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International Journal of Current Research
Vol. 10, Issue, 03, pp.66483-66486, March, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EVALUATION OF CYTOTOXICITY OF POLYETHERETHERKETONE (PEEK) AS A DENTAL IMPLANT MATERIAL

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ARTICLE INFO

Article History:
Received 29th December, 2017
Received in revised form
24th January, 2018
Accepted 09th February, 2018
Published online 28th March, 2018

Key words:
PEEK (Polyetheretherketone),
Dental Implant,
Cytotoxicity,
Fibroblasts,
Colony Formation Assay.

ABSTRACT

Purpose: PEEK (polyetheretherketone) is a synthetic polymer being used increasingly as a dental implant material due to its iso-elastic nature and enhanced mechanical properties. This study analysed the cytotoxicity and biocompatibility of PEEK which helps to improve its bioactivity and ensure widened clinical prospects in future.

Methods: Samples of PEEK dental implant material were added to murine T3T fibroblasts which were cultured in Dulbecco's modified eagle medium at 37ºc. After 24 hours incubation at 37ºc and 5% carbon dioxide, the medium was replaced with 200 micro litre of medium which contained extracts of PEEK implant material. Cell morphology was analysed using Motic Inverted Microscope.

Results: The result for biocompatibility of PEEK as a dental implant material when evaluated using Colony-forming unit fibroblast assay, was positive showing no signs of cytotoxicity.

Conclusion: With analogous physical and mechanical properties to bone, PEEK has proved to be a potent biocompatible dental implant material.

Citation: Dr. Rhea Mahajan, Dr. Roseline Meshramkar, Dr. Lekha, KP., Dr. Ramesh Nadiger and Dr. Sandeep Mahajan, 2018. "Evaluation of cytotoxicity of polyetheretherketone (PEEK) as a dental implant material.", *International Journal of Current Research*, 10, (03), 66483-66486.

INTRODUCTION

PEEK (Polyetheretherketone) is a synthetic thermoplastic, tooth coloured polymer. Due to its prime property of iso-elasticity with bone and its radiolucent nature, PEEK has benefited the field of orthopaedics in the form of spine and hip implants and it shows comparable promise as a dental implant material mainly because of its superior physical properties such as stress shielding. In dentistry, PEEK is presently being used in a variety of applications ranging from fabrication of fixed crowns and bridges, components of removable partial dentures, implant abutments and dental implants. Among these, there is maximal degree of interplay with bio-mechanical requirements in case of replacing a tooth in toto or multiple teeth using dental implants (Ramamoorthi *et al*., 2015). Some case reports have suggested prevalence of allergy positive reactions against titanium reagents along with similar reports on materials such as chromium, mercury, palladium and nickel (Hosoki *et al*., 2016). Ceramic based materials are also seen to exhibit inconsistent biologic behaviour as dental restorations (Kerem Kilic *et al*., 2013). Although such reports are scanty, they give us all the more reason to evaluate the cytotoxicity of PEEK as an exceptional, bio inert substitute to these conventional implant materials such as titanium and zirconia. Recognition of an implant material as biocompatible nowadays depends on a large number of factors, such as: Absence of cytotoxicity, mutagenicity and carcinogenicity. The exclusion of its allergenic properties, physical and chemical stability and biological 'inertia' in a biological environment is essential (Katzer *et al*., 2002). Although the mechanical performance of such materials can be assessed readily, in vivo performance and biocompatibility must be scrutinized before prosthesis can be safely implanted (Morrison *et al*., 1995). Therefore, it becomes essential for us to assess the cytotoxic and biocompatible properties of a relatively new biomaterial such as PEEK to ensure its long term safety as a dental implant material.

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MATERIALS AND METHODS

Test sample preparation

T3T mouse connective tissue fibroblastic cell line was used to study the cytotoxicity of PEEK dental implant material in vitro. PEEK was acquired in the form of granules (2 press Bio HPP clear-granules SP Dental, Pune, India). Three test samples each containing four sterilized (autoclaved) PEEK granules with equal mass by volume ratio were taken. The comparison regarding fibroblastic cell viability was done by analysing test samples against untreated murine (T3T) fibroblasts which were used as a control group (Figure 1).

Figure 1. Autoclaved PEEK granules

Cell culture

The PEEK granules were immersed in 7 ml of culture medium for 24 hours at 37ºC to extract any cytotoxic substances. Murine normal fibroblast cells (NCCS, Pune) were cultured at 37˚C under a humidified atmosphere of 5% CO_2 and 95% air and were grown in DMEM (Dulbecco's modified eagle medium), High Glucose medium (HIMEDIA Laboratories, Mumbai) supplemented with 10% fetal bovine serum (HIMEDIA Laboratories, Mumbai) and 1% Antibiotic Antimycotic solution (HIMEDIA Laboratories, Mumbai) (Figure 2).

Figure 2. Murine t3t fibroblasts cultured with PEEK extract

Colony formation assay

A qualitative assessment of the cell culture groups was performed using the Colony Formation Assay. The fibroblastic cells were grown up to 80% confluence and were trypsinized and seeded in 6-well plates in triplicate at a density of 500 cells/well for 2 days at 37°C (Figure 3).

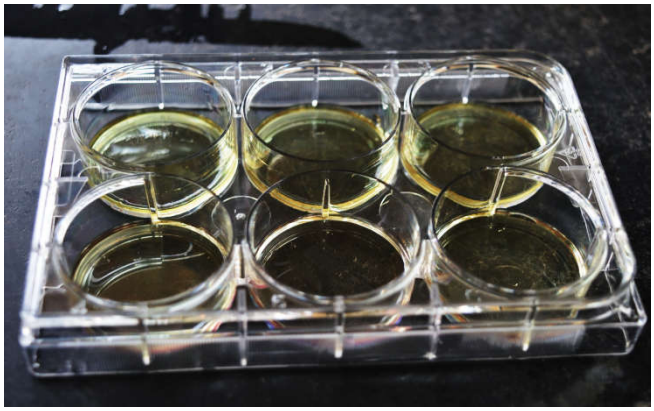

Figure 3. Colony formation assay carried out in 6 -well plates

Analysis of cell morphology

After 48 hours of incubation, the changes in the cell morphology were captured under objective of Motic Inverted Microscope with 10MP resolution camera with the help of Motic Image PLUS 2.0 (Figure 4, 5).

RESULTS

Comparison between optical density of fibroblastic cells seen in control group and test samples (PEEK group) was done under the Motic inverted microscope. The microscopic picture of the murine cells exposed to the test sample exhibited no morphological alterations or a significant reduction in cell number and cell death.

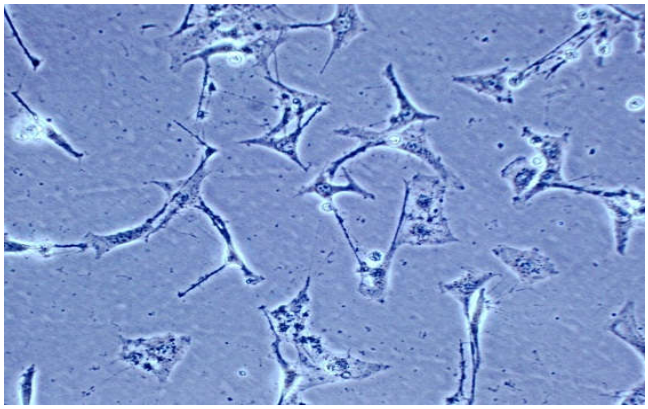

Figure 4. Viable fibroblasts with control group (without PEEK)

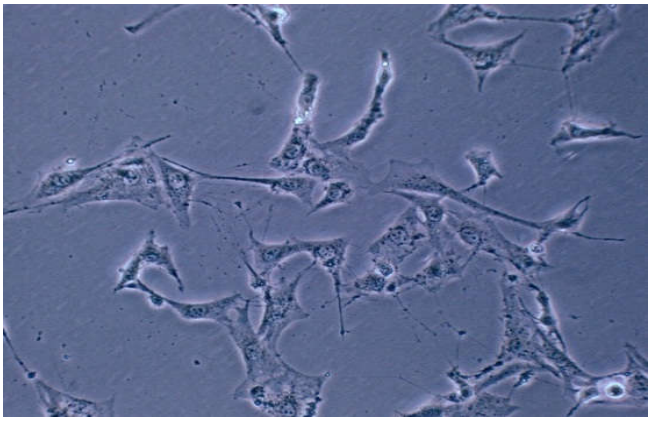

Figure 5. Viable t3t fibroblasts with PEEK test sample

The qualitative evaluation showed no statistical difference in the cell number between the control and the test specimen.

DISCUSSION

The physical and biologic properties of the implant materials adjudge the long term success of implants. Considering that oral implants thrive on close contact between implant surface and oral epithelium, it is crucial for us to evaluate the cytotoxic effect of any novel bio material (Miura *et al*., 2012). The conventionally used implant materials such as Titania (TiO_2) and Zirconia (ZrO_2) are still not up to scratch in terms of an ideal material which replaces hard biological tissue (Marchi *et al*., 2010), whereas PEEK is emerging as a feasible candidate for the same. Usually, for *in vitro* toxicity tests, some cells are plated in a well of a cell-culture dish where they attach, forming the so-called test system.

The material to be tested is then placed in this test system. If the material is not cytotoxic, the viable cells will remain attached to the well with time (Gociu *et al*., 2013). Colony formation assay (CFA) also known as colony formation unit-fibroblast (CFU-F), is one of the most popular and standard recognized qualitative test for determination of cytotoxic effects of a given material, among many other tests such as MTT assay, cell proliferation assay, cell transformation assay.Even though CFA poses a tiring and time consuming attempt at counting the number of colonies manually or evaluating them under the microscope, it is still regarded as a gold standard test (Katz *et al*., 2008). To exclude the possibility of any inconsistency with the results, it was made sure that the granules used for the samples were of similar size and comparable mass by volume ratio.

Using the colony formation assay, we analysed the results for cytotoxicity of PEEK. It was observed that the fibroblasts showed no remarkable morphologic alterations. The cell viability observed in the test sample was neither increased because of the presence of PEEK granules nor decreased as in comparison to the control group. The in vitro interaction of mice fibroblasts with untreated PEEK showed no overt cytotoxic or mutagenic effects. Various studies affirm the biocompatibility of PEEK using tests for mutagenesis like Ames test (Katzer *et al*., 2002). This makes untreated PEEK not only a biocompatible, but also a bio-inert material. Morrison *et al* conducted a similar study comparing biocompatibility of PEEK and epoxy resin, which confirmed that it showed no significant cytotoxicity when it was assessed quantitatively in terms of cell protein content, leakage of cytosolic lactate dehydrogenase (LDH) activity through damaged cell membranes, intracellular reduced glutathione (GSH) content and MTT assay (Morrison *et al*., 1995). In 2002, another study used SV40 rat osteoblasts and 3T3 mouse fibroblasts in direct contact with PEEK material and revealed that there were no effects on the morphology of the osteoblasts nor was there any evidence of a negative influence on the 3T3 proliferation rate or cytotoxic effects on the osteoblasts in the MTT assay. On the contrary, there was even evidence of stimulation of the osteoblast protein content which has resulted in discussion that PEEK might have a favourable effect on bone growth (osseointegration) (Katzer *et al*., 2002).

Despite the stable chemical nature of PEEK which makes it an attractive endo-prosthetic material, chemical surface inertness does not account for a sound interfacial biocompatibility and PEEK requires a surface modification prior to its application in vivo (Briem *et al*., 2005). Studies have proved that silane-coupled PEEK-HA had in general improved biomechanical properties than untreated PEEK and did not show cytotoxicity in vitro (Rashidi *et al*., 2015). Another study showed improved biocompatibility of PEEK modified specifically through the methods of plasma technology (Briem *et al*., 2005). Therefore, to evaluate future scope of PEEK as a dental implant material, further research is required to analyse satisfactory results for more desirable physical properties along with minimal cytotoxic effects.

Conclusion

By the course of this experimental study we concluded that PEEK can be utilized as a suitable biomechanical and chemically stable dental implant material. When studied under colony formation assay with living fibroblasts, it showed negligible alterations to the cell morphology or number clearly indicating that it is not cytotoxic in nature.

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