requires a crucial initial step—animal studies. Animals make excellent subjects for ethically and carefully controlled research on the biological responses, osseointegration processes, and potential adverse consequences of various implant materials due to their anatomical and physiological parallels to humans [19, 20]. Prior to proceeding with human trials, these studies offer researchers invaluable understanding into the biocompatibility, biomechanics, and durability of dental implants.

Although PEEK is also a good alternative and both are from same aryl group, PEKK was chosen as this material is more mechanically stable. The sulfonation percentage was taken through reference articles and therefore highest percentage given in literature was taken [21]. The study aimed to enhance the surface roughness of PEKK implants through sulfonation to increase their hydrophilicity and evaluate the osteogenic potential both in vitro and in animal models. The null hypothesis proposed that there would be no significant difference in osteogenic potential between sulfonated polyetherketoneketone (SPEKK) and titanium (Ti) implants.

Material and method

Study settings and sample size determination

This investigation was done on the basis of three groups. Group 1 being Titanium (Ti), group 2 and group 3 being polyetherketoneketone (PEKK) and sulfuric acid-modified PEKK (SPEKK), respectively, the control group being Ti. The study was done in two phases: Phase 1 was in vitro, and Phase 2 was in vivo (Animal model). The study was carried out in a deemed dental institute with approval (BRULAC/SDCH/SIMATS/IAEC/01–2023/05) of the scientific review board. Sample size was calculated to be 45-disc samples (9 for surface topography and 36 for contact angle and MTT) and 24 animals using G*Power software 3.0.10 [22]. In order to conduct an animal study based on prior research, a total of 24

animals were randomly split into three groups (Ti, PEKK, SPEKK), each with eight samples.

Sample preparation

For in-vitro analysis PEKK (INTAMSYS, China) disc shaped samples of dimensions 10 mm in diameter and 2 mm in width, were 3D printed using thermal resin 3D printer (FUNMAT PRO 410, INTAMSYS, China). Titanium samples were printed using Direct Metal Laser Sintering (DMLS) technology (EOS, Munich, Germany). Titanium DMLS samples were fabricated using an EOS (Munich, Germany) metal 3D printer. The samples were produced from Ti–6Al–4 V (Grade 23) titanium alloy (EOS GmbH, Munich, Germany) using the EOS M290 printer, with a layer thickness of 30 µm, laser power of 400 W, and a scanning speed of 1200 mm/s. For animal study sample of screw (3 mm diameter, 3 mm length) design was made in sketch app software and STL (Standard Tessellation Language) file was exported to make 3D printed (FUNMAT PRO 410, INTAMSYS, China) PEKK sample and Titanium DMLS (EOS, Munich, Germany) sample.

Surface modifications

To ensure the sterility and cleanliness of all samples, a rigorous cleaning protocol was implemented. Initially, all samples, regardless of their material, were autoclaved. This was followed by disinfection using acetone for 2 min, after which they were exposed to ethanol for an equal duration [23]. Next, the samples were washed for 10 min on a shaker equipped with a magnetic pellet. As part of the preparation, samples underwent sulphonation for 5 min using 98% sulfuric acid as the reagent [21]. Following this step, the samples were placed in a hot air oven at 50 °C for 30 min. After modification of samples were cleaned again using the same detailed protocol and autoclaved once more to ensure sterility before use in the study.

Invitro evaluation

Scanning electron microscopy

Surface topography was observed by Scanning Electron Microscopy (0.7 mm SEM TEM EDS; Hitachi High Technologies Co., Tokyo, Japan). Scanner electron microscopy was used to observe the surface topography. The sample was first analyze using secondary electron imaging (SE) at a 7 kV acceleration voltage. TIFF grayscale files containing digital images with a resolution of 1424 × 968 and 8 bits per pixel were quickly created. The same specimen was then subjected to additional processing. After achieving a smooth surface through polishing, a high-precision diamond-coated disk was used to slice it exactly along its longitudinal axis. The material was then implanted in glycol methacrylate using a Technovit 7200 VLC (Heraeus Kulzer, Hanau, Germany) apparatus. Following the embedding process, it was mounted using the same technique and carbon coated using an Emitech K250 flash evaporator from Emitech located in Montigny Le Bretonneux, France. The investigation proceeded using the same FEG SEM, but with backscattered electron (BSE) imaging and an acceleration voltage of 15 kV.

Energy dispersive X-ray (EDX)

The chemical composition of the SPEKK was semi-quantitatively analyzed using an EDX analyzer (FEA-USA (S.E.A) PTE LTD [Figure 1 A].

Contact angle

The wettability of a solid surface by a liquid is gauged by the contact angle. It is the angle that forms at the point where a solid surface and a droplet of liquid collide. An important measure of the interactions between a liquid and a solid substance is this angle, which measures how a liquid spread over a solid surface. The contact angle was measured with the help of a Goniometer. The surface of the device was cleaned and the samples were cleaned using ultrasonic cleaning. Contact angle measurements were performed with deionized water at 22 °C using the static sessile drop method [22]. A Kruss needle (model NE62, outer diameter = 1 mm, inner diameter = 0.82 mm) was used to suspend deionized water, which was then let to fall freely onto the substrate surface. Proper lighting was ensured for the assessment of the drop and the device was aligned with the camera to get proper images. Measurements of the contact angle were used to assess the surface qualities. The objective of this study was to evaluate the wettability of PEKK and modified PEKK samples by measuring the contact angle between water and the samples.

Surface roughness

In non-contact mode, Atomic Force Microscopy (AFM; PSIA XE–100) was used to measure surface roughness using a silicon tip with a resonance frequency of 32 kHz and a force constant of 42 N/m [12]. With a resolution of 515 × 515 pixels, pictures were taken with a scan rate of 0.5 Hz. This technique reduced tip-sample interaction and produced high-precision surface topography measurements, guaranteeing accurate surface roughness evaluation [Figure 1B].

MTT assay

In vitro materials and method Materials and Procedure for In vitro Stem Cell Isolation: At Saveetha Dental College and hospitals, human SCAP cells are extracted from the dental roots of molar teeth that have been extracted as a result of orthodontic treatment. The Institutional Ethics Committee authorized the study procedure, and each

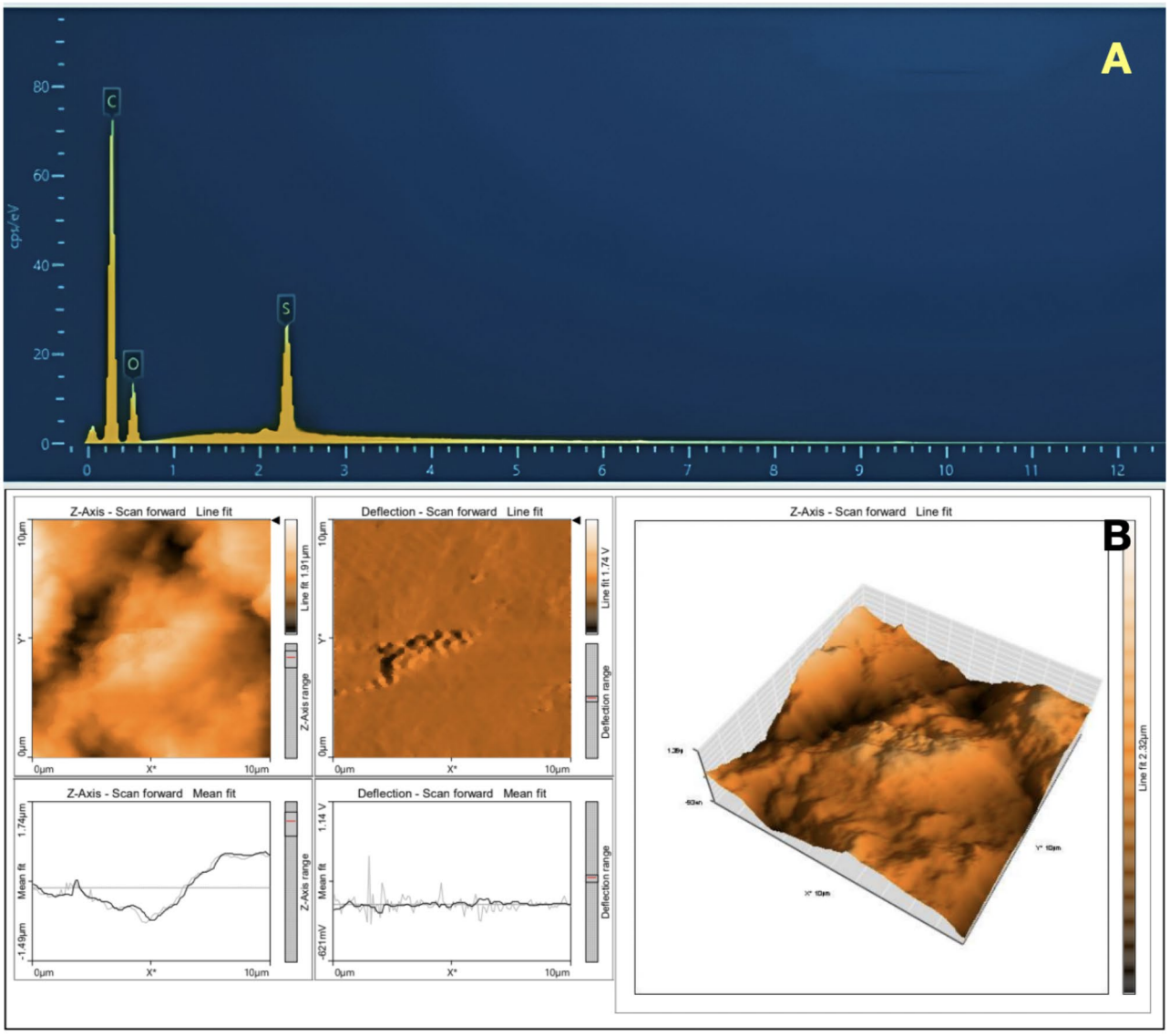

Fig. 1 **A**. Elemental analysis from EDX, **B**. Surface roughness from AFM

participant provided informed permission. After rinsing the extracted tooth's apical portion with phosphate buffered saline (PBS), it was placed in a digesting solution that contained 1 mL of PBS and 3 mg/mL of type II collagen. It was then incubated for one hour at 37 °C. The output of centrifugation was then poured into a T25 flask filled with DMEM-F12 medium. The resultant samples were incubated at 37 °C with 5% CO2. Every three days, the culture media was replaced, and the subculture was carried out at 80% confluence. For this investigation, all in vitro tests involved seeding cells in culture dishes at passage 2.

Cell viability/ MTT assay The MTT reagent was used to determine the biocompatibility of the experimental and control groups (C.Q. included carrageen hydrogel 2.5% and 5%). During a day 1 and day 3 incubation period, the biocompatibility of C.Q. integrated carrageen hydrogel 2.5% and 5% hydrogels was assessed by looking at DPSc attachment and growth. Following elution from C.Q., 2.5% and 5% hydrogels containing carrageen were incubated on stem cells planted on 96-well culture plates for 24 and 72 h, respectively. In order to calculate the percentage of viable cells, 10 μl of stock MTT dye (10 mg/ml) was added to each well of the post-incubated cells, and the plate was then incubated for an additional 4 h at 37 °C. Using a Synergy hybrid Multi-Mode Reader (BioTek, Winooski, VT, US), absorbance was measured at 570 nm after the medium was changed to 100 µl DMSO in each well to dissolve the formazan crystals. We used the following formula to determine the percentage of cell viability:

$$Cell\ viability\ (\%) = O.D.\ of\ cells\ treated\ with\ CLC\ NPs / O.D.\ of\ control\ cells \times 100$$

Using freshly prepared GIC in vitro cell culture, live/dead labeling was carried out two days after incubation to determine which cells were alive and which were dead. Using a Live/Dead Viability/Cytotoxicity kit (Calcein-AM dye, Invitrogen, USA) with minor adjustments was done in accordance with the manufacturer's instructions. Briefly, 6-well plates were used to seed the stem cells at a density of 1 × 106 cells/well. Calcein-AM dye was added after 24 h of culture, incubated for 30 min, and then rinsed with 1x PBS. Inverted Phase Contrast Fluorescence Microscopy (Invitrogen, evos) was also used to check the cells. Calcein-AM was used exclusively to stain viable cells that were showing green fluorescence. In order to determine the ratio of living to dead cells for each cell state, live and dead labeled cells were manually counted. The Fijian Analyze Particles measurement was used to determine the cellular aspect ratios from thresholded LIVE/DEAD pictures [Figure 2].

Animal study

Sample selection

Male Wistar Albino rats were selected, aged 3–5 months and weighed 200–300gms. In accordance with the moral principles guiding animal research, the Institutional Animal Ethics Committee approved the use of rats as animal models. 30 male rats were selected of which 24 were randomly selected by manual randomization process and used for the experiment (8 /group). It was triple blinded study. The author, operator and the researcher were not aware of the groups.

Selection criteria

Inclusion Criteria: Species & Strain, Age Range, Weight Range, Gender, Health Status, No Previous Surgery/Intervention.

Exclusion Criteria: Signs of Disease or Injury, Pre-existing Conditions, Prior Medications/Treatments, Pregnancy or Lactation, Recent Surgery or Trauma, Abnormal Weight/Growth.

Surgical procedure

Every surgical procedure will be carried out in a sterile animal laboratory operating room, following strict hygiene protocols. Rats will be put to sleep using intraperitoneal injection (i.p.) ketamine hydrochloride (Anaket, Neon Laboratories Ltd., India) and intramuscular (IM) xylazine (Rompun, Bayer, Germany) at doses of 75 mg/kg and 10 mg/kg body weight, respectively based on previous literature. After shaving, the ventral portion of the neck is aseptically prepped with a benzidine solution. On the anterior neck, a single median vertical skin incision of 2 cm length is done to reveal the muscles and fascia below. The mandibular bone is revealed as these tissues withdraw. On one side of the jaw, a standardised round through-and-through osseous defect of 3 mm in diameter was made in a similar manner using a motor regulator-controlled straight hand-piece drill equipped with a trephine bur. The tissues are frequently irrigated

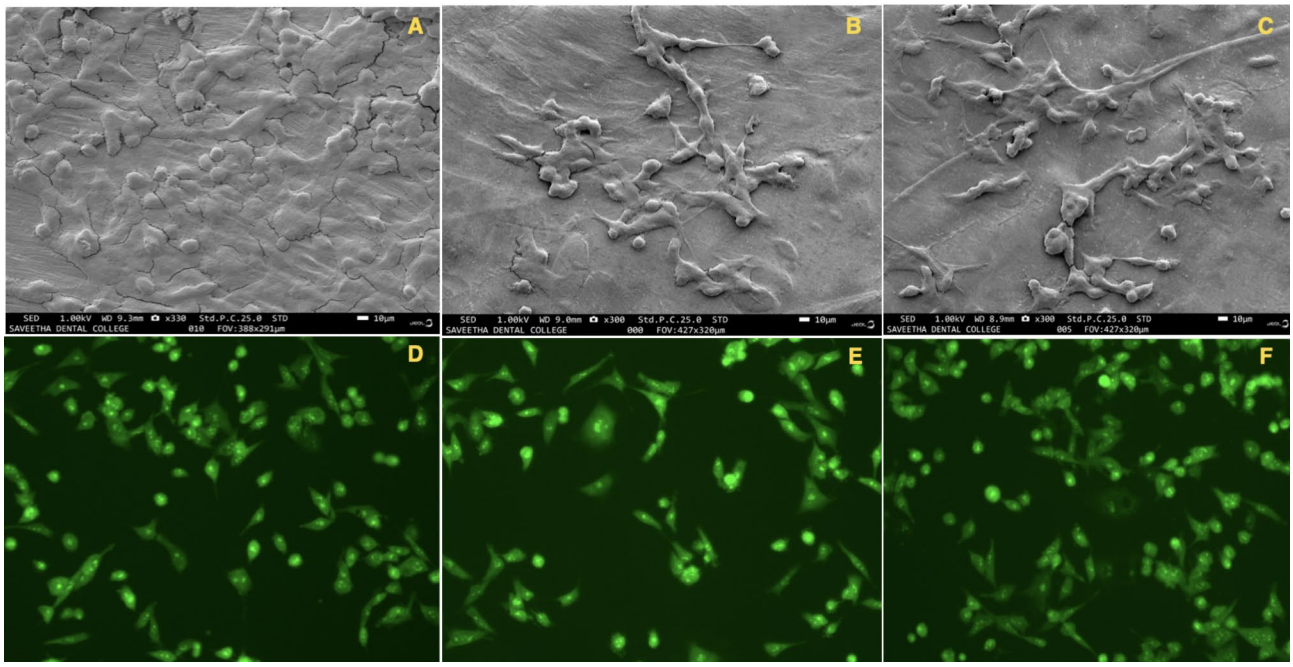

Fig. 2 MTT assay showing cell viability under SEM and Fluorescent Microscopy **A**, **B**, **C**. Showing cells under Scanning electron Microscopy and **D**, **E**, **F** showing cells under fluorescent microscopy for Ti, PEKK AND SPEKK respectively

with saline water during the drilling operation. During the procedure, great care was taken to avoid damaging the vessels. Following that, all groups of implants were manually screwed into the drilled site in a clockwise motion to gradually insert the implant into the bone. All the groups (Ti, PEKK, SPEKK) underwent the operation. Following the application of betadine ointment to the sutured area and the suturing of the tissue flaps using resorbable suture threads (Vicryl 5/0, Ethicon®, Somerville, NJ, USA), the rats are placed in separate cages. 1.5 ml of blood was drawn by cardiac puncture [Figure 3].

Post operative management

As a postoperative analgesic, subcutaneous Meloxicam (Artaz, Torrent Pharmaceuticals, India) (1 mg/kg body weight) was administered. The rats were given boluses made of a regular pellet diet (VRK Nutritional solutions, India).

Humane end-point

Rats were healthy and euthanasia was performed for the rats after 6 weeks by putting them to sleep in a CO2 chamber. The rats were then sectioned and the parts were used for Nano CT and histology purposes. Under sedation that was induced, the animals were put to death in a CO2 chamber. Mandible was sectioned and was preserved for 48 h at 4 °C in a 10% CaCO3-buffered formalin solution (pH 7.4). They were then stored at 4 °C in 70% ethanol until the nanoCT examination.

Biochemical tests

Serum preparation

Before euthanasia cotton wool saturated in chloroform fumes was used to anaesthetise the rats. They were taken out of the jar as soon as they passed out. Rats' eyes were used to draw blood, and the rats were kept head down while the blood was allowed to flow into capillary tubes that had been cleaned, dried, and corked. The rats were then allowed to clot, and the serum was allowed to develop for ten minutes at room temperature. After spinning at 3000 rpm for 5 min, the serum was collected using a Pasteur pipette, frozen, and used within 12 h of collection for the various assays of liver and kidney function.

Renal function test and liver function test

Creatinine and urea level was checked for renal function test (RFT) and SGOT and SGPT level was checked

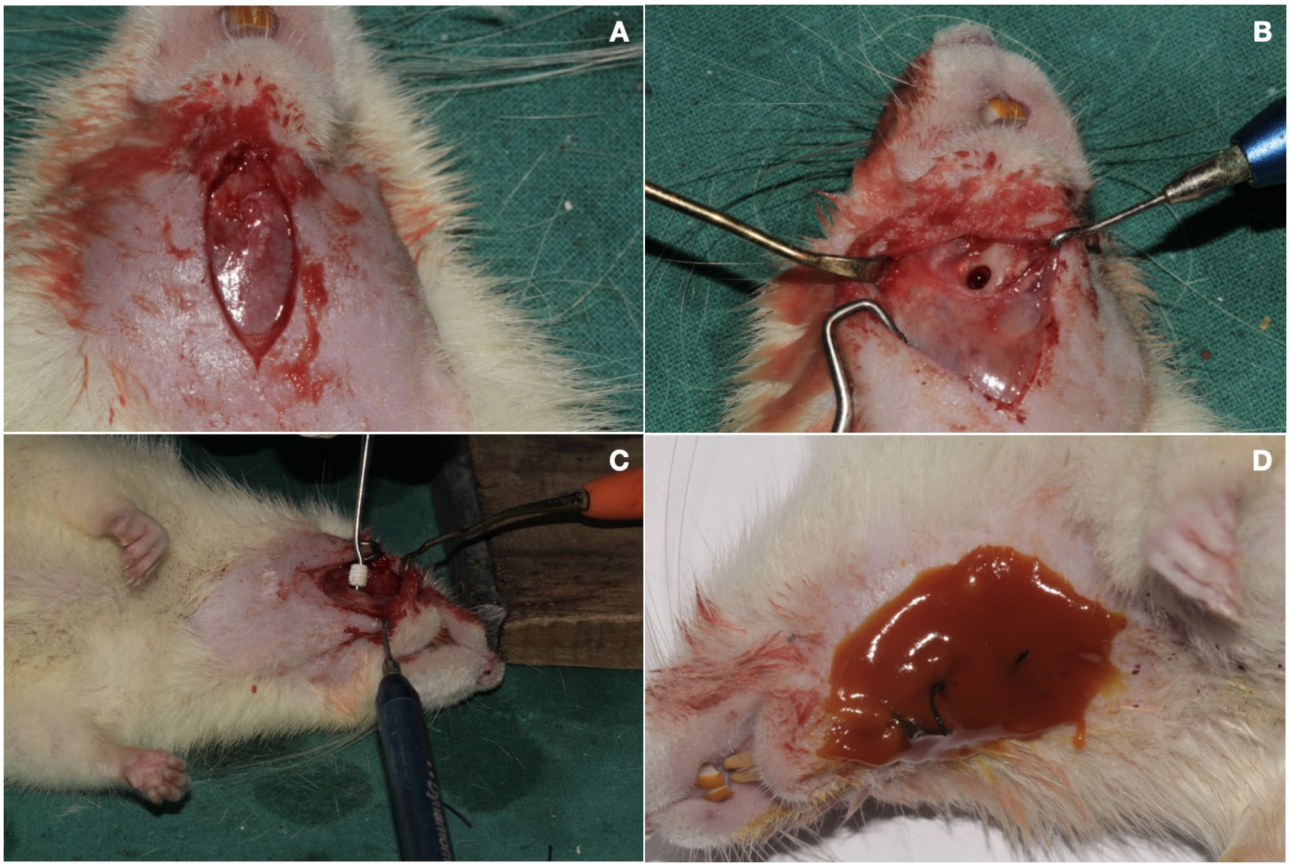

Fig. 3 Surgical procedure: **A**- Incision, **B**- Osteotomy site preparation, **C**- Implant placement in the mandible, **D**- Closure of the site and antiseptic solution application

in liver function test (LFT). The Tietz et al. [23] method was used to assess the creatinine concentration, and the Kaplan method [24] was used to calculate the serum urea concentration. The liver function was assessed using IFCC techniques, and the results were analysed using 365 nm Genesis 20 spectrophotometer. Blood plasma was combined using a reagent kit at room temperature (37 °C). A total of 1000uL of blood plasma was combined with a reagent kit in 100 µL.

Nano CT imaging

The high-resolution nano-CT scanner Skyscan 2214 (Bruker, Kontich, Belgium) was intended to be used with this protocol. The scanning specifications included a 1 mm Al filter, 80 kV X-ray voltage, 90 µA electric current, and 20.0 µm pixel size. NRecon software was used to rebuild the data sets (SkyScan, Aartselaar, Belgium). 2D and 3D imaging were obtained from the nano CT imaging by sectioning the bone sample and measuring the bone volume and trabecular thickness in the sectioned portion [Figure 4].

Histological evaluation

Following Nano-CT analysis, the samples were embedded in poly methyl methacrylate after being dehydrated in alcohol at progressively higher concentrations (75 to 100%). Using a microtome (SAT–001, AoLiJing, China), the implanted specimens were longitudinally cut into sections about 100 µm thick. These sections were then polished and ground down to a final thickness of about 25 µm. The resulting slices were observed histologically using a light microscope (Bx60, Olympus, USA) fitted with a digital charge-coupled device camera after being stained with methylene blue, basic fuchsin, and toluidine blue. Using Image-Pro Plus software (Media Cybernetics, USA), the obtained microscope pictures were histologically assessed for a quantitative examination of freshly produced bone tissue. The area of new bone in the implant's macropores was measured quantitatively during the procedure using the "segmentation" tool.

Statistical analysis

To evaluate differences within and between the groups, the collected data was subjected to the relevant statistical analysis. The statistical study was carried out utilising IBM Corp.'s 2011 SPSS software. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.], where all statistical tests are performed at a significance level of $p < 0.05$. One way ANOVA was done to compare among groups (Ti, PEKK, SPEKK) and for pairwise comparison Post hoc Tukey test was performed to check the difference between groups.

Results

Micromorphology

The PEKK samples exhibited a comparatively smoother surface morphology than the SPEKK samples. The hydrosulphuric acid treatment effectively increased surface roughness and porosity, which contributed to improved cell adhesion on the SPEKK surface by providing a larger surface area and porosity for cell attachment [Figure 5]. Energy dispersive X-ray analysis identified key elements in SPEKK as C, O, S (Carbon, Oxygen, Sulphur). This confirms the formation of the Sulphur group in PEKK to form SPEKK.

Contact angle

The contact angle measurements showed that the Ti group (60.80° ± 3.00) and the SPEKK group (61.47° ± 9.95) had comparable values, whereas the PEKK group exhibited a noticeably higher contact angle (80.26° ± 4.41). The differences between the groups were statistically significant ($p = 0.01$) [Table–1&2]. Decrease in

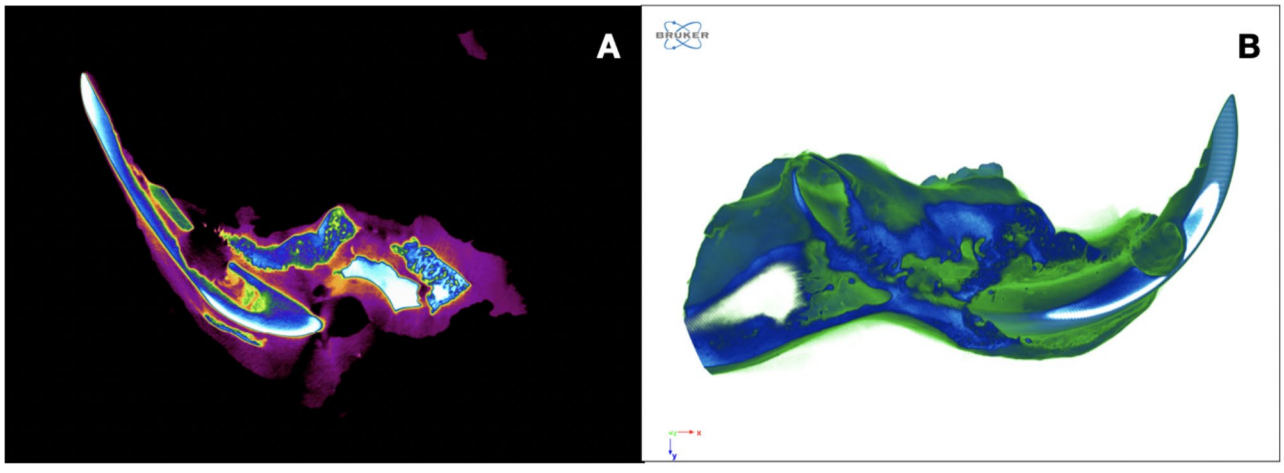

Fig. 4 NanoCT imaging (**A**) 2D Nano-ct image of rat mandible; (**B**) 3D Nano-ct image of rat mandible

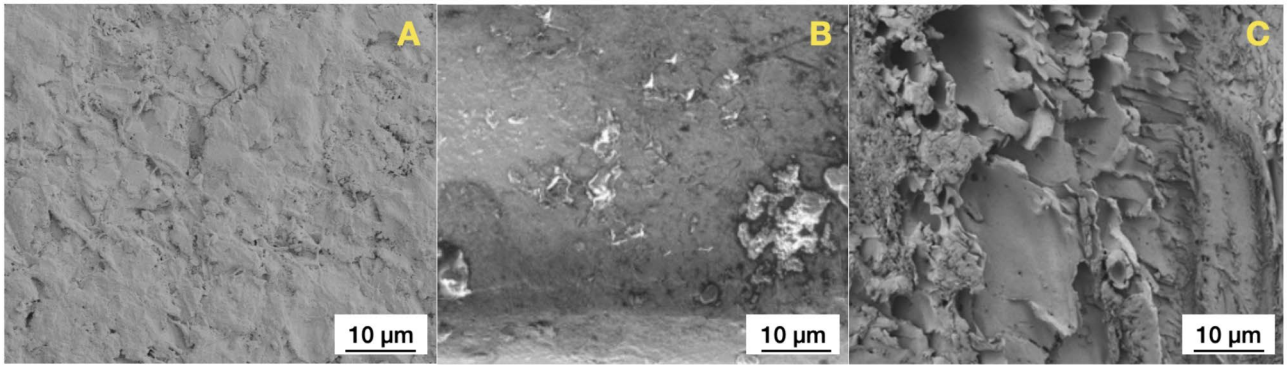

Fig. 5 SEM imaging **A**. Titanium; **B**. PEKK; **C**. SPEKK implants

Table 1 Comparison among groups (Ti, PEKK and SPEKK) based on contact angle and surface roughness

PARAMETER	GROUP	MEAN ± SD	95% CI LOWER	95% CI UPPER	SE	F value	*p* value
Contact Angle	Ti	60.80 ± 3.00	58.90	62.71	0.86	89.34	0.001*
	PEKK	80.26 ± 4.41	77.46	83.07	1.27		
	SPEKK	61.47 ± 9.95	58.58	64.36	1.31		
Surface Roughness (Ra)	Ti	120.65 ± 7.96	115.59	125.72	2.30	14.31	0.001*
	PEKK	111.17 ± 7.56	106.36	115.98	2.18		
	SPEKK	127.20 ± 6.52	123.06	131.35	1.88		

P value was derived from a one-way ANOVA test; *significant at 0.05

Table 2 Pairwise comparison between the groups (Ti, PEKK and SPEKK) based on contact angle and surface roughness

PARAMETER	GROUP	MEAN Difference	95% CI LOWER	95% CI UPPER	SE	*P* Value
Contact Angle	Ti vs. PEKK	19.45	15.40	23.51	1.65	0.001*
	Ti vs. SPEKK	0.66	–4.71	3.39	1.65	0.915
	PEEK vs. SPEKK	18.79	14.73	22.84	1.65	0.001*
Surface Roughness (Ra)	Ti vs. PEKK	9.48	2.09	16.87	3.01	0.009*
	Ti vs. SPEKK	6.55	1.84	13.94	3.02	0.091*
	PEEK vs. SPEKK	16.03	8.64	23.42	3.00	0.001*

P value was derived from Post hoc Tukey test; *significant at 0.05

contact angle from PEKK to SPEKK was indicating the increased wettability of SPEKK implant material.

Surface roughness

The mean roughness of the Ti group was 120.65 ± 7.96, the mean roughness of the PEKK group was 111.17 ± 7.56, and the mean roughness of the SPEKK group was 127.20 ± 6.52, according to the surface roughness measurements. As seen by the statistically significant differences between the groups (p-value = 0.001), the surface roughness of the materials varied significantly. The surface roughness values of the Ti and SPEKK groups were comparable, although the mean roughness of PEKK was marginally lower than those of the other two groups. The differences between the Ti and SPEKK groups, as well as between the PEKK and SPEKK groups, are statistically significant [Table–1&2].

Cell viability

The results showed that all groups (Ti, PEKK, and PEKK-S) exhibited good cell viability, with values of 80%, 82%, and 86%, respectively, compared to the control group (without any substrate), which demonstrated 100% cell viability. Although the PEKK-S group had the highest cell viability, the differences between the groups were not statistically significant. This suggests that while PEKK-S showed a slight advantage, all tested materials had comparable effects on cell viability.

Bone volume and trabecular thickness

At 6 weeks after the surgery, the site had healed well and there was new bone formation around the implant in titanium and sulfonated PEKK groups. One-way analysis of variance (ANOVA) revealed that the new bone formation in SPEKK was significantly higher (280.09 ± 12.03 µm) than those of Ti and PEKK (265.12 ± 11.08 µm and 266.52 ± 7.28 µm) and the difference among group was

Table 3 Comparison among groups (Ti, PEEK and PEKK) based on Bone volume surrounding implant and Trabecular thickness

PARAMETER	GROUP	MEAN ± SD	SE	95% CI LOWER	95% CI UPPER	F Value	*P* Value
Bone volume surrounding implant	TI	265.12 ± 11.08	3.20	258.08	272.17	7.66	0.002*
	PEKK	266.52 ± 7.28	2.10	261.89	271.15		
	S PEKK	280.09 ± 12.03	3.47	272.44	287.73		
Trabecular thickness	TI	0.634 ± 0.03	0.014	0.597	0.671	1.46	0.244
	PEKK	0.658 ± 0.04	0.017	0.613	0.704		
	SPEKK	0.659 ± 0.03	0.015	0.617	0.698		

P value was derived from one way ANOVA test; *significant at 0.05

Table 4 Pairwise comparison between the groups (Ti, PEKK and SPEKK) based on Bone volume surrounding implant

PARAMETER	GROUP	MEAN Difference	95% CI LOWER	95% CI UPPER	SE	*P* Value
Bone volume surrounding implant	Ti vs. PEKK	1.39	−8.95	11.75	4.22	0.94
	Ti vs. SPEKK	14.96	4.60	25.32	4.22	0.003*
	PEKK vs. SPEKK	13.56	3.20	23.92	4.22	0.00

P value was derived from Post hoc Tukey test; *significant at 0.05

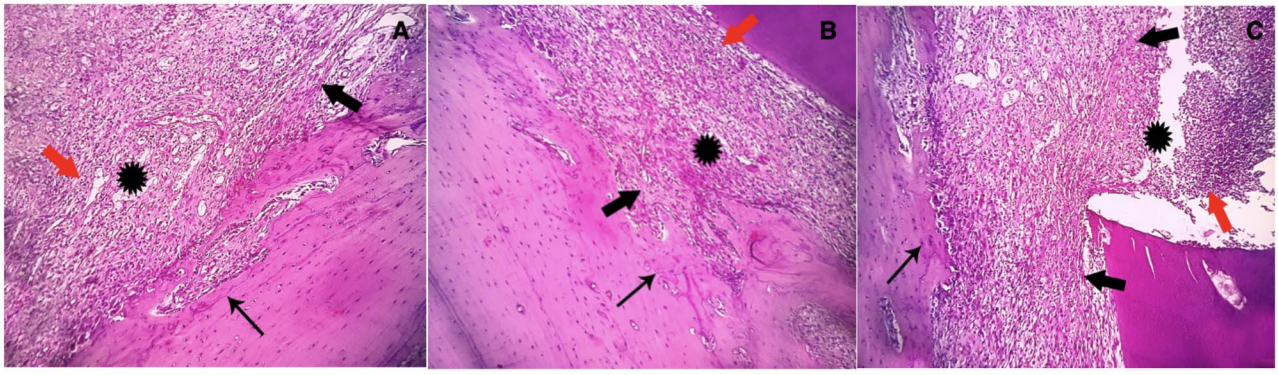

Fig. 6 Histo-morphology (**A**) New bone formation around Ti implants; (**B**) New bone formation around PEKK implants; (**C**) New bone formation around SPEKK implant

statistically significant ($P = 0.002$). SPEKK was showing the maximum thickness (0.065 ± 0.03) in the defect area from Nano CT but there was no significance difference amongst the groups (Ti, PEKK and SPEKK) in Trabecular thickness ($p = 0.0244$). The unit is measured in micrometer(um). The equation used is Control bone volume - test bone volume = new bone formation. Percentage is compared with respect to control bone [Tables–3&5].

Histological analysis

Histopathological analysis shows that both the control and experimental groups' mandibular bones have a defect region (shown by asterisks). The mandibular bone's walls are made of compact bone with a regular architecture, known as black thin arrows. Red, thick arrows point to the implant in the mandibular defect area where inflammatory cells have infiltrated. The development of a network of interwoven bone, represented by Black Thick arrows, shows the progression of bone remodeling, as well as the transformation and maturation of the endochondral ossification, at the defect site. When compared to unmodified PEKK, it was found that SPEKK had exceptional bone regeneration and the existence of woven bone growth, demonstrating osseointegration potential of SPEKK as implant biomaterial [Figure 6].

Toxicity analysis

The data analysis of serum creatinine, urea, SGOT and SGPT levels across the different groups (Titanium, PEKK, and SPEKK) revealed no significant differences ($p < 0.05$). Regarding kidney function, Creatinine levels showed no significant variation between the groups ($p = 1.093$), with mean values of 0.92 ± 0.40 for Titanium, 0.85 ± 0.29 for PEKK, and 1.13 ± 0.18 for SPEKK. Similarly, Urea levels did not show any significant differences ($p = 0.36$), with values of 28.75 ± 5.22 for Titanium, 30.75 ± 3.93 for PEKK, and 29.72 ± 4.86 for SPEKK. In relation to the liver functions, SGOT levels also showed no statistical difference ($p = 0.350$), with means of 15.38 ± 3.10 for Titanium,

Table 5 Comparison among groups (Ti, PEEK and SPEKK) based on creatinine, urea, SGOT and SGPT

PARAMETER	GROUP	MEAN ± SD	SE	95% CI LOWER	95% CI UPPER	F Value	*P* Value
Creatinine	TI	0.92 ± 0.40	0.14	0.58	1.25	1.78	0.193
	PEKK	0.85 ± 0.29	0.10	0.60	1.09		
	SPEKK	1.13 ± 0.18	0.06	0.97	1.2		
Urea	TI	28.75 ± 5.22	1.84	24.38	33.11	0.36	0.701
	PEKK	30.75 ± 3.93	1.39	27.46	34.03		
	SPEKK	29.72 ± 4.86	1.72	25.65	33.79		
SGOT	TI	15.38 ± 3.10	1.09	12.78	17.98	1.10	0.350
	PEKK	17.2 ± 1.64	0.58	15.82	18.57		
	SPEKK	18.07 ± 5.33	1.88	13.61	22.53		
SGPT	TI	26.77 ± 2.41	0.85	24.75	28.79	2.77	0.085
	PEKK	28.54 ± 5.66	2.00	23.80	33.27		
	SPEKK	22.85 ± 5.95	2.10	17.87	27.82		

P value was derived from One Way ANOVA test

17.2 ± 1.64 for PEKK, and 18.07 ± 5.33 for SPEKK. SGPT levels also did not differ significantly between groups ($p = 0.085$), with values of 26.77 ± 2.41 for Titanium, 28.54 ± 5.66 for PEKK, and 22.85 ± 5.95 for SPEKK. These results indicate that the PEKK and SPEKK modifications do not induce toxicity or affect liver and kidney function (Table 5).

Discussion

Micromorphology testing is a critical method for assessing surface modifications in materials used for orthopedic and dental implants [25]. In this study, micromorphology tests were conducted to evaluate the surface characteristics of polyetherketoneketone (PEKK) and its sulphonated variant (SPEKK). The goal was to understand how surface alterations impact key properties like roughness, porosity, and hydrophilicity, which are essential for successful osseointegration. Techniques such as Scanning Electron Microscopy (SEM), contact angle measurement for wettability, and nano-CT analysis were utilized to capture detailed surface attributes [26, 27]. SEM revealed significant differences in surface texture, with SPEKK exhibiting increased porosity and roughness compared to unmodified PEKK [28]. Contact angle measurements demonstrated enhanced hydrophilicity in SPEKK, which is attributed to the introduction of sulfonic acid groups during the sulphonation process. These functional groups improve surface bioactivity, facilitating better cell adhesion and proliferation. Histopathological analysis further validated these findings by showing superior bone growth and integration with SPEKK compared to unmodified PEKK, especially at six weeks post-implantation. Biomechanical tests corroborated these results, indicating stronger bonding between SPEKK and the surrounding bone tissue. The enhanced performance of SPEKK can be explained by the combination of its smoother morphology and improved surface chemistry, which collectively provide an optimal environment for cellular interactions. The results underscore the importance of sulphonation in enhancing the bioactivity of PEKK, making it a promising material for implant applications. These modifications are crucial for addressing the limitations of unmodified PEKK, such as its bioinert nature and limited capacity to bond with bone tissues.

The findings of the study underscore the positive impact of sulfonated polyether ketone ketone (SPEKK) in implant applications, aligning with the established body of literature. Specifically, sulphonation introduces hydrophilic functional groups onto the PEKK surface, which significantly improves cell adhesion and proliferation, as highlighted by Bo Yuan et al. [18]. These properties are critical for enhancing osseointegration—the process by which the implant bonds to the surrounding bone tissue—making sulphonation an essential surface modification technique for implants. The hydrophilicity imparted by sulphonation facilitates better interaction between the implant surface and biological fluids, as well as between the surface and surrounding cells. This improved interaction is crucial for accelerating the healing process and enhancing the bonding between the implant and bone. Hydrophilic surfaces are known to encourage the formation of a more stable and biologically active interface, leading to faster tissue integration and potentially better clinical outcomes. Moreover, the increased porosity and roughness observed in sulphonated PEKK surfaces are in line with principles of biomaterials science, which suggest that such features create additional anchoring points for cells. The rougher and more porous a surface, the greater the potential for cellular attachment, which in turn supports more effective osseointegration. These characteristics are widely accepted as beneficial for implant surfaces, as they promote cell attachment, migration, and differentiation, thereby enhancing the long-term success of the implant. Despite the promising results, there are

some cautionary perspectives in the literature regarding the potential variability in the performance of sulphonated surfaces. For instance, the effects of sulphonation may vary depending on the biological model used (e.g., in vitro versus in vivo) and the specific testing conditions. While short-term studies often show improved cell interactions, there is less clarity on how these modifications perform over the long term, particularly under dynamic physiological conditions such as mechanical loading and fluid flow. Further concerns have been raised about the uniformity of the sulphonation process and its potential impact on the mechanical properties of the material. In some cases, excessive sulphonation could degrade the material or alter its mechanical integrity, which may compromise the implant's performance, especially in load-bearing applications. This could be a limiting factor for the widespread adoption of SPEKK in certain high-stress environments, such as joint implants or spinal devices. However, the majority of the existing evidence supports the view that sulphonation significantly enhances the bioactivity of PEKK, making it a promising option for implant technology. The ability to modify the surface properties to improve cellular interactions, osseointegration, and healing while maintaining the material's structural integrity offers substantial potential for advancing implant technologies in clinical settings.

While this study highlights the benefits of sulphonated PEKK, certain limitations persist. A key challenge is the reliance on animal models, which differ anatomically and physiologically from humans in aspects like bone density, healing mechanisms, and biomechanical forces [29]. These discrepancies, along with distinct oral microbiota and hygiene practices, can affect outcomes and limit the applicability of results to human scenarios [30, 31]. Additionally, the study lacks consideration for comorbidities, aging, and critical parameters such as implant stability, wear resistance, and long-term durability, which are vital for comprehensive clinical evaluation [32].

A key limitation of this study is the lack of mechanical property testing for SPEKK, PEKK, and titanium implants. Given that the sulfonation process may influence the mechanical properties of SPEKK, further studies are required to evaluate its compressive strength, tensile strength, and flexural strength. Understanding these properties is crucial for optimizing the manufacturing process and ensuring the long-term clinical performance of SPEKK-based implants. Additionally, future research should focus on clinical trials involving diverse human populations to assess the real-world applicability of these materials, considering variables such as age, health conditions, and lifestyle factors. Long-term studies should assess SPEKK's performance under dynamic loading conditions, ensuring suitability for load-bearing implants [33, 34]. Exploring controlled sulphonation techniques could optimize bioactivity while maintaining mechanical integrity, expanding SPEKK's applications. Innovations like 3D printing for patient-specific implants and antimicrobial surface modifications could further enhance outcomes, positioning SPEKK as a next-generation material for orthopedic and dental implants.

Conclusion

The study demonstrates that PEKK implants treated with sulfuric acid (SPEKK) exhibit enhanced hydrophilicity compared to untreated PEKK and titanium implants, reflecting a stronger affinity for water that may promote improved biological interactions. Notably, SPEKK implants showed no signs of cytotoxicity, confirming their compatibility with biological systems. Moreover, bone formation around the sulfuric acid-treated PEKK implants was significantly better than that observed with titanium and untreated PEKK implants. This indicates that the PEKK implant with surface modification will not only enhance the hydrophilic nature but also creates a more conducive environment for bone integration, highlighting SPEKK as a viable and innovative alternative to titanium in dental and orthopedic applications.

Abbreviations

AFM	Atomic Force Microscopy
ANOVA	One-way analysis of variance
EDX	Energy Dispersive X-ray
H_2SO_4	Sulfuric acid
LFT	Liver Function Test
PBS	Phosphate Buffered Saline
PEKK	Polyetherketoneketone
RFT	Renal Function Test
SEM	Scanning Electron Microscopy
SPEKK	Sulfuric acid-modified Polyetherketoneketone
Ti	Titanium

Supplementary Information

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Supplementary Material 1

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Author contributions

Conceptualization, S.M; methodology, B.N, S.M, JP; software, BN, L.S, AH; validation, SM, MKG; formal analysis, SM, JP and A.H.; investigation, B.N., M.K.G, S.M; resources, B.N. and A.H.; data curation, B.N, S.M, M.K.G, J.P, L.S and A.-H.; writing—original draft preparation, B.N, SM, M.K.G; writing—review and editing, J.P, L.S and A.H.; visualization, S.M; supervision, S.M, M.K.G; project administration, S.M and A.H. All authors have read and agreed to the published version of the manuscript.

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Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Ethical approval
Ethical approval has been obtained from the Institutional Animal Ethics Committee (IAEC) (BRULAC/SDCH/SIMATS/IAEC/01–2023/05) of Saveetha Institute of Medical and Technical Sciences.

Informed consent
Not applicable.

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