Materialistic characterization, thermal properties, and cytocompatibility investigations on acrylic acid-functionalized nSiO$_2$-reinforced PEEK polymeric nanocomposite

Thanigachalam Mugilan$^1$ · Muthusamy Subramanian Aezhisai Vallavi$^1$ · Durai Sugumar$^2$

Received: 9 June 2022 / Revised: 6 August 2022 / Accepted: 11 August 2022 / Published online: 20 August 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
Polyether ether ketone (PEEK) is a biocompatible alternative to metallic biomaterials because of its unique properties and biocompatibility. Its bioinert nature may lead to implant failure from inadequate osseointegration. Therefore, this research aims to develop the nSiO$_2$ ceramic particle-reinforced PEEK (nSiO$_2$@PEEK) polymer nanocomposite. The particle size of nanoparticles was measured as 43.6 nm using the particle size analyzer (PSA). The morphology of the fabricated composite was analyzed using FESEM. The structural characteristic of nSiO$_2$@PEEK was investigated using XRD and FTIR. Thermal stability was examined using TGA thermograms and DSC curves. Minimum toxic level (grade: slight, 1–20%) was observed by in vitro cytotoxicity assessment using direct and indirect methods. Excellent cell viability was found as 83.6% through MTT assay. The MG-63 cell adhesion study was conducted subsequently excellent cell growth and cell morphology were monitored using SEM analysis. This investigation found the nanocomposite to be biocompatible. It is a promising biomaterial for medical implants.

Keywords Biomaterials · Polymer nanocomposite · In vitro · Cell adhesion · FESEM

Introduction
PEK and PEEK are thermoplastics with outstanding thermal properties as well as remarkable tensile and compressive strengths. PEEK was invented by the ICI in 1982. There are many industrial and medical uses for the PEEK linear aromatic polymer, and it is also widely considered to be the best thermoplastic material available. PEEK is characterized by its repeating monomers that include two ether and ketone groups [1, 2]. According to previous studies, PEEK has great potential in dentistry as additional or alternative material to more conventional metals and ceramics [3, 4]. PEEK has been used in a variety of dental devices, including dental implants, healing caps, orthodontic braces, and denture prosthetic frames [5]. The PEEK’s enhanced processing capabilities make it an ideal biomaterial for making a patient-specific prosthesis, which might be significant potential for the biomaterial. Compared to other polymers, the PEEK polymer exhibits excellent fracture resistance [6–8]. PEEK composite is utilized in clinical dentistry and orthopedic scaffolds because of its excellent strength-to-weight ratio. PEEK composites low bonding energy has been attributed to their chemical inertness and reduced surface energy [9–12].

There has been a dramatic rise in bone fracture and trauma prevalence across industrialized and developing countries over the past several decades. Because of their osteoconductive, osteoinductive, and biocompatible qualities, bioactive glasses, particularly those based on silica, are poised to play a critical role in this sector. They found that silica and bioactive-glass-rich microspheres were more effective in stimulating bone regeneration than those made of polyactic-co-glycolic acid (PLGA). Using silica- and bioactive-containing microspheres to replace natural bone tissue has been suggested.

In vitro measurements of CCK-8 cell cytotoxicity and qualitative comparisons of inverted fluorescence microscopy...
images of cell morphology showed that adding Al$_2$O$_3$ decreased the cell survival of PEEK slightly. A particular surface topography (defined roughness equal to approximately Ra = 0.30 microns), which provides the best potential survivability of human osteoblasts, was found in 30 nm Al$_2$O$_3$-reinforced composites. There is evidence that current glass-reinforced PEEK or Ti-6Al-4 V manufactured using fast prototyping technology can be utilized to construct implants that can be used in clinical situations [13]. PEEK implants may be improved in cytocompatibility, soft tissue integration, and osseointegration by using TiO$_2$ nanostructures [14]. The L929 mouse cell line was exposed to extracts of PVA-PEG-HAp thin membrane for 24 h. The findings showed a maximum of 99 and 93% cell viability with 1 and 7% cytotoxicity of different weight percentages [15]. The culture medium (cell line MG-63) with direct cytotoxicity reactivity showed a maximum of 71 and 57% cell viability and a minimum of 29 and 43% moderate cytotoxicity [16]. In the first test on extract-cotton seed oil of 10 ml and 20 ml extract, the 40% of SS particulate-filled HDPE, which is between grades 3 and 4 (21–50%), was shown to be a minimum cytotoxic effect. Given that the amount of reactivity was below a moderate level, it was acceptable for the clinical circumstances [17].

The 30 wt % HA/PEEK composite was selected in the cytotoxicity experiments. Alkaline phosphatase (ALP) activity was shown to be greater in PEEK composite samples than in UHMWPE and pure PEEK, as evidenced in the cell assays. After 7 days of immersion in SBF, the HA/PEEK composite was covered with apatite growth, which continued to grow over extended periods. In animal experiments, there was higher bone contact and bone growth around the HA/PEEK (HA-hydroxyapatite) composite than around UHMWPE or pure PEEK [18]. The PEEK and CFR-PEEK (CFR-carbon fiber reinforcement) were machined and injection moulded, as well as polished (Ra = 0.200 microns) and rough (Ra = 0.554 microns) cpTi were all considered. On PEEK (Ra = 0.095 microns) and CFR-PEEK (Ra = 0.350 microns) injection moulded versions, osteoblast adhesion at 4 h was equivalent to titanium. Both PEEK and CFR-PEEK materials were much less machined than their natural counterparts (Ra = 0.902 and 1.106 microns) and determined at 48 h. As a result, the maximum thymidine incorporation was found in the injection moulded unfilled PEEK, which was much greater than the rough titanium control [19]. When applied to the PEEK disc implant, the HA coating adhered strongly and formed a homogeneous layer that was simple to clean. Cell adhesion and viability were both increased in early cell adhesion and viability tests performed on the material. It was shown that cells grown on HA-coated PEEK discs had increased ALP activity and calcium concentration and that they had a higher calcium concentration. The expression of osteoblast development indicators such as ALP, bone sialoprotein, and runt-related transcription factor was also increased in these cells [20], as well as the expression of other genes. The mesenchymal stem cell proliferation experiment results revealed that the treated layer had more significant cell proliferation when comparing treated and untreated PEEK. The apatite formation data revealed the presence of HA growth on the treated PEEK. However, there was no evidence of HA development on the untreated PEEK even after 2 weeks of testing [21].

The success or failure of implants is primarily determined by their ability to integrate with the surrounding bone, which is a significant function of their biocompatibility with the surrounding bone. As a result, nanoparticles of metal oxides have lately become widely employed in composites to improve the topographical and biological characteristics of the materials. The objective of this current work is to investigate the biocompatibility of the nSiO$_2$-reinforced PEEK nanocomposite. The mechanical properties of the functionalized nSiO$_2$-filled PEEK nanocomposites with different weight percentages were investigated with detailed discussions in the previous work [22]. Based on the conclusion the best combination was selected as 12 wt % nSiO$_2$-reinforced PEEK nanocomposite.

Therefore, the present research aims to develop the functionalized ceramic nanoparticle-reinforced PEEK polymer nanocomposite. The acrylic acid-functionalized nSiO$_2$ particles were used as reinforcement and PEEK as a matrix material. The composite was fabricated through the vertical injection moulding process. The morphology of the developed composite sample was investigated using field emission scanning electron microscope (FESEM) analysis. The various elements present in the fabricated composite were analyzed using energy dispersive X-ray analysis (EDAX) and the elemental mapping technique. The material was further characterized with the help of X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), thermal gravimetric analysis (TGA), and differential scanning calorimetry (DSC) analysis. The biocompatibility of the newly developed nanocomposite has been investigated through in vitro direct and indirect cytotoxicity investigations. Cell viability and cell adhesion studies were carried out to confirm biocompatibility. The MG-63 cell adhesion was investigated using SEM micrographs.

Material and methodology

PEEK and nSiO$_2$

The PEEK polymer is utilized as a matrix material to develop the polymer nanocomposite. The melting point temperature of PEEK is around 343 °C and was purchased from Engineered Polymers Ind Pt Ltd. The ceramic nSiO$_2$
was purchased from AD Nano Technologies Pvt Ltd., Karnataka, India. It was selected for incorporation into the polymer matrix as reinforcement, and the melting point of nSiO$_2$ is about 1710 °C. The MFI (Melt Flow Index) of pure PEEK polymer at 400°C is 3 g/10 min. The flow diagram of the methodology of the current research work is shown in Fig. 1.

The ceramic nSiO$_2$ nanoparticles were used in the functionalization process (using acrylic acid) [23–25]. The nSiO$_2$ particles were combined with 30 g of acrylic acid. It was incorporated into a second mixture, which contains 32 g of a diluted hexane solution of water, and successfully mixed. The produced mixture was sonicated for 20 min at room temperature using an ultra probe sonicator, after which they were allowed to cool to room temperature. The mixture was centrifuged to remove surface-adsorbed residues and separate functionalized nSiO$_2$. Sigma 216KL centrifuge at 13,000 rpm and 23 °C for 2 h with 3 intervals. Each interval was 7–10 min to control excess heat generation during centrifugation. The functionalized nanoparticles were obtained by drying the nSiO$_2$ particles under a vacuum.

**Particle size analyzer**

To determine the particle size of functionalized nSiO$_2$, the ZETASIZE NANO Zs90 type PSA was used at a constant temperature of 25 °C. The nSiO$_2$ particles sample refractive index and absorption values are 2.5 and 0.1, respectively. The dispersant used in PSA is water with a refractive index of 1.330.

**Fabrication of nanocomposite using injection moulding**

A vertical plastic injection moulding procedure was used to fabricate a functionalized ceramic nSiO$_2$ particle-reinforced PEEK polymer nanocomposite. The ratio of 10:1.2 weight percentage was selected to develop the nSiO$_2$@PEEK polymer nanocomposite. The PEEK polymer was combined with preheated nSiO$_2$ particles in the proper ratio before being fed into the moulding machine. As a result of this, the PEEK polymer was blended with the nanoparticles at between 90 and 100 °C. Due to the melt blending phenomenon inside the chamber, the reinforcement was mixed with the PEEK matrix. The semi-pressurized blended PEEK matrix and ceramic reinforcement were injected into the die cavity are injected and the pressure maintained in the machine was around 45–50 bar [26]. Then the die was opened, and the composite specimens were separated.

**FESEM and EDAX analysis**

The FESEM (Model: Tescan MIRA-3) was used to examine the surface morphological characteristics of the fabricated nSiO$_2$@PEEK nanocomposite specimen. The elemental composition of the developed nSiO$_2$@PEEK was also determined using energy dispersive X-ray analysis (EDAX), which allowed for validation of the existence of elements in the injection moulded specimen.

![Fig. 1 Schematic flow diagram of a methodology of the current research work](image)
FTIR

When the nSiO₂@PEEK polymer nanocomposite sample was examined using the FTIR (PerkinElmer Frontier, L160000A) method, it was discovered that it included both organic and inorganic components. The infrared absorption frequency range 400–4000 cm⁻¹ was used to identify the actual functional groups contained in the sample, which was calculated using spectrum data collected by the automated spectroscopy software. The incident laser of 1 mW with a resolution of 5 cm⁻¹ was used to record spectra to improve the signal-to-noise ratios.

XRD

X-ray diffractometry (Model: Miniflex, Rigaku corporations) is a technique used to identify the underlying crystal structure of a material; it allows for the verification of the crystallinity and structure of the nSiO₂@PEEK polymer nanocomposite sample. The fitting of XRD patterns was used to calculate the lattice parameters. The XRD analysis was carried out using Rigaku Corporations, MiniFlex model machine with the 2θ range from 10° to 90°. The XRD patterns of concrete specimens are identified using a standard database (the JCPDS database) for XRD patterns, which contains information on a wide range of crystalline phases.

Thermal properties

Thermal stability by TGA and melting behavior by DSC

TGA is a technique for determining the thermal stability of materials, particularly polymers. The developed polymer nanocomposite sample was heated in an air environment under a nitrogen atmosphere from 50 to 500 °C at a rate of 20 °C/min. The data on thermal behavior was gathered through the use of the setup program. Experiments were conducted on samples with an average mass of 20 mg and a purge gas flow rate of 20 mL/min, with the samples being weighed before each experiment. In this approach, changes in the weight of a specimen are measured while the temperature of the specimen is raised over time. TGA is also used to determine the moisture and volatile contents of a sample.

The DSC analysis determines the amount of energy required to raise the sample’s temperature compared to the reference material. The sample and reference material temperatures are kept almost constant, with the heat flux generated during the analysis making up the difference. DSC experiments were conducted on polymer nanocomposite samples on a NETZSCH model, 1100 M with Q50 type thermobalance under a nitrogen atmosphere. A sample with an average mass of 10 mg was heated from 10 to 500 °C at a rate of 20 °C/min.

Biocompatible assessment

MG-63 cell line and cell culture

The MG-63 cell line was used for in vitro studies, obtained from the National Centre for Cell Science (NCCS) in Pune, India. The cell line is human, has bone origins, and has the shape of osteosarcoma. An established and well-characterized cell line, MG-63, can produce consistent findings. Fetal bovine serum was added to a minimum essential medium to create a cell culture medium. As an appropriate serum, it has more growth factors that may be used in several uses for cell culture since it has the lowest level of antibodies in the market [27]. One of the most prominent cell lines in osteogenesis investigations is the human osteoblastic line MG-63. For cell biology inquiry, the cells must stay stable in their phenotypic throughout numerous passages in cell culture. MG-63 cells can be employed to give all the fundamental insights into cell material interaction. Hence, the MG-63 cell line has been used for this current study.

In vitro direct and indirect cytotoxicity assay

The ethylene oxide (ETO) sterilization is used to sterilize the specimens to test for in vitro cytotoxicity. For a pressure of 5 bar and a temperature of 50 °C, the samples were first preconditioned for 20–30 min and then humidified for another 20–30 min. The sample was exposed to 100% ETO dose for the next 7 h, followed by aeration for the next 12 h [28]. Finally, an ETO-sterilized sample was prepared and used. The experiments were conducted at South India Textile Research Association (SITRA, Coimbatore, India). For in vitro direct and indirect cytotoxicity, ISO 10993:5 standard was followed [29]. For the in vitro direct and indirect cytotoxicity evaluation, liquid extracts of material should be prepared according to ISO 10993–12. By adding 15–20 μl of cell suspension using a P-20 pipetman between the hemocytometer and cover glass. A total of 100–200 cells/square are desired. Divide the number of cells into the four outer squares by four (mean cells/square). Cells/ml suspension Equals cells/square \times 10^4. This protocol works for trypsinized adherent MG-63 cells. It was calculated with cell concentration using the following Eq. (1). The percentage of cytotoxic effect was calculated by using the following Eq. (2).
Cell viability assay

Cells derived from human osteosarcoma (MG-63 osteoblast-like cells) were cultured in a standard culture medium at 37 °C, the cultures were kept in a humidified environment with 5% CO₂ that was replaced every 2 days. The mitochondrial dehydrogenase activity was used to measure the proliferation and viability of cells colonizing the samples using MTT assay. MG-63 cells were planted in well plates with 5% CO₂ to examine the nSiO₂@PEEK polymer composite sample’s cytotoxicity. Then it was placed for 4 h at 37 °C with MTT solution on top of the well in a 1 ml serum-free medium. The plate was shaken for 15 min after removing the solution before the absorbance was measured with an ELISA microplate reader. The percentage of viable cells was then calculated with control [30–32]. The cell viability was calculated by using Eq. (3).

\[
\text{Viability of MG-63 cells} \, (\%) = \left\{ \frac{\text{Treated}}{\text{Control}} \right\} \times 100 \tag{3}
\]

Results and discussion

Particle size analysis

The purchased nSiO₂ powder was introduced into functionalization through acrylic acid to improve properties and enhance bio integration of material into the human system. The functionalized nSiO₂ particle size is analyzed using the particle size analyzer and obtained an average particle size of about 43.6 nm. Dispersions generally offer phase stability, dimensional stability, and hardness, which can be used to enhance the properties of the nanoparticles and control their functions as well. When a dispersant adsorbs on the surface of the particles, it affects the electrostatic interactions. Hence, the water has been chosen as a dispersant, to perform the particle size analyzer. The histogram shown in Fig. 2 gives the binomial lognormal distribution of particle sizes.

![Histogram analysis of particle size distribution](image)
FESEM morphology analysis

The presence and distribution phenomena of nSiO₂ reinforcement on the polymer matrix were observed using FESEM morphological analysis. Figure 3a reveals the proper distribution of nanoparticles with the matrix. Also, the functionalized nanoparticles were thoroughly combined with the PEEK matrix was observed due to the acrylic acid functionalization. And also confirms the proper mixing of nanoparticles with the PEEK matrix during the plastic injection moulding process. In a few regions, the agglomeration of nanoparticles was also observed due to covalent bond formation [35]. The region of agglomeration is shown in Fig. 3b. The suitable temperature helps to bind the nanoparticles with PEEK completely, which is clearly observed from the FESEM analysis. The presence of nSiO₂ particles in the developed nanocomposites was focused with the help of the cross-sectioned specimen’s FESEM image shown in Fig. 3c. From the above observations, the interfacial force between the functionalized nSiO₂ particles with the PEEK matrix promoted the uniform dispersion of nanoparticles in the developed nSiO₂@PEEK polymer nanocomposite.

EDAX and elemental mapping

The element presence and the confirmation were investigated through the EDAX and elemental mapping technique. The presence of nSiO₂ particle was confirmed with the help of EDAX spectrum results are shown in Fig. 4a. From the EDAX analysis, the elements like carbon (C), oxygen (O), and silica (Si) were identified. The weight percentages of C, O, and Si elements are 68.54%, 19.32%, and 12.14%, respectively. The atomic percentages of C, O, and Si elements are observed as 77.68%, 16.42%, and 5.89%, respectively. The elemental mapping was carried out to verify the dispersion of elements on the surface of the developed nanocomposite, which is shown in Fig. 4b, c. Various colors were used to record the specific elemental mappings for each element as shown in Fig. 4d–f.

FTIR

FTIR analysis is an effective technique that is sensitive to interfacial interaction, intermolecular interaction, etc. To identify the presence of polymer and filler, monitor the absorption peak shifts and interfacial interactions in a specific region to determine the functional groups interaction related to the PEEK, nSiO₂. FTIR spectra of nSiO₂@PEEK polymer composite were taken from 400 to 4000 cm⁻¹ and shown in Fig. 5. The spectrum shows carbonyl group (C=O) stretching at 1759 cm⁻¹, which is an important characteristic of the ketone bond for PEEK [36]. The sharp peaks were observed at 3692 and 1496 cm⁻¹, confirming the strong O–H stretch functional group. The broad peak observed between 2850 and 3250 cm⁻¹ confirmed the strong C=C-H group at the wavenumber of 3052 cm⁻¹. The presence of SiO₂ was confirmed with the help of the Si–O–Si group at 981 cm⁻¹ and Si–O at 504 cm⁻¹ [37] in the FTIR spectra. The ether functional group (R-O-R) was identified at 1209 cm⁻¹ wavenumber [38], which supports confirming the PEEK in the FTIR spectra of nSiO₂@PEEK polymer composite.

XRD

The phase structure and crystallinity of the developed nSiO₂@PEEK polymer composite were investigated by
XRD pattern, as shown in Fig. 6. The sharp prominent peak revealed the presence of PEEK in the nSiO$_2$@PEEK polymer composite, which was indicated by a green color symbol. The sharp and minor fine peaks in the XRD pattern of nSiO$_2$@PEEK polymer composite indicated the presence of crystalline nSiO$_2$. The broad peak of PEEK signified the amorphous nature of the PEEK phase. Based on the XRD pattern, the diffraction peaks were observed at 18.53°, 22.35°, and 24.52°, which indicates the presence of PEEK with lattice parameters (hkl) are (110), (110), and (200) respectively. The presence of nSiO$_2$ was confirmed with the help of peaks observed at 27.31°, 42.32°, 54.49°, and 64.76° with (101), (201), (210), and (212) planes. The observed broader peak in the XRD pattern pronounced the more significant amount of amorphous polymer compound present in the nanocomposite matrix.

**TGA and DSC**

The TGA thermogram of nSiO$_2$@PEEK polymer composite is shown in Fig. 7. This analysis was carried out to investigate the change in mass loss of the sample concerning temperature changes under the nitrogen gas condition. According to the TGA thermogram, the developed composite sample showed three degradation stages. In the first thermal degradation stage, the volatile components like the moisture of the material were lost, and at the end of the first stage, 2.67% of mass loss was observed. In the second stage of the thermal degradation, the mass loss was monitored at about 3.87% between 93.65 and 209.32 °C. This is due to the decomposition of the organic component present in the composite sample. Further thermal decomposition was monitored between
Fig. 5 FTIR spectrum of developed nSiO$_2$@PEEK polymer composite

Fig. 6 XRD pattern of nSiO$_2$@PEEK polymer composite
Fig. 7  TGA thermogram for nSiO$_2$@PEEK polymer composite

Fig. 8  DSC curve for nSiO$_2$@PEEK polymer composite
The mass loss was found as 4.48% due to the decomposition of a few carbon residuals. The total mass loss was found as 11.02% in the TGA analysis, which shows the developed nanocomposite’s relatively higher thermal stability.

DSC was adopted to investigate the influence of nSiO₂ on the crystallization and melting behaviour of PEEK in the developed nanocomposite. The DSC curve of nSiO₂@PEEK polymer composite was observed and depicted in Fig. 8. The crystallization temperature of net PEEK was about 310 °C. Interestingly, the reinforcement of nSiO₂ leads to a slight improvement in melting temperature, which confirms the thermal stability of nSiO₂@PEEK polymer composite. The peak melt temperature was monitored as 341.13 °C, and the melt onset temperature was 342.72 °C. The nanoparticle loaded with PEEK signified the higher thermal stability up to the temperature of 348.56 °C. The energy observed during the functionalized nSiO₂-reinforced PEEK polymer composite analysis was about −13.72 J/g. This energy absorption happened due to the endothermal reaction of the sample in heating conditions. The further increasing the temperature, the composite reached the amorphous state.

**Direct cytotoxicity assessment**

The composite sample was sterilized with ETO under PBS incubation conditions at 37 ± 1 °C for 24-h duration. The cultured MG-63 line cell line was replaced with a new fresh medium. The liquid extract of the developed nanocomposite sample was introduced into the cell culture. Then the MTT assay was added to all the wells, and the incubation of 4 h was conducted. After that, minimum amount of organic sulfur and dimethyl sulfoxide was added to the wells. Finally, the cytotoxicity assessment results of three replicates were observed with the help of a photometer. The percentage of cytotoxic effect was calculated by using the following Eq. (1).

The cytotoxicity percentage was found as 16.4%, as shown in Fig. 9. It was compared in the standard reactivity level reference table [27]. The grade was assigned as slight cytotoxic reactivity between the cytotoxicity level of 1–20%. The inverted phase-contrast microscopic images of control and sample dishes were monitored, as shown in Fig. 9a, b. From this observation, the living and non-living cells were identified to measure the cytotoxic effect of the composite sample. The maximum number of living cells was addressed through this assessment. Thus, the minimum toxic level was confirmed for the developed nanocomposite material.

**Indirect cytotoxicity assessment**

The MG-63 cell culture was prepared after 48-h culture period, and then the cells interacted with the specimen using an in vitro indirect method of cytotoxicity evaluation. Further 24 h, the grade of cytotoxic reactivity was measured and compared with the standard reference table [29]. The viable and nonviable cells were monitored using
microscopic image observations. The live cells are identified with fibroblast structure and dead cells are identified with a round shape that is differentiated and shown with different color circles. The viable (living) and nonviable (non-living) cells are shown in Fig. 10, with different color dotted circles. From this observation, the maximum number of viable MG-63 cells was noticed in the test culture, which interacted with the developed composite specimen. The level of cytotoxicity fell between grade 2 (slight reactivity level) during the measurement of cytotoxicity by indirect method. This reactivity level was lying in an acceptable range while deciding on the material as a biocompatible one for medical applications.

Cell viability assay

The biocompatibility of nSiO₂@PEEK polymer composite in MG-63 type cells was evaluated using the MTT test assay method. The composite sample was subjected to MG-63 cells that were cultured for 48 h. After interaction with the cell line, the cell viability was calculated by using Eq. (2). The cell viability of the developed nSiO₂@PEEK composite was achieved as 83.6% from the MTT test assay, as shown in Fig. 11. This is due to the surface functionalization of nanoparticle reinforcement into the PEEK matrix. The addition of nSiO₂ influenced the viability and growth of cells in the assessment. Therefore,
functionalized nSiO$_2$ in PEEK polymer nanocomposite promotes good cell viability. Thus, it was confirmed that developed composite material could be a suitable candidate for medical implant application with excellent biocompatibility.

**Cell adhesion study**

The cell adhesion study has been conducted after confirmation of good cytotoxicity and excellent cell viability through in vitro direct, indirect cytotoxicity assessment and cell viability tests. In this study, the MG-63 cell morphologies and adhesion of cells growth have been observed through the SEM analysis. After proper sterilization of composite samples by autoclave, the 7-day growth MG-63 cells were attached to the surface of the composite samples. Gold-sputtered cells attached to the samples underwent SEM investigation to get the morphological details of MG-63 cells on the surface. Also, the cell growth and proliferation of adhered cells were monitored using SEM observations. The polygon-shaped MG-63 cells [39, 40] and spindle-shaped cell morphologies were identified in Fig. 12a–c. The filopodia shape was identified clearly and confirmed the excellent MG-63 growth, as shown in Fig. 12d–f. The observed hairline structure spread throughout the entire surface of the sample helped to confirm the growth of living cells on the composite surface. These confirmations reveal the excellent osteointegration of cell-implant interaction. Thus, the addition of nSiO$_2$ greatly signified the biological reaction of the cell enhancement to the cell adhesion samples. Hence, it has been proven that the developed nSiO$_2$@PEEK nanocomposite has good cytocompatibility.

**Conclusion**

The outcomes of the present research work are as follows. To overcome the short comes of the metal implants, the functionalized ceramic nSiO$_2$-reinforced polymer nanocomposite was developed using an injection moulding process. The uniform distribution and presence of nSiO$_2$ particles in the fabricated composite were investigated using FESEM morphologies and EDAX analysis. The presence of functional groups and crystallinity conditions have been analyzed by FTIR and XRD respectively. The presence of nSiO$_2$ was confirmed with Si–O–Si at 981 cm$^{-1}$ and the PEEK matrix was confirmed with an R-O-R functional group at 1209 cm$^{-1}$. The improved thermal stability of the nSiO$_2$@PEEK composite was found due to the addition of nSiO$_2$ particles. The melting behaviour of the composite was improved up to 348.56 °C, which was monitored using
DSC analysis. The in vitro cytotoxicity evaluations reveal the excellent biocompatibility of the polymer nanocomposite material. The grade I (slight: minimum toxic level) cytotoxic reactivity level (16.4%) has been achieved from both direct and indirect cytotoxicity assessments. The filopodia shape of MG-63 cells was identified through the cell adhesion study, which confirms the excellent cell growth on the developed nSiO$_2$@PEEK polymer nanocomposite. Thus, the above results confirmed that the developed composite could be a promising alternative for metal dental implants.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Babu RD, Prakash P, Devaprapkasam D (2017) Review on emerging applications of nanobiomaterials in dentistry and orthopaedics. Trends Biomater Artif Organs 31:164–171
2. Li S, Jin Y, Wang Z et al (2019) Preparation and characterisation of nickel-plated carbon fibre/polyether ether ketone composites with high electromagnetic shielding and high thermal conductivity. Colloid Polym Sci 297:967–977. https://doi.org/10.1007/s00396-019-04522-5
3. Liao C, Li Y, Tjong SC (2020) Polyetheretherketone and its composites for bone replacement and regeneration. Polymers (Basel) 12:1–48. https://doi.org/10.3390/polym12122858
4. Mishra S, Chowdhary R (2019) PEEK materials as an alternative to titanium in dental implants: a systematic review. Clin Implant Dent Relat Res 21:208–222. https://doi.org/10.1111/cid.12706
5. Najeeb S, Khurshid Z, Zohaib S, Zafar MS (2016) Bioactivity and osseointegration of PEEK are inferior to those of titanium: a systematic review. J Oral Implantol 42:512–516. https://doi.org/10.1563/aoi-d-16-00072
6. Lesiuk G, Sawicka A, Correia J, Prątczak R (2017) Fracture resistance analysis of PEEK-polymer. Eng Struct Tech 9:207–213. https://doi.org/10.3846/2028882x.2017.1417062
7. Ulgey M, Gorler O, Karahan Gunduz C (2021) Effects of laser modalities on shear bond strengths of composite superstructure to zirconia and PEEK infrastructures: an in vitro study. Odontology 109:845–853. https://doi.org/10.1016/j.sod.2021.00608-1
8. Oldapao BI, Zahedi SA, Ismail SO et al (2021) 3D printing of PEEK–cApat scaffold for medical bone implant. Bio-Des Manuf 4:44–59. https://doi.org/10.1016/j.bdim.2020.04089-0
9. Shimizu T, Fujibayashi S, Yamaguchi S et al (2016) Bioactivity of sol-gel-derived TiO$_2$ coating on polyetheretherketone: in vitro and in vivo studies. Acta Biomater 35:305–317. https://doi.org/10.1016/j.actbio.2016.02.007
10. Sargin F, Erdogan G, Kanbur K, Turk A (2021) Investigation of in vitro behavior of plasma sprayed Ti, TiO$_2$ and HA coatings on PEEK. Surf Coat Technol. https://doi.org/10.1016/j.surfcoat.2021.126965
11. Oldapao BI, Ismail SO, Ikumapayi OM, Karagiannidis PG (2022) Impact of cG:coated PEEK and lattice on bone implant. Colloids Surf, B 216:112583. https://doi.org/10.1016/j.colsurfb.2022.112583
12. Kruse HV, Lewin WT, Suchowerska N et al (2022) Plasma immersion ion-implanted 3D-printed PEEK bone implants: in vivo sheep study shows strong osseointegration. Plasma Processes Polym. https://doi.org/10.1002/ppap.202100244
13. Olesik P, Godzierz M, Koziot M et al (2021) Structure and mechanical properties of high-density polyethylene composites reinforced with glassy carbon. Materials 14:1–10. https://doi.org/10.3390/ma14144024
14. Gu X, Sun X, Sun Y et al (2021) Bioinspired modifications of PEEK implants for bone tissue engineering. Front Bioeng Biotechnol 8:1–15. https://doi.org/10.3389/fbioe.2020.631616
15. Rajan S, Marimuthu K, Ayyanan CB, Hoque ME (2022) Development and in-vitro characterization of HAP blended PVA/PEG bio-membrane. J Market Res 18:4956–4964. https://doi.org/10.1016/j.jmrt.2022.04.130
16. Rajan S, Marimuthu K, Balaji Ayyanan C et al (2022) In-vitro cytotoxicity of zinc oxide, graphene oxide, and calcium carbonate nanoparticles on titanium. J Biomater Res 35:305–317. https://doi.org/10.1007/j.10808-021-00452-5
17. Chinnappan BA, Krishnaswamy M, Thanigachalam M et al (2022) Fabrication, characterization and in vitro assessment of Laevistrombus canarium-derived hydroxyapatite particulate-filled polymer composite for implant applications. Polymers (Basel). https://doi.org/10.3390/polym14050872
18. Geringer J, Tatkiewicz W, Rouchouse G (2011) Wear behavior of PAEK, poly(aryl-ether-ketone), under physiological conditions, outlooks for performing these materials in the field of hip prosthesis. Wear 271:2793–2803. https://doi.org/10.1016/j.wear.2011.05.034
19. Sagomonyants KB, Jarman-Smith ML, Devine JE et al (2008) The in vitro response of human osteoblasts to polyetheretherketone (PEEK) substrates compared to commercially pure titanium. Biomaterials 29:1563–1572. https://doi.org/10.1016/j.biomaterials.2007.12.001
20. Ceen B, Kozaci D, Yuksel M et al (2015) Biocompatibility of MG-63 cells on collagen, poly-L-lactic acid, hydroxyapatite scaffolds with different parameters. J Appl Biomater Funct Mater 13:10–16. https://doi.org/10.5301/jabfm.5000182
21. Almasi D, Lau WJ, Rasaei S et al (2020) Fabrication of a novel hydroxyapatite/polyether ether ketone surface nanocomposite via friction stir processing for orthopedic and dental applications. Prog Biomater 9:35–44. https://doi.org/10.1016/j.pbiom.2020.020-00130-7
22. Muthusamy Subramanian AV, Thanigachalam M (2022) Mechanical performances, in-vitro antibacterial study and bone stress prediction of ceramic particulates filled polyether ether ketone nanocomposites for medical applications. J Polym Res 29:318. https://doi.org/10.1007/s10965-022-03180-6
23. Delfi M, Ghomi M, Zarrabi A et al (2020) Functionalization of polymers and nanomaterials for biomedical applications: antimicrobial platforms and drug carriers. Prosthesis 2:117–139. https://doi.org/10.3390/prosthetics2020012
24. Chen S, Yang L, Li K et al (2018) Carboxylic acid-functionalized tin$_2$ oxide nanoparticle-loaded pmma/ peek copolymer matrix as a dental resin for 3d complete denture manufacturing by stereolithographic technique. Int J Food Prop 21:2557–2565. https://doi.org/10.1080/10942912.2018.1534125
25. Raju SS, Srinivasa Rao G, Samantha C (2019) Wear behavioral assessment of Al-CSAp-MMCs using grey-fuzzy approach. Measurement 140:254–268. https://doi.org/10.1016/j.measurement.2019.04.004
26. Ayyanan CB, Dharshini MD, Marimuthu K et al (2022) Design, fabrication, and characterization of natural fillers loaded HDPE composites for domestic applications. Polym Compos. https://doi.org/10.1002/pc.26806
27. Ayyanar CB, Marimuthu K, Gayathri B, Sankarrajah (2020) Characterization and in vitro cytotoxicity evaluation of fish scale and seashell derived nano-hydroxyapatite high-density polyethylene composite. Polym Polym Compos. https://doi.org/10.1177/0967391120981551

28. Thanigachalam M, Muthusamy Subramanian AV (2021) In Vitro Cytotoxicity Assessment And Cell Adhesion Study Of Functionalized nTiO2 reinforced PEEK biocompatible polymer composite. Poly-Plast Technol Mater. https://doi.org/10.1080/25740881.2021.2005093

29. Balaji Ayyanar C, Marimuthu K (2020) Investigation on the morphology, thermal properties, and in vitro cytotoxicity of the fish scale particulates filled high-density polyethylene composite. Polym Polym Compos 28:285–296. https://doi.org/10.1177/0967391119872877

30. Malvindi MA, Brunetti V, Vecchio G et al (2012) SiO2 nanoparticles biocompatibility and their potential for gene delivery and silencing. Nanoscale 4:486–495. https://doi.org/10.1039/c1nr1269d

31. He M, Wang X, Wang Z et al (2017) Biocompatible and biodegradable bioplastics constructed from chitin via a “green” pathway for bone repair. ACS Sustain Chem Eng 5:9126–9135. https://doi.org/10.1021/acs.suschemeng.7b02051

32. Bengalli R, Ortelli S, Bloisi M et al (2019) In vitro toxicity of tio2:SiO2 nanocomposites with different photocatalytic properties. Nanomaterials. https://doi.org/10.3390/nano9071041

33. Thanigachalam M, Muthusamy Subramanian AV (2021) Evaluation of PEEK-TiO2- SiO2 nanocomposite as biomedical implants with regard to in vitro biocompatibility and material characterization. J Biomater Sci Polym Ed. https://doi.org/10.1080/09205063.2021.2014028

34. Venkatesan J, Anil S (2021) Hydroxyapatite derived from marine resources and their potential biomedical applications. Biotechnol Bioprocess Eng 26:312–324. https://doi.org/10.1007/s12257-020-0359-0

35. Shi G, Cao Z, Yan X, Wang Q (2019) In-situ fabrication of a UHMWPE nanocomposite reinforced by SiO2 nanospheres and its tribological performance. Mater Chem Phys 236:121778. https://doi.org/10.1016/j.matchemphys.2019.121778

36. Liu B, Hu W, Robertson GP, Guiver MD (2008) Poly(aryl ether ketone)s with carboxylic acid groups: synthesis, sulfonation and crosslinking. J Mater Chem 18:4675–4682. https://doi.org/10.1039/b806690f

37. Eddy DR, Ishmah SN, Permana MD, Lutfi Firdaus M (2020) Synthesis of titanium dioxide/silicon dioxide from beach sand as photocatalyst for Cr and Pb remediation. Catalysts 10:1–11. https://doi.org/10.3390/catal10111248

38. Kumar M, Gnansounou E, Thakur IS (2020) Synthesis of bioactive material by sol–gel process utilizing polymorphic calcium carbonate precipitate and their direct and indirect in-vitro cytotoxicity analysis. Environ Technol Innov 18:100647. https://doi.org/10.1016/j.eti.2020.100647

39. Sarker B, Singh R, Silva R et al (2014) Evaluation of fibroblasts adhesion and proliferation on alginate-gelatin crosslinked hydrogel. PLoS ONE 9:1–12. https://doi.org/10.1371/journal.pone.0107952

40. Chen L, Hu J, Ran J, Shen X, Tong H (2016) Synthesis and cytocompatibility of collagen/hydroxyapatite nanocomposite scaffold for bone tissue engineering. Polym Compos 37(1):81–90

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.