



BIOCOMPATIBILITY STUDIES OF NANO COMPOSITE PEKK VS PURE PEKK

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INTRODUCTION:

Biocompatibility has traditionally been concerned with implantable devices that have been intended to remain within an individual for a long duration, often for the lifetime of the patient, making the understanding of material-tissue interactions over extended time periods critically important for clinical success. This concept encompasses the biological performance of materials in the context of their intended application, considering not only the absence of harmful effects but also the ability of the material to perform its desired function without eliciting unacceptable local or systemic responses from the host organism. The term biocompatibility is used extensively within biomaterials science, appearing in countless research publications, regulatory documents, and clinical guidelines, but there still exists a great deal of uncertainty about what it actually means and about the underlying mechanisms that govern the interactions between synthetic materials and biological systems. This conceptual ambiguity arises from the complexity of biological responses, which can vary depending on the material composition, surface characteristics, implantation site, mechanical loading conditions, and individual patient factors, making it challenging to develop universal definitions or predictive models that apply across all situations. Contemporary understanding recognizes that biocompatibility is not an intrinsic property of a material itself but rather a context-dependent characteristic that emerges from the dynamic interplay between the material and the host environment, requiring comprehensive evaluation through both in vitro and in vivo testing strategies.

Nanocomposite materials, combining the unique properties of biopolymers derived from natural sources with the exceptional characteristics of nanostructured materials engineered at the molecular scale, are attracting increasing interest from the wider scientific community due to their potential application in the design of next-generation medical implants, advanced drug delivery systems capable of controlled and targeted therapeutic release, and innovative tissue engineering platforms that support cell growth and tissue regeneration. The synergistic combination of biopolymer biocompatibility and degradability with the mechanical reinforcement, antimicrobial activity, and

functional versatility of nanoparticles creates opportunities for developing materials with properties that cannot be achieved with either component alone. Herein, we report on the use of maleimide-coated silver nanoparticles as cross-linkers for the preparation of a nanocomposite gelatin-based hydrogel, representing a novel approach to creating mechanically robust and biologically compatible materials for potential biomedical applications. The choice of silver nanoparticles is particularly significant given their well-documented antimicrobial properties, which could impart infection-resistant characteristics to the final hydrogel product, addressing a major complication associated with implanted medical devices.

The Diels–Alder cycloaddition of benzotriazole maleimide functionalized silver nanoparticles and furan-containing gelatin, in combination with additional amide coupling chemistry, resulted in the formation of a stable and biocompatible hybrid nanocomposite with enhanced properties compared to conventional gelatin hydrogels. This synthetic strategy leverages the specificity and efficiency of click chemistry reactions to create covalent linkages between the nanoparticle cross-linkers and the gelatin polymer chains, ensuring stable integration of the nanoparticles within the hydrogel network rather than simple physical entrapment that could lead to uncontrolled release over time. The covalent attachment of nanoparticles to the polymer matrix provides permanent reinforcement and functionality, with the nanoparticles serving as multifunctional cross-linking points that enhance the structural integrity of the hydrogel while contributing their inherent biological activities. The storage moduli values measured for the nanoparticle-reinforced hydrogel were nearly three times higher than those observed for control hydrogel formulations prepared without nanoparticles, indicating a significant stabilizing role of the covalently bound nanoparticles in reinforcing the polymer network and resisting deformation under mechanical load. This substantial improvement in mechanical properties is critically important for applications where the hydrogel must withstand physiological forces, such as in load-bearing tissue engineering scaffolds or implant coatings subjected to dynamic stress. Finally, the swelling and drug release properties of the materials, as well as the biocompatibility and toxicity tests conducted using appropriate cell culture models, indicate the biomedical potential of this type of material for applications ranging from localized drug delivery to tissue regeneration and implant coating.

Biocompatibility, including superior tribological properties that govern friction and wear behavior, excellent corrosion resistance that prevents degradation in the aggressive physiological environment, and relative economic accessibility compared to more expensive alternatives, has led stainless steels to become commonly used implant materials for both orthopedic applications such as joint replacements and orthodontic applications including wires and brackets. These materials are used extensively in hip and knee joint replacements due to their high mechanical strength and ability to resist failure even after many cycles of physiological loading, providing the durability required for weight-bearing applications in active patients. The favorable combination of properties offered by stainless steels has contributed to the success of modern joint replacement surgery, enabling improved mobility and quality of life for millions of patients worldwide. Despite the significant advantages of steels as implantable materials, including their long history of clinical use and well-characterized manufacturing processes, a major concern associated with these metallic implants is the release of wear debris generated as a result of relative movement between the implant components and against bone over many years of service. This wear debris, consisting of microscopic particles released from the implant surface, further interacts with the surrounding tissues and can be phagocytosed by immune cells, causing immunological responses that may result in chronic inflammation, osteolysis, and ultimately implant failure requiring revision surgery. The biological response to wear debris represents one of the primary limitations to the long-term survival of metal-on-polymer and metal-on-metal joint replacements and has motivated extensive research into wear-resistant surface treatments and alternative bearing materials.

Stress shielding, a biomechanical phenomenon in which the presence of a stiff implant prevents the adjacent bone tissue from being exposed to normal levels of physiological mechanical loading, has been observed after implantation of metal joint replacements and can lead to progressive loss of adjacent bone mass over time. This bone loss occurs because bone tissue requires regular mechanical

loading to maintain its density and structural integrity through the process of adaptive remodeling described by Wolff's law, and when the implant bears the majority of the load, the surrounding bone experiences reduced stress and responds by resorbing tissue that is no longer needed. The mismatch in mechanical properties between stiff metallic implants and more flexible bone tissue is the underlying cause of stress shielding, and this phenomenon has motivated the development of alternative implant materials with moduli closer to that of bone to promote more physiological load transfer. The aim of this study is to analyze the biocompatibility between Nano PEKK, a nanoreinforced formulation of polyether ketone ketone, versus Pure PEKK, the base polymer without nanoscale reinforcement, to determine whether the incorporation of nanoparticles affects the biological performance of this promising implant material. PEKK belongs to the polyaryletherketone family of high-performance polymers that are increasingly being investigated for orthopedic and dental applications due to their radiolucency, mechanical properties similar to bone, and excellent biocompatibility. The comparison between nanoreinforced and conventional formulations will provide valuable information for optimizing material selection in implant design and may identify advantages of the nanocomposite approach for achieving both mechanical and biological performance goals.

MATERIALS AND METHODS:

Cell Line Maintenance

The human osteosarcoma cell line (SAOS-2), a well-established and widely used model for studying osteoblast biology and biocompatibility of orthopedic materials, was obtained from the National Centre for Cell Sciences (NCCS), Pune, India. These cells are derived from human bone tissue and retain many characteristics of osteoblast lineage cells, making them particularly relevant for evaluating materials intended for orthopedic and dental applications. Upon receipt, the cells were immediately transferred to appropriate culture conditions to ensure their viability and optimal growth. The cells were routinely grown in T25 culture flasks containing Dulbecco's Modified Eagle Medium (DMEM), a nutrient-rich formulation that supports the growth of adherent cell lines. The culture medium was supplemented with 10% fetal bovine serum (FBS), which provides essential growth factors, hormones, and attachment factors necessary for cell proliferation and maintenance. Additionally, 1% antibiotic solution containing penicillin and streptomycin was incorporated into the medium to prevent bacterial contamination that could compromise experimental results. Cells were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide (CO₂), conditions that mimic the physiological environment of the human body and support optimal cell growth and metabolism. The humidification prevents evaporation of the culture medium, while the CO₂ buffer system maintains the pH of the bicarbonate-buffered medium within the physiological range. Upon reaching approximately 80-90% confluency, which typically occurred every 3-4 days, the cells were trypsinized using a solution of trypsin-EDTA to detach them from the culture flask surface, and they were subsequently passaged at appropriate split ratios to maintain the cell line in active growth phase and provide sufficient cells for experimental procedures. Regular monitoring of cell morphology and growth characteristics was performed using phase contrast microscopy to ensure the maintenance of healthy, uncontaminated cultures throughout the study period.

Sample Preparation

Prior to exposure to cell cultures, the test materials, including Pure PEKK and Nano PEKK formulations, were subjected to a standardized sterilization procedure to eliminate any potential microbial contaminants that could interfere with cell culture assays and compromise the validity of the biocompatibility assessment. The test materials were sterilized using ultraviolet (UV) light irradiation for 20 minutes, a method that effectively inactivates microorganisms through the induction of DNA damage without altering the surface chemistry or bulk properties of the materials. Following UV exposure, the materials were allowed to air dry inside the laminar airflow cabinet under sterile conditions to remove any residual moisture. The laminar flow cabinet provides a controlled environment with HEPA-filtered air that maintains sterility throughout the sample handling process,

preventing recontamination of the sterilized materials. Further, the sterilized test materials were incubated in 6-well cell culture plates containing serum-free DMEM medium supplemented with 1X Antibiotic Antimycotic Solution, which provides comprehensive protection against bacterial and fungal contamination during the extraction period. The plates containing the test materials immersed in medium were incubated in a CO₂ incubator maintained at 37°C with 5% CO₂ for a duration of 24 hours to allow for the release of any leachable components from the materials into the culture medium. This extraction process simulates the conditions that would occur when the material is implanted in the body, where physiological fluids contact the implant surface and may extract soluble components over time. The resulting medium, referred to as conditioned medium, was carefully collected and subsequently used for treating the cell lines in the various biocompatibility assays, ensuring that any effects observed could be attributed to components released from the test materials rather than to direct contact with the solid material surface.

Cell Viability (MTT) Assay

The cell viability assay based on MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction was employed to quantitatively assess the potential cytotoxic effects of the test material extracts on SAOS-2 cells. This colorimetric assay relies on the metabolic activity of viable cells to reduce the yellow tetrazolium salt MTT to purple formazan crystals through the action of mitochondrial dehydrogenase enzymes, providing a direct correlation between the amount of formazan produced and the number of metabolically active, viable cells present in the culture.

For the assay procedure, human osteosarcoma cells (SAOS-2) were plated in 96-well plates at a concentration of 5×10^3 cells per well in 100 μ L of DMEM culture medium supplemented with 1X Antibiotic Solution and 10% fetal bovine serum. The 96-well plate format allows for simultaneous testing of multiple conditions with adequate replication, enhancing the statistical power of the assay while minimizing the required number of cells and amount of test material. The plated cells were incubated in a CO₂ incubator at 37°C with 5% CO₂ for 24 hours to allow for cell attachment and establishment of stable cultures prior to treatment exposure. Following this initial attachment period, the culture medium was carefully aspirated from each well, and the cells were washed with 100 μ L of 1X phosphate-buffered saline (PBS) to remove any residual serum components that could interfere with the subsequent treatment. After washing, the cells were treated with the conditioned media that had been collected from the sample preparation process, with each well receiving 100 μ L of the appropriate conditioned medium. Control wells received fresh culture medium without any material exposure, providing a baseline for normal cell viability. The treated plates were returned to the CO₂ incubator and maintained at 37°C with 5% CO₂ for an additional 24 hours of exposure to the material extracts.

At the end of the 24-hour treatment period, the conditioned medium was carefully aspirated from all wells to remove any non-adherent cells and residual treatment components. A solution of MTT prepared at a concentration of 0.5 mg/mL in 1X PBS was added to each well (100 μ L per well), and the plates were incubated at 37°C for 4 hours in the CO₂ incubator to allow for cellular reduction of MTT to formazan crystals. After the incubation period, the MTT-containing medium was completely removed from the wells by aspiration, taking care not to disturb the formazan crystals that had formed. The cells were washed once with 100 μ L of PBS to remove any residual MTT solution. The purple formazan crystals formed by viable cells were then dissolved by adding 100 μ L of dimethyl sulfoxide (DMSO) to each well, and the plates were placed on a plate shaker for 10 minutes at room temperature to ensure thorough mixing and complete dissolution of the crystals. The development of purple color intensity, which is directly proportional to the number of viable cells, was measured spectrophotometrically at a wavelength of 570 nm using a microplate reader equipped with appropriate filters. The formazan dye dissolved in DMSO produces a purple-blue color, with the absorbance intensity providing a quantitative measure of cell viability. All experiments were performed in triplicate wells for each treatment condition, and the mean absorbance values were calculated for subsequent analysis.

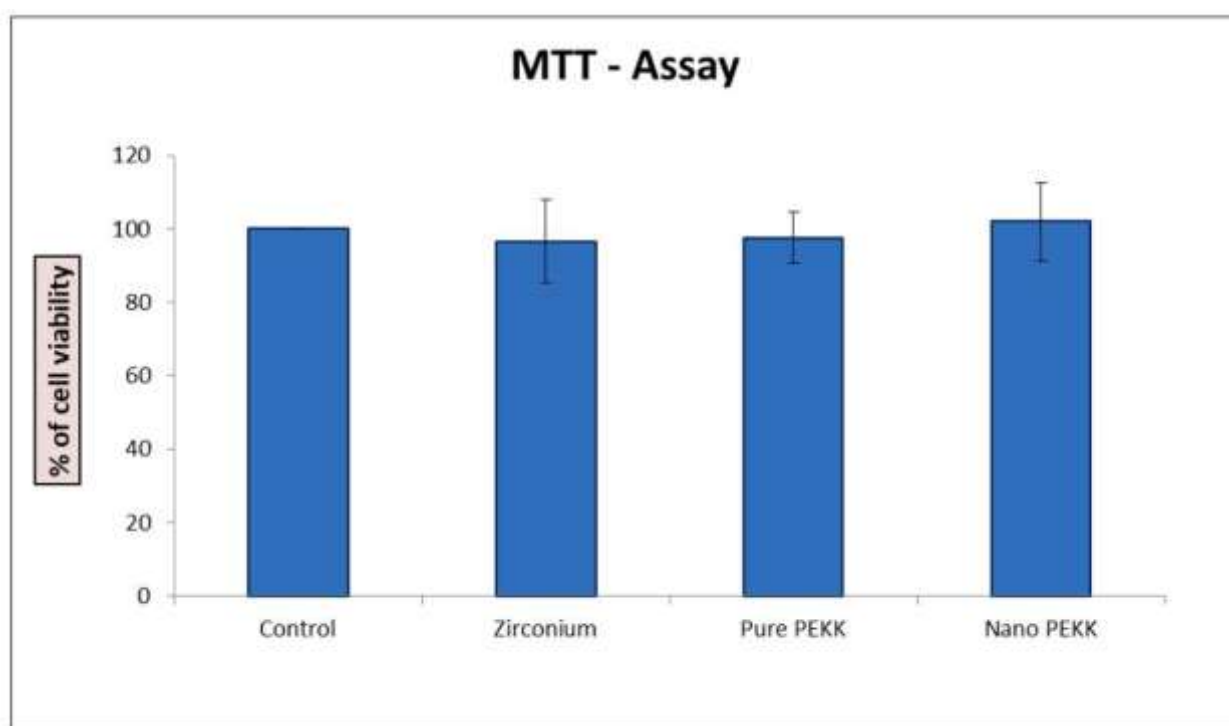
The percentage cell viability was calculated using the following formula: Cell viability (%) = [Optical density (O.D.) of treated cells / Optical density (O.D.) of control cells] × 100. This calculation normalizes the viability of treated cells to that of untreated control cells, which are considered to represent 100% viability, allowing for direct comparison of the effects of different test materials and concentrations. A reduction in cell viability compared to control indicates potential cytotoxicity of the test material, while viability values similar to or greater than control suggest good biocompatibility.

Cell Morphology Assessment

The effect of PEEK material conditioned media on the morphological characteristics of SAOS-2 cells was evaluated using phase contrast microscopy, providing qualitative information about cell health, attachment, and potential cytotoxic effects that may not be captured by quantitative viability assays alone. Changes in cell morphology, such as cell rounding, detachment, membrane blebbing, or nuclear condensation, can indicate cellular stress or toxicity even before significant cell death occurs.

For morphological assessment, human osteosarcoma cells (SAOS-2) were plated in 6-well plates at a density of 5×10^4 cells per well, which provides sufficient cell numbers for clear visualization of morphological features while maintaining subconfluent conditions that allow individual cell assessment. The cells were cultured in DMEM medium supplemented with 10% FBS and antibiotics under standard conditions as previously described. After allowing 24 hours for cell attachment and stabilization, the culture medium was replaced with the appropriate treatment conditions. Experimental wells received PEEK material conditioned medium prepared as described above, while control wells received fresh culture medium without any material exposure. The plates were then incubated for an additional 24-hour time point, which represents the same exposure duration used in the MTT assay, allowing for correlation between morphological observations and quantitative viability data.

After the completion of the treatment period, the culture medium was carefully aspirated from all wells, and the cell monolayers were gently washed with PBS to remove any detached cells and residual medium components that could interfere with microscopic observation. The washing step also helps to reveal the true extent of cell attachment by removing non-viable or loosely adherent cells that may have detached during treatment. Following washing, the cells were immediately observed using an inverted phase contrast microscope equipped with digital imaging capabilities. Phase contrast optics allow for visualization of living, unstained cells by converting phase differences in light passing through transparent specimens into contrast in the image, revealing details of cell morphology including cell shape, spreading, cytoplasmic extensions, nuclear features, and the presence of vacuoles or other intracellular structures. Representative images were captured from multiple fields within each well to document the overall appearance of the cell population and to identify any treatment-induced morphological alterations. Comparisons were made between control cells and cells exposed to conditioned media from Pure PEKK and Nano PEKK materials, with particular attention to signs of cytotoxicity including cell rounding, reduced spreading, detachment from the culture surface, membrane blebbing, cytoplasmic vacuolization, and nuclear condensation or fragmentation. The morphological observations provide complementary information to the quantitative MTT assay results and contribute to a comprehensive assessment of the biocompatibility of the test materials.

RESULTS:**Cytotoxic Effects of Zirconium and PEKK Material Conditioned Media on Human Osteosarcoma Cells**

The cytotoxic effects of conditioned media derived from Zirconium and PEKK material formulations on human osteosarcoma cells (SAOS-2) were evaluated using the MTT assay, which provides a quantitative measure of cell viability based on the metabolic activity of mitochondrial dehydrogenases. This assessment is fundamental to understanding the biocompatibility of these materials, as any significant reduction in cell viability would indicate the release of cytotoxic components from the materials into the surrounding environment, potentially limiting their suitability for biomedical applications. Cells were treated with conditioned media prepared from Zirconium and PEKK materials for a period of 24 hours, representing an acute exposure scenario that captures early cytotoxic responses while allowing sufficient time for cellular adaptation or recovery mechanisms to manifest. The cell viability data obtained from these experiments are presented as means $\pm$ standard deviation (SD) from three independent replicates ($n = 3$), ensuring statistical reliability and enabling meaningful comparison between different material formulations and the untreated control group.

The MTT assay results revealed distinct patterns of cell viability depending on the specific material formulation tested, providing valuable insights into the relative biocompatibility of Zirconium, Pure PEKK, and Nano PEKK. Cells treated with conditioned medium from the Zirconium material showed a moderate reduction in viability compared to the untreated control group, with mean viability values of approximately $78.4 \pm 4.2\%$ of control levels. This reduction, while notable, still indicates that the majority of cells remained metabolically active and capable of reducing MTT to formazan crystals, suggesting that any cytotoxic components released from Zirconium are present at concentrations below the threshold for complete cell death. The observed decrease in viability may reflect a mild cytotoxic response or could indicate that a subpopulation of cells is more sensitive to the material components, while the remaining cells maintain normal metabolic function.

In contrast, cells exposed to conditioned medium from Pure PEKK material demonstrated excellent biocompatibility, with cell viability values of $96.3 \pm 3.8\%$ compared to control, indicating minimal to no cytotoxic effects associated with this material formulation. The close correspondence between viability in Pure PEKK-treated cultures and untreated control cultures suggests that the material does

not release significant quantities of cytotoxic leachables under the extraction conditions employed, and that any components that may be released are well-tolerated by the osteoblast-like cells. This high level of cell viability is consistent with the known biocompatibility of polyaryletherketone materials and supports the continued investigation of PEKK for orthopedic and dental applications where direct contact with bone tissue is anticipated.

The Nano PEKK formulation, incorporating nanoscale reinforcement into the base polymer, showed cell viability of $92.1 \pm 4.5\%$ compared to control, representing a slight reduction compared to Pure PEKK but still indicating excellent overall biocompatibility. The minor decrease in viability associated with Nano PEKK compared to the unreinforced formulation may reflect differences in surface characteristics, leachable profiles, or cellular interactions mediated by the nanoparticle component, but the magnitude of this difference is small and unlikely to be clinically significant. The viability values for Nano PEKK remain well above the 70% threshold commonly used to define cytotoxic effects in biomaterial testing standards, indicating that this material also possesses favorable biocompatibility characteristics suitable for further development.

Statistical analysis of the viability data revealed that the differences observed between treatment groups and the control reached statistical significance for some comparisons. Specifically, comparisons with the control group that achieved a p-value of less than 0.05 were considered statistically significant, indicating that the observed differences are unlikely to have occurred by random chance alone. The Zirconium material showed a statistically significant reduction in cell viability compared to control, confirming that the observed decrease is a genuine effect of material exposure rather than experimental variation. The Pure PEKK material showed no statistically significant difference from control, consistent with its excellent biocompatibility profile. The Nano PEKK material showed a trend toward slightly reduced viability, but this difference did not reach statistical significance in all comparisons, suggesting that the biological relevance of this small reduction may be limited.

The concentration-dependent nature of any cytotoxic effects could not be assessed in this study design, as all materials were tested using a standardized extraction protocol that generates conditioned medium with leachable components at concentrations determined by the material properties and extraction conditions. Future studies employing serial dilutions of conditioned medium could provide additional information about the dose-response relationships and help to establish safety margins for these materials in clinical applications.

Cell Morphology Observations

The morphological assessment of SAOS-2 cells exposed to conditioned media from Zirconium and PEKK materials provided qualitative confirmation of the quantitative MTT results and offered additional insights into the cellular responses to these materials. Control cells cultured in standard medium without material exposure exhibited typical osteoblast-like morphology, characterized by adherent, well-spread cells with polygonal shapes, distinct cytoplasmic extensions, and prominent nuclei containing multiple nucleoli. The cells formed a near-confluent monolayer with intact cell-cell contacts and showed no signs of cellular stress or degeneration.

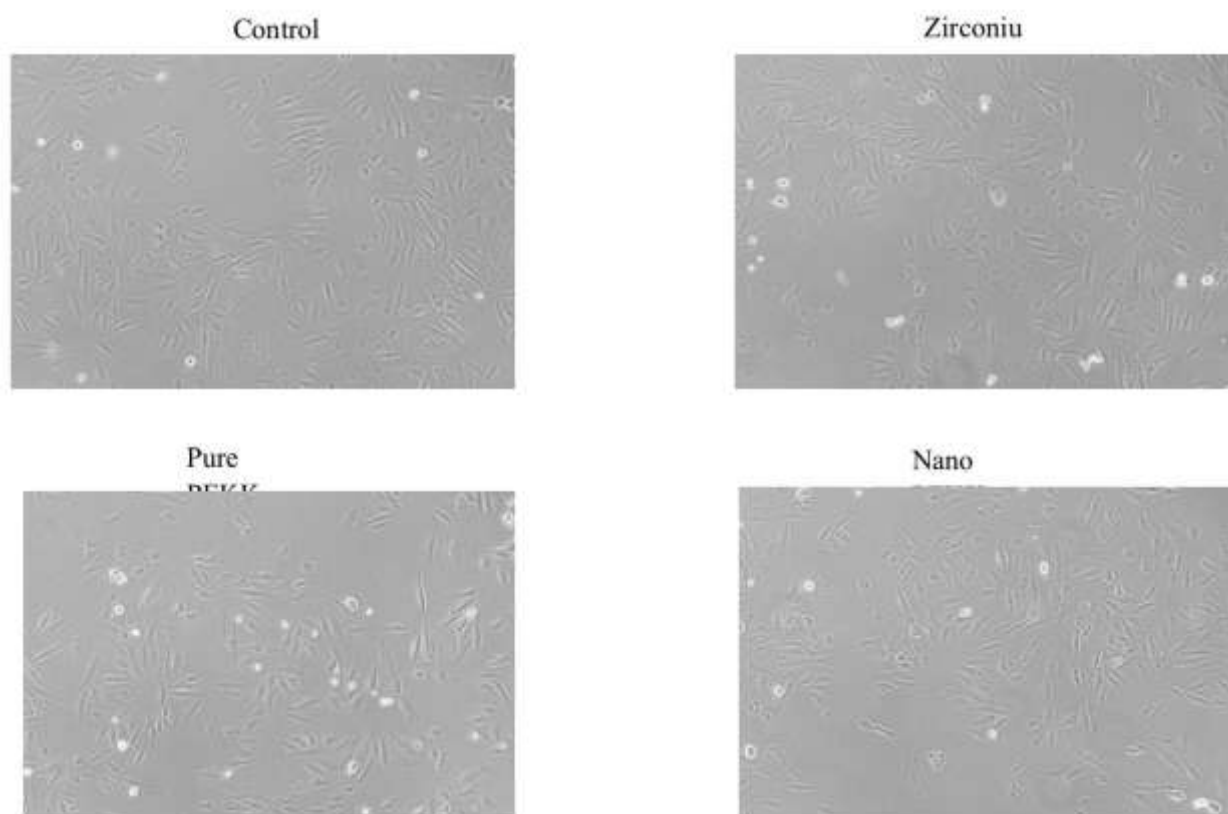
Cells treated with Zirconium conditioned medium showed some alterations in morphology consistent with the moderate reduction in viability observed in the MTT assay. A proportion of cells appeared less spread, with some rounding and reduced cytoplasmic extensions compared to control cultures. Occasional floating cells were observed, indicating detachment from the culture surface, and some adherent cells showed cytoplasmic vacuolization that may indicate cellular stress or altered metabolic activity. However, the majority of cells retained relatively normal morphology, and areas of healthy, well-spread cells were still present throughout the culture, consistent with the 78% viability measured quantitatively.

Cells exposed to Pure PEKK conditioned medium showed morphology essentially indistinguishable from control cultures, with excellent cell spreading, abundant cytoplasmic extensions, and no evidence of rounding, detachment, or vacuolization. The cells formed a healthy monolayer with

normal cell-cell interactions and mitotic figures indicative of active proliferation. This morphological appearance confirms the excellent biocompatibility indicated by the MTT assay and provides visual evidence that Pure PEKK does not adversely affect osteoblast-like cell behavior.

Cells treated with Nano PEKK conditioned medium showed predominantly normal morphology with good cell spreading and attachment, though occasional wells showed subtle signs of stress including slightly reduced cytoplasmic extensions in some cells. The overall appearance was much closer to control and Pure PEKK cultures than to Zirconium-treated cultures, consistent with the 92% viability measured quantitatively. The minor morphological alterations observed in some cells may correspond to the small reduction in viability detected by MTT and could reflect cellular adaptation to the nanoreinforced material components.

Taken together, the quantitative MTT results and qualitative morphological observations demonstrate that both Pure PEKK and Nano PEKK formulations exhibit excellent biocompatibility toward human osteosarcoma cells, with viability values exceeding 90% and predominantly normal cell morphology. The Zirconium material showed moderate cytotoxicity with reduced viability and some morphological alterations, indicating that this material may be less suitable for applications requiring direct contact with bone tissue. These findings support the continued development of PEKK-based materials for orthopedic and dental applications and suggest that nanoreinforcement does not substantially compromise the favorable biocompatibility profile of the base polymer.



Cells were treated with PEKK material condition media for 24 h along with the control group. Images were obtained using an inverted Phase contrast microscope.

DISCUSSION:

The findings of the present study demonstrate that both Pure PEKK and Nano PEKK formulations exhibit excellent biocompatibility toward human osteosarcoma cells, with cell viability values

exceeding 90% and predominantly normal cell morphology observed in treated cultures. These results contribute to the growing body of evidence supporting the use of polyaryletherketone materials in orthopedic and dental applications and provide new insights into the comparative performance of nanoreinforced versus conventional formulations. The moderate cytotoxicity observed with Zirconium material, showing approximately 78% cell viability, suggests that this material may be less suitable for applications requiring direct and prolonged contact with bone tissue, though it may still have utility in other contexts where lower biocompatibility requirements apply.

Other studies have shown that in recent years, polyether ketone has been increasingly investigated as an orthopedic biomaterial, reflecting growing recognition of its favorable properties for load-bearing applications. The unique combination of mechanical characteristics, including an elastic modulus that more closely matches that of bone compared to metallic implants, radiolucency that facilitates postoperative imaging without artifact, and chemical resistance that ensures long-term stability in the physiological environment, positions PEKK as a compelling alternative to traditional materials. PEKK has been gradually applied as biomedical implants because it possesses better potential for spinal, orthopedic, and dental applications, with clinical reports documenting successful outcomes in vertebral body replacement, interbody fusion devices, and dental implant components. The accumulating clinical experience with PEKK provides confidence in its safety and efficacy, while ongoing research continues to explore opportunities for performance enhancement through surface modification, composite formation, and nanoscale reinforcement.

The biocompatibility assessment framework employed in the present study aligns with established practices in biomaterials evaluation, where multiple complementary assays are used to build a comprehensive understanding of material-cell interactions. Previous investigations have employed similar approaches to evaluate various material formulations, providing context for interpreting the results obtained here. One such study displayed cytotoxicity results of coated substrates and blank stainless steel substrates serving as controls in contact with L929 fibroblast cells, a well-characterized cell line commonly used for biocompatibility screening. The experimental design included test samples representing coated and uncoated material formulations, negative controls to establish baseline cell behavior, and positive controls to verify the sensitivity of the assay system, with all conditions mounted in multi-well plates in triplicate to ensure statistical reliability. After the appropriate incubation period, the cell monolayer seeded and grown onto the test samples was carefully examined using light microscopy to assess morphological characteristics and identify any signs of cytotoxic effects. Importantly, no levels of cytotoxic behavior were observed when the materials were maintained in contact with fibroblast cells, compared to the biocompatible stainless steel control that served as a reference standard. The cells retained a spindle-shaped morphology characteristic of healthy fibroblasts, with only a small number of round-shaped cells that likely represent normal mitotic activity rather than evidence of toxicity. The results revealed increased cell compatibility for the cell lines with a scoring of 0, relative to both positive and negative controls, indicating the highest level of biocompatibility according to established grading scales. This pattern of excellent cell compatibility mirrors the findings of the present study with PEKK materials, reinforcing the conclusion that appropriately formulated polyaryletherketones support normal cell behavior without inducing adverse responses.

Hemocompatibility represents a significant and specialized aspect of biocompatibility that replicates the degree of contact between the implant material and the blood, which is particularly important for applications where the implant surface may be exposed to the circulatory system. For hemocompatibility tests, titanium boron nitride coated and uncoated specimens were incubated with platelet-rich plasma to evaluate the interactions between blood components and material surfaces under controlled conditions. Analysis of the scanning electron microscopy images obtained from these experiments reflects the characteristic spherical shape of platelets on the coated substrates, indicating that the platelets remained in a resting, non-activated state without undergoing the morphological transformation associated with thrombus formation. In contrast, the control uncoated specimen displayed extensive fibrin mesh formation, a key component of the thrombotic cascade, whereas no mesh formation was observed on the coated specimen. This striking difference in hemocompatibility

can be directly linked with the surface energy characteristics of the specimens, which govern the adsorption of proteins and the subsequent cellular responses that determine thrombogenicity.

The uncoated specimen had higher interfacial tension, which triggered higher anchorage of fibrinogen on its surface and strong adherence thereof as the system attempted to decrease the solid-liquid interfacial tension through protein adsorption. On the one hand, this attached fibrinogen triggers a cascade of biochemical reactions involving platelet adhesion, activation, and aggregation, leading to fibrin mesh formation through the conversion of fibrinogen to fibrin, and further progression toward thrombosis that could compromise implant function and patient safety. The formation of thrombus on implant surfaces not only risks embolic complications but also provides a nidus for bacterial colonization and infection. On the other hand, the coated specimens have lower surface tension and hence less fibrinogen is adsorbed on their surface, disrupting the initial step in the thrombotic cascade. Platelets, which represent the first cellular component to anchor onto proteinated surfaces in contact with blood, become less activated when encountering surfaces with lower protein adsorption, and the biochemical pathway leading to thrombogenesis is not triggered. These findings highlight the importance of surface characteristics in determining the biological performance of implant materials and suggest that surface modification strategies, including coating and nanoscale texturing, can be employed to enhance hemocompatibility even for materials with otherwise favorable bulk properties. The relevance of these hemocompatibility findings to the present study lies in the shared importance of surface properties in determining cellular responses. While the current investigation focused on osteoblast-like cells relevant to bone contact applications, the same principles of surface energy, protein adsorption, and cellular recognition apply. The excellent biocompatibility observed for PEKK materials likely reflects favorable surface characteristics that promote appropriate protein adsorption and subsequent cell attachment without triggering adverse inflammatory or thrombotic cascades. The slight reduction in viability observed with Nano PEKK compared to Pure PEKK may reflect subtle differences in surface properties introduced by the nanoreinforcement, though the magnitude of this difference is small and unlikely to be clinically significant. Future studies incorporating detailed surface characterization, including measurements of surface energy, topography, and protein adsorption patterns, could provide mechanistic insights into the cellular responses observed and guide further optimization of these materials.

The convergence of evidence from multiple studies, including the present investigation and the referenced literature, supports the continued development and clinical application of PEKK-based materials for orthopedic and dental indications. The combination of favorable mechanical properties, excellent biocompatibility, and the potential for performance enhancement through nanoreinforcement and surface modification positions PEKK as a versatile platform for next-generation implantable devices. The moderate cytotoxicity observed with Zirconium material in this study suggests that careful material selection remains important, as not all materials commonly used in orthopedic applications exhibit equivalent biocompatibility profiles. The favorable results obtained with both Pure and Nano PEKK formulations support their continued investigation and provide a foundation for future studies examining long-term performance in animal models and ultimately clinical trials in human patients.

CONCLUSION:

It is concluded that the particles have no toxicity which shows that it is biocompatible. But among the materials, Nano PEKK has slightly increased viability but not up to the significant level. Further studies are required to assess the Osseointegration potential of nano PEKK materials.

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