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Assessing the Biocompatibility of Composite Consisting from Polyether Ether Ketone Reinforced by Silicon Carbide Nanofiller

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ABSTRACT

Background and Objective: The comparison between commercially pure titanium and polyetheretherketone (PEEK) highlights significant differences in their mechanical properties and biological interactions, influencing their suitability as dental implant materials. The present study aims to conduct a biological evaluation of PEEK and PEEK-SiC composite dental implants, focusing on their biocompatibility and cytotoxicity.

Methods: In this laboratory study, PEEK composites (PEEK and SiC) were categorized into five groups, with particular weight percentage ratios of (0% wt., 1.5%wt., 2.5%wt., 4%wt. in 24h and 48h) via a compounding by melt blending by Internal Mixer at 365ºC for 5min. Samples were prepared in the form of sheets, cutting and machining into desired shapes. All tests were done according to (ISO 10993 2009). The test involves various steps including sample preparation, sterilization, cell culture, MTT cytotoxicity test, morphology and cell attachment observation in addition to EDS analysis.

Findings: The findings from the experiments demonstrated that PEEK and its nanocomposite variants exhibited no toxic effects on the cells. Cell viability in the control group was 94.46±0.432 and in the 2G group was 90.73±0.411 in 48 hours. In addition, the cells demonstrated strong adhesion to these materials, underwent significant proliferation, and established a compact cell layer.

Conclusion: The results of this study showed that PEEK and PEEK-SiC nanocomposite did not cause any toxic effects on cells. Moreover, surface characteristics of both the polymer and nanocomposite samples presented biocompatible environments conducive to optimal cell growth.

Keywords: *SiC, PEEK, Nanofiller, Biocompatibility*.

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Introduction

Numerous biomaterials have been formed and established across the past decades aimed for rebuilding tissues to reestablish some of its functions, outmoded metallic orthopedic medical appliances, comprising Titanium (Ti), Stainless steel, cobalt-chromium (Co-Cr), and others, which have been widely used due to their excellent corrosion resistance, high mechanical strength and load-bearing, cytocompatibility, and ability to promote bone integration. However, growing concerns regarding release of metal ions, mismatched modulus of elasticity between metals and human bone, and radiopacity have led to the need for identification of alternative ways (1).

Polyetheretherketone (PEEK) a basic item of the polyaryletherketones (PAEK) group. PEEK is a thermoplastic that has gained popularity for orthopedic, spinal, dental, and trauma therapies (2). Moreover, PEEK implants can be used for the constructions of calvarias bone (3). Such findings introduce PEEK as a replacement for titanium as dental endosseous implant substance (2).

Ceramics made from Silicon carbide (SiC) used in a number of industrial therapeutical products are famous for high values of mechanical potence, low level thermal expansion coefficient, high chemical inertness, oxidation and corrosion resistance (4). Bulk porous silicon carbide ceramics have recently attracted attention in medical products as materials with high biocompatibility, and suitable for the fabrication of orthopedic and dental implants. PEEK composites were produced for different subjects of applications. Load bearing implant is assumed to be one of the most important applications (5). The present study aims to conduct a biological evaluation of PEEK and PEEK-SiC composite dental implants, focusing on their biocompatibility and cytotoxicity.

Methods

This experimental study was conducted in the Department of Prosthodontics, College of Dentistry, University of Baghdad, Iraq. Ethical approval was obtained from the University of Baghdad Ethics Committee (Ethical code #79, November 13, 2019), and the research adhered to the principles of the Declaration of Helsinki.

The samples were prepared and categorized into five groups. The preparation method includes preparing the polymer composite of PEEK and Silicon carbide (SiC) with particular weight percentage ratios of (0% wt., 1.5%wt., 2.5%wt., 4%wt.).

The polymer composite was made by mixing PEEK polymer with SiC nano filler with following proportions [(Pure PEEK, G1 group (1.5%wt. SiC), G2 group (2.5%wt. SiC and G3 group (4%wt. SiC)]. The polymer composite was fabricated using a sequence of processes as follow: Mixing, compounding and compression molding (6).

The bioactivity test was done and achieved according to (ISO 10993 2009), which involves various steps starting with samples preparation. The samples were cut into rectangular specimens for entire cell experiments; samples had dimensions of (10 mm ×10 mm × 250 μm, length, width and height, respectively) (6). The used samples in this test are neat PEEK, selected from the groups of polymer nanocomposite samples.

In preparation for the experiments, all nanocomposite specimens underwent sterilization by immersion in 70% (v/v) ethanol for 30 minutes, followed by a 2-hour treatment in phosphate-buffered saline (PBS) with a pH of 7.4. This PBS solution contained 100 μg/ml of streptomycin and 100 units/ml of penicillin to inhibit bacterial growth. Subsequently, the samples were washed with Dulbecco's Modified Eagle's

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Medium (DMEM) supplemented with 10% (v/v) fetal calf serum and antibiotics. For the sample extraction process, adherence to British Standard ES5736 part 10 was maintained. The extraction was performed using conical flasks placed in a vibrating water bath at 37 °C for a duration of 48 hours.

For cell culture study, L-929 mouse fibroblast cells were employed. These cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) enriched with 10% fetal bovine serum (FBS) in a controlled humidified setting of 5% CO_2 and 95% air at 37°C, utilizing a 96-well plate format. The culture medium was refreshed every 3 days to ensure optimal growth conditions.

Cytotoxicity evaluation has been successfully carried out using the MTT assay. It is crucial to choose the appropriate cell type to ensure biocompatibility, which allows for the extrapolation of a specific host response related to the implanted material. The biological properties of fibroblast cells cultured on plain PEEK and its nanocomposite were investigated to assess their proliferation rates, attachment capabilities, and cellular morphology. These results were then compared to those from a control sample.

The experimental results confirmed that neither PEEK nor the PEEK nanocomposite exhibit any cytotoxic effects on fibroblast cells. The fibroblast cells displayed strong adhesion to both materials, effectively proliferated, and established a dense cellular layer. Additionally, the surfaces of both the polymer and the nanocomposite created biocompatible environments that facilitated cell growth.

In vitro cell culture assays were conducted using mouse fibroblast L-929 cells cultivated on the chosen samples, including neat PEEK and polymer nanocomposite samples. To assess cytotoxicity, the MTT assay was employed, which involves the reduction of yellow MTT solution (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to insoluble purple formazan crystals by viable cells. Initially, the samples were placed in a 96-well polystyrene cell culture plate. A medium consisting of DMEM, supplemented with antibiotics (0.3 ml fungin, 0.6 ml plasmocin, and 0.6 ml normocin) and 10% fetal bovine serum, was added to all samples and incubated for 2 hours to enhance sample wettability prior to adding L-929 cells. Following this incubation, 100 µL of L-929 cell suspension (5×10^4 cells) was plated onto rectangular specimens measuring 0.5 mm × 0.5 mm × 250 µm, which were situated in the 96-well plate. The specimens were incubated at 37 °C in a humidified atmosphere containing 5% CO2 and 95% air for 24 hours. Subsequently, the culture media was replaced with 100 µL of 10% fetal bovine serum, and the medium was refreshed every 3 days. After an additional 24 hours, 10 µL of sterilized MTT solution was introduced, followed by incubation. Finally, 100 µL of isopropanol was added to each sample in the 96-well plate. The last column of the cell culture plate served as a control, containing only media and cells. Optical absorbance was measured at 570 nm using a microplate reader (STAT FAX 2100, USA), with additional tests conducted over different incubation durations (24 and 48 hours) to assess results (7, 8).

The evaluation of cytotoxicity was carried out through the MTT assay, highlighting the necessity of selecting appropriate cell types to establish biocompatibility. This selection is vital for accurately extrapolating specific host responses associated with the application of implanted materials. This test was conducted according to ISO 10993. The cell viability is calculated as follow:

$$\text{Viab \%} = \frac{100 \times OD_{570e}}{OD_{570b}}$$

Where:
Viab. %: The percentage of living cells
OD_{570e}: is the measured average value of the sample optical density.
OD_{570b}: is the measured average value of the control sample optical density.

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This assessment with four times repetition, due to having four specimens, were used for each type of the samples used in this test, and the final result represents the average value for the four tested specimens. The morphology and cell attachment were observed at the end of the specified time for cell culture time, which is (24, 48) hours of cell culture in vitro, and inverted microscope used to evaluate the morphology and cell attachment.

Primarily, the specimens were bathed three times using phosphate-buffered saline (PBS) followed by fixation with 4% glutaraldehyde (pH: 7.4) for thirty minutes aiming to remove the dead cell in any remaining solution (9).

Afterward, the samples were quickly washed with (PBS) three times, and each plate was examined with a phase contrast microscope to evaluate and confirm that the cell growth is comparatively even across the microliter plate.

In the presented study, the vitro test steps were achieved in the In vitro Lab/Polymeric Biomaterial Department/Iran Polymer and Petrochemical Institute/Tehran-Iran.

Energy Dispersive X-Ray Analysis for Elemental Research (EDX) is an x-ray technique known as (EDX) or (EDS) that is used to map the investigated sample elements. The EDX-produced data consists of spectra with peak appearances that are associated with the constituents making up the specified specimen. Table 1 show the analysis condition that were used in this study.

Table 1. Analysis Conditions

Accelerating Voltage (kV)	**15.0**
Beam Current (nA)	10.000
Magnification	134
Live Time (s)	45
Preset Time (s)	45
Nb Channels	1024
Ev/Channel	20
Offset (keV)	0
Width (keV)	20

Results

Cytotoxicity and biocompatibility evaluation: Table (2) shows the MTT assay results for neat PEEK, the polymer composite (G1), (G2) and (G3) for two days' incubation compared with a control (G0). The findings were obtained by comparing the MTT assay with TPS (tissue culture polystyrene), which represents the control. This procedure shows that the Yellow water-soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium-bromide) metabolically shrank in viable cells to a blue-violet insoluble formazan. The viable cell count is associated with the color intensity settled by photometric measurements after formazan dissolve in alcohol.

Results showed that there is an ongoing raise in the light absorbance corresponding with the culture time for all polymer composite samples (Figure 1 and 2).

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Table 2. Cell viability and proliferation of L-929 cells cultured for control, neat PEEK (G0), PEEK composite (G1), (G2) and (G3) for various 24hr, 48hr of incubation period

Variable	Control Mean±SD	PEEK Mean±SD		G1 Mean±SD		G2 Mean±SD		G3 Mean±SD	
		24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
Cell viability	94.46± 0.432	85.74± 0.416	87.88± 1.232	88.12± 0.048	89.31± 0.803	88.36± 0.808	90.73± 0.411	86.46± 0.412	89.31± 0.403
Cell proliferation	0.140± 0.044	0.120± 0.037	0.123± 0.039	0.123± 0.035	0.125± 0.040	0.124± 0.039	0.127± 0.040	0.121± 0.039	0.125± 0.040

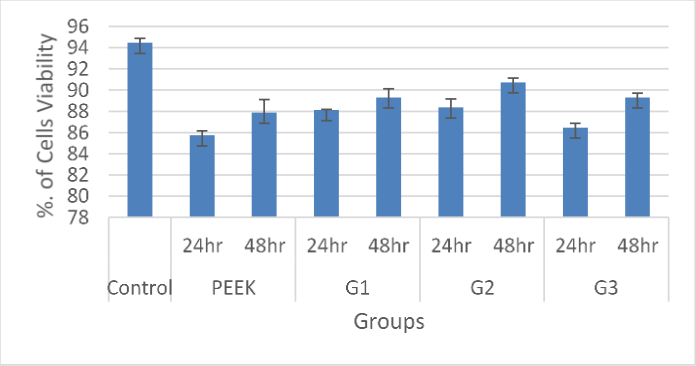


Figure 1. Cell viability of L-929 cells cultured on different samples (a). Control sample (b). Neat PEEK (c). G1 (d). G2 (e). G3 group sample

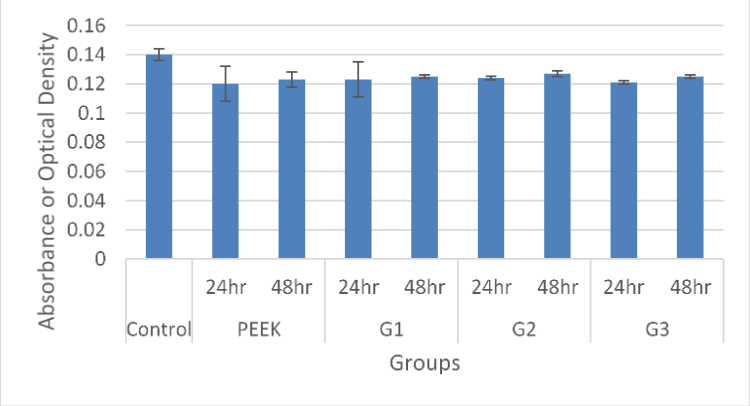


Figure 2. Cell proliferation of L-929 cells cultured on different samples (a). Control sample (b). Neat PEEK (c). G1 (d). G2 (e). G3 group sample

The groups (G1), (G2), and (G3) nanocomposites seeded with the mouse fibroblasts have the same behavior in match up with plain PEEK, and all of the nanocomposites exhibit high degree absorbance values. Moreover, the chart reveals a lesser difference for the cell growth among peek and nanocomposite groups after 2 days. This is represented in next levels as it was compared to the control.

For more information on cellular adhesion, optical microscope inspection was done to observe the of L-929 cell line on neat PEEK, the polymer composite (G1), (G2) and (G3) groups and morphology of cultured cells after 24 and 48 hours for all groups is shown in Figure 3.

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The optical SEM images shown at intimate contact of fibroblasts with the nanocomposite's samples along with neat PEEK (in the absence of nanoparticle powders).



Figure 3. Optical SEM morphologies for the cells in dissimilar statuses of cell growth comprising division and flattening after 24 hour (left) and 48 hours (right) where: A- control sample B- neat PEEK (G0), C- (G1), D- (G2), E- (G3)

Elemental analysis (EDX) generates spectra that display peaks associated with the elemental constituents, reflecting the precise composition of the material being analyzed. The data obtained by EDX analysis comprises spectra that exhibit peaks corresponding to the elemental components comprising the accurate composition of the analyzed material. Figure 4 and Table 3 represents the EDX pattern of the control group of neat PEEK (G0), G1, G2, and G3.

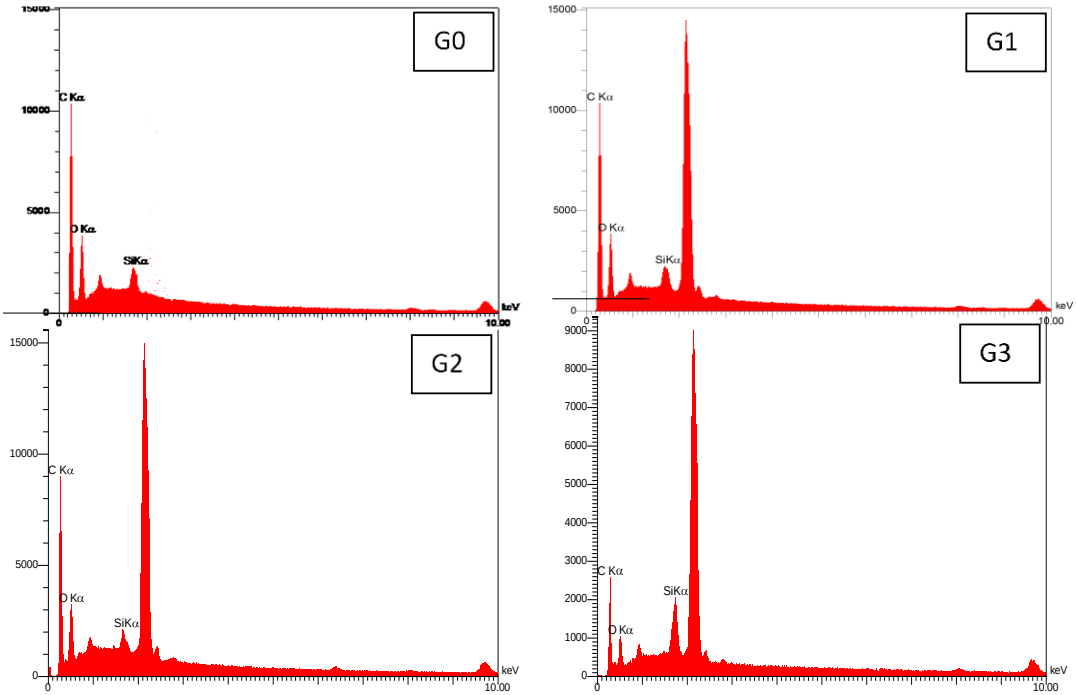


Figure 4. EDX of neat PEEK and PEEK composite

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Table 3. Elemental Analysis of the PEEK and PEEK-Composite (elemental concentration)

	Elt	Line	Int	Error	K	Kr	W%	A%	ZAF
	C	Ka	440.2	1692.0411	0.4150	0.1441	45.46	55.03	0.3171
G0	O	Ka	326.9	395.5826	0.3131	0.1088	42.77	38.87	0.2543
	Si	Ka	263.0	692.3677	0.2719	0.0944	11.78	6.10	0.8018
	C	Ka	440.2	1692.0411	0.4150	0.1441	45.46	55.03	0.3171
G1	O	Ka	326.9	395.5826	0.3131	0.1088	42.77	38.87	0.2543
	Si	Ka	263.0	692.3677	0.2719	0.0944	11.78	6.10	0.8018
	C	Ka	372.9	1414.1818	0.4007	0.1383	46.45	56.42	0.2977
G2	O	Ka	262.3	405.0862	0.2864	0.0988	40.17	36.63	0.2460
	Si	Ka	265.6	541.1298	0.3130	0.1080	13.38	6.95	0.8070
	C	Ka	163.6	86.9309	0.3396	0.1186	50.66	62.43	0.2342
G3	O	Ka	85.0	142.4974	0.1792	0.0626	29.08	26.90	0.2153
	Si	Ka	211.3	381.8471	0.4812	0.1681	20.26	10.67	0.8298

Discussion

The results shows that the absorbance of light increase gradually along with culture time for all polymer composite samples, and this denotes that the nanocomposites afford encouraging sites for cell proliferation on surface, but at the same time, the difference between the three groups of nanocomposite samples are not very significant. Thus, the nanocomposites have higher cell activity or biocompatibility have no cytotoxic effect as according to ISO 10993 part 5, according to which if cell viability for a given material is lower than 70%, the material is considered as toxic substance (reduction of cell viability above 30% is regarded as cytotoxic effect). Thus, the cell viability in this study is above 85%, so the peek composite is considered as non-toxic and biocompatible, which agrees with the results obtained by Wenz et al. (10). These essayists resolved that the PEEK composite displayed “excellent” in vitro biocompatibility in the ASTM standard cell culture patterns. This implies that the PEEK nanocomposites provide encouraging surface housings for cell multiplying and growth, and optical microscopy showed the distribution of L-929 cell line on neat PEEK, the polymer composite (G1), (G2) and (G3) groups and morphology of cultured cells after 24 and 48 hours for all groups.

The optical images at intimate contact of fibroblasts with the nanocomposites samples along with neat PEEK (in the absence of nanoparticle powders), the images of L-929 fibroblast cells on nanocomposites samples after 24hour, and the spread of cells found in control showed that the cell spread in neat PEEK, the polymer composite (G1), (G2) and (G3) groups 24 hours and 48 hours, indicating that PEEK and PEEK nanocomposite did not cause any toxic effect on cells. Moreover, results showed attachment of cells to these materials, and proliferation, and formation of a layer with dense cell in conjunction with numerous cell-cell contacts (the optical images at intimate contact of fibroblasts with the nanocomposites samples along with control [in the absence of nanoparticle powders]). This agrees with other results previously reported by Josset et al. (11). These results mean that SiC nanoparticle does not show any sign of cytotoxicity.

Based on similar optical charts or diagrams, it may be recognized that the exterior surface for polymer and nanocomposite samples afford biocompatible site to enable the cell growing of the mouse fibroblastic cell. This result indicates that increase in cell adhesion may be due to the incorporation of the Nano Silicone carbide to the polymer, leading to changes that show nanoscale modification of the PEEK surface, including improved PEEK surface wettability, proven by a lessened water contact angle and increase in surface roughness detected by the AFM test, which was previously mentioned. This predicts that nanocomposites

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afford encouraging sites on the surface for cell proliferation correspondingly granted with Yakimova et al., (12), whose results display that (SiC) material had an effective biocompatibility level and SiC (cubic 3C poly type) used in this study shows that its highly consistent and has an approvingly encouraging cellular reaction regardless of the kind of cell, and we determined to go in for that material expand in the area of biocompatibility, which agrees with Saddow (13). Also, these results are in an appropriate unity and agreement with the other researchers' outcomes, Zhao et al. (14) and Chandar et al. (15).

This animal study was done in Iraq with approval by Animal Ethics Committee in the College of dentistry, Baghdad University; all experiments were done along with the institutional guidelines and regulations of the Iraqi Veterinary Medical Syndicate in Iraq, and the experiments were done at the Center of AL-Dyhaa for Agricultural and Veterinary Services in Baghdad.

PEEK and PEEK-SiC nanocomposite did not cause any toxic effect on cells. Moreover, the attachment of cells to materials formed and proliferated a dense cell layer and the polymer surfaces and nanocomposite samples afford biocompatible surfaces permitting the mouse fibroblastic cell growth.

Conflict of Interest: The authors and planners have disclosed no potential conflicts of interest, monetary or else.

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