Effect of Sterilization Methods on the Morphological, Molecular, and Biocompatibility Characteristics of Nanofibrous PEEK Layers

Nikolai Ivanovich Vatin[1,2*],*G V V* Satyanarayana[3], Kalluri Anil[4], *Anup* Singh Negi[5], *Nittin* Sharma[6], *Manvinder* Brar[7], *Diksha* Sharma[8]

[1]Peter the Great St. Petersburg Polytechnic University, Saint Petersburg 195251, Russian Federation
[2]Lovely Professional University, Phagwara, Punjab, India
[3]Department of Civil, GRIET, Bachupally, Hyderabad, Telangana, India.
[4]Department of Mechanical Engineering, KG Reddy College of Engineering and Technology, Chilkur(Vil), Moinabad(M), Ranga Reddy(Dist), Hyderabad, 500075,Telangana, India.
[5]Uttaranchal University, Dehradun - 248007, India
[6]Centre of Research Impact and Outcome, Chitkara University, Rajpura- 140417, Punjab, India
[7]Chitkara Centre for Research and Development, Chitkara University, Himachal Pradesh-174103 India
[8]Faculty of Pharmaceutical Sciences, Research & Incubation Centre, Rayat Bahra University, Chandigarh-Ropar NH 205, Greater Mohali, Punjab, 140103, India
*Corresponding Author: vatin@mail.ru

Abstract: In this work, the polyether ether ketone (PEEK) nanofibrous layers and their sterilized methods influence on molecular properties, structural properties, and biocompatibility have been discussed.Some instrument employed were gamma radiation, ultraviolet light, Ethylene oxide (EtOx) and an autoclave to sterilised the layers developed through melt-electrospinning process.umber of instruments used such as gamma radiation, ultraviolet light, ethylene oxide (EtOx), and an autoclave was used to sterilise the layers prepared via melt-electrospinning process. The SEM result unveiled that the sterilization processes changed the fibre form slightly as well as the diameter which was modified slightly as well.However, the sterilization processes did not alter the molecular weight of PEEK fibers, as far as GPC study was concerned.d biocompatibility has been explored. A number of instruments used such as gamma radiation, ultraviolet light, ethylene oxide (EtOx), and an autoclave was used to sterilise the layers prepared via melt-electrospinning process. The scanning electron microscopy (SEM) showed that the sterilization processes slightly altered the fiber's form along with small variations in

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*Corresponding Author: vatin@mail.ru

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diameter. While, the sterilization processes did not achanged the molecular weight of PEEK fibers, according to gel permeation chromatography (GPC) research. In addition, the murine fibroblasts (3T3) were assessed for in vitro biocompatibility using MTT test. While cell adhesion and proliferation rate were the same across all the layers in the sterile control sample, the UV-sterilized material significantly reduced the level of cell viability. Consequently, the employed methods, namely the autoclave, EtOx, and gamma radiation were determined to be efficient to sterilise PEEK nanofibrous layers. However, solar radiation with the method may also reduce their biocompatibility.

Keywords: PEEK, nanofibrous layers, sterilization, autoclave, ethylene oxide, UV radiation, morphology, biocompatibility, electrospinning

1 Introduction

Polyetheretherketone (PEEK) has become one of the most preferred polymers in biomedical field thanks to their beneficial properties including chemical inertness, strong mechanical properties, and good biocompatibility. Due to itshigh strength to weight ratio and biocompatibility, titanium is applied in orthopedics, spinal devices, and the latest dental implants [1–3]. This has been due to the recent new opportunities arising from the novel applications of nanotechnology sized PEEK nanofibers within the filed of drug delivery and biomedical applications. The employs of PEEK nanofibers are possible to serve as drug delivery system providing an opportunity to investigate the multifaceted organization and functioning of the skeletal system.The focus of ongoing evaluations is to determine which types of medical products can be implanted for different therapeutic functions knowing trends of biomaterial/biologic tissue interactions[4–6].bustness and longevity make it highly suitable for applications in orthopedics, spinal devices, and advanced dental implants [1–3]. The recent advancements in nanotechnology filed have opened new doors for PEEK nanofibers, specifically in drug delivery and biomedical areas. The PEEK nanofibers are considered as efficient candidate in enabling precise drug administration and provide a pathway to better understand the complex architecture and functionality of the skeletal system. The aim of current assessments is to classify the types of medical devices that can be safely implanted for various therapeutic purposes, taking into account the unique interactions between biomaterials and biological tissues[4–6]. In clinical practice, sterility of biomaterials is one of the most crucial requirements and the most important measure of compatibility with biological systems.On the other hand, PEEK itself and its uses have been the subject of many of these studies, but the effects of sterilization on the properties and performance of PEEK nanofibers have been scarce [7–9].llent biocompatibility. Its robustness and longevity make it highly suitable for applications in orthopedics, spinal devices, and advanced dental implants [1–3]. The recent advancements in nanotechnology filed have opened new doors for

PEEK nanofibers, specifically in drug delivery and biomedical areas. The PEEK nanofibers are considered as efficient candidate in enabling precise drug administration and provide a pathway to better understand the complex architecture and functionality of the skeletal system. The aim of current assessments is to classify the types of medical devices that can be safely implanted for various therapeutic purposes, taking into account the unique interactions between biomaterials and biological tissues[4–6]. In clinical practice, sterilizing biomaterials is paramount for ensuring safety and compatibility with biological environments. In other hand, the significant research has been devoted to PEEK and its applications, but it is limited data on sterilization affects the structural and functional properties of PEEK nanofibers [7–9]. In microbiology sterilization can be defined as the process of eradication of bacteria, virus and other microorganisms on surfaces or inside the structure of a material through physical, chemicals or biological means. The frequently used sterilizing methods are autoclaving, gamma radiation, UV flash, and ethylene oxide which have been regarded as effective methods for removing interferences. Nevertheless, sterilization raises specific problems for polymers such as PEEK which is susceptible to high thermal conductivity and may be degraded by most established heat sterilization procedures. Also, gamma radiation and ethylene oxide present other methods for achieving the sterilization; however, the primary usage of these methods is limited because of high cost [16-18]. Moreover, it has a low ability in penetrating deep or complicated grooves which are very useful.

This paper seeks to fill the foregoing knowledge gap by assessing the effect of sterilization on PEEK nanofiber mats produced by electrospinning. For microbial decontamination effectiveness of autoclaving, gamma radiation, ultraviolet, ethylene oxide sterilization has been discussed[10–12]. The observations made were incorporated in a methodical way in order to investigate changes in fibre morphology, molecular integrity and biological interactions after sterilization. Surprisingly, there was the no impact on the structure and chemical composition of the nanofibers, but gamma radiation significantly reduced the thermal stability. However, the UV oxidation light revealed high efficiency in surface sterilization but the ability of UV to penetrate may be shallow, which may not be desirable for certain medical application.

In addition, this work investigates the process of PEEK nanofiber sterilization and provides comparative analysis of its microbiological properties against the background of other industrial polymers[18–21]. One of the aims of the present work is to optimize the process of PEEK sterilization and to achieve the maximum cost efficiency of the method for biomedical applications without compromising the strength properties of the material. Additionally, this work evaluates how PEEK nanofiber derivatives can be used in biological systems to determine their ability to support various medical applications. This study by revealing the sterilization effects on PEEK nanofibers supports the creation of advanced and safe biomaterials in the subsequent advances in medical applications[22–24]. The main purpose of this work consists in the identification of the costs and benefits of sterilization of the given PEEK polymer and the attempts to preserve some of its essential mechanical properties. A new research is in progress to understand the possibility of utilising compounds from PEEK nanofibers for the biological fields.

2 Materials and Methods

2.1 Preparation of the Materials

This fibrous material was created using (PEEK; Victrex, UK; Mn: 150,000 g/mol).. The PEEK was melted and then extruded into nanofibers after being heated to 370°C. The fibers were collected in an aluminum collector that was grounded around 150 mm from the bottom[25]. Achieving uniform nanofibrous structures requires fine-tuning the extrusion and collecting rates. A custom-built environmental room was used to control climatic factors including temperature and relative humidity in order to attain uniformity during melt-electrospinning. A 30 g/mµ density polypropylene nonwoven substrate (PF Nonwovens, Czech Republic) was covered with thin layers of nanofibrous PEEK.

Table 1. **S**pecific melt-electrospinning conditions used for the preparation of PEEK nanofibrous layers.

Distance between collector and nozzle [mm]	Voltage [kV]	Extrusion speed [mm/min]	Collector speed [mm/s]	Temperature [°C]	Relative humidity [%]	Area weight [g/m²]
150	35	200	150	370	30	30.2

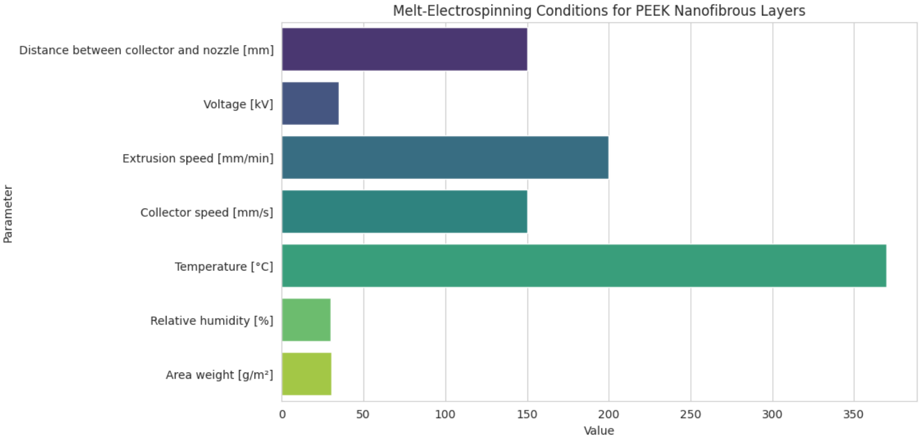


Fig. 1. The specific melt-electrospinning conditions used for the preparation of PEEK nanofibrous layers

2.2 Morphology Assessment

To investigate the structure of the fibrous PEEK layers, scanning electron microscopy (SEM, JEOL JSM-IT500, Japan) was used. For this, the samples were sputter-coated with a 5 nm layer of platinum to protect them from charge. ImageJ (National Institutes of Health, USA) is employed to evaluate structural homogeneity and fiber diameter, At least 300 fiber diameters have to be measured for every sample in order for the results to be considered statistically significant. Our statistical analysis was conducted using GraphPad Prism v8. The non-normal distribution of the data necessitated the use of the Kruskal-Wallis test for statistical comparison; a p-value of 0.05 or less was considered significant. Results are presented as means with corresponding 95% CIs.

2.3 Analysis of Molecular Weight

Differential variations in molecular weight were evaluated by gel permeation chromatography (GPC) of the PEEK nanofibrous layers both before and during sterilization. A concentration of 2 mg/ml of trichlorobenzene (TCB) was used to dissolve pure granulated PEEK and nanofibrous layers for laboratory investigation. An investigation of Geophysical Photoluminescence (GPC) was performed using an Agilent PL-GPC 50 system using a refractive index detector and a PLgel 10 µm MIXED-B column (300 × 7.5 mm, Agilent, USA). The TCB mobile phase was heated to a maximum temperature of 135 degrees Celsius at a rate of one millilitre per minute. The molecular weight distribution was determined by tracking the shift in the peak of the chromatogram, which revealed the molecular weight fraction represented by the greatest abundance. The observations of shorter elution and retention periods suggest a decrease in molecular weight.

2.4 In Vitro Study

The 3T3 murine fibroblasts (ATCC, USA) were seeded onto 15 mm disks of sterile PEEK nanofibrous material. A medium from Sigma-Aldrich in the USA was used to cultivate the cell cultures. In it were diluted solutions of fetal bovine serum (ten percent), glutamine (one percent), and penicillin-streptomycin (one percent). The MTT metabolic test was used to evaluate cell adherence and proliferation on PEEK surfaces after 1 and 7 days of incubation. For our statistical analysis, we evaluated two sterilization procedures side by side using the Mann-Whitney U test. Average absorbance at 570 nm and 95% CIs for proliferation were shown in the data.

3 RESULTS AND DISCUSSION

3.1 Morphology

The nanofibrous PEEK layers were successfully fabricated using melt-electrospinning. SEM images of both non-sterile and sterilized materials are presented in Figure 2, highlighting the morphology before and after different sterilization methods. The fiber diameters of the PEEK layers before and after sterilization are shown in Figure 2. The non-sterile PEEK fibers had an average diameter of 410 ± 25 nm. The smallest fiber diameter was observed in the UV-sterilized samples (370 ± 22 nm), while the largest fiber diameter was noted for the gamma radiation sterilized samples (452 ± 18 nm). As with other polymers, such as PVDF, slight variations in fiber diameters were observed, which can be attributed to production variability during melt-electrospinning. The SEM analysis indicated that none of the sterilization methods caused significant structural changes to the nanofibrous PEEK layers. No bead defects, fiber coalescence, or foil-like structures were detected, indicating that the chosen sterilization methods are suitable for PEEK nanofibrous materials without compromising their morphology.

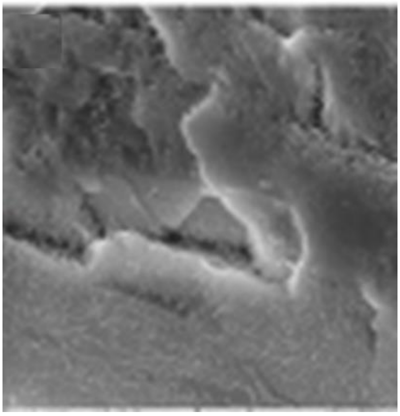


Fig. 2. SEM image of PEEK layer

3.2 Molecular Weight Analysis

The molecular weight of the PEEK nanofibrous layers was evaluated before and after sterilization using gel permeation chromatography (GPC). The GPC analysis showed that the molecular weight of PEEK remained stable across all tested sterilization methods. Figure 2 shows the elution curves of the PEEK samples, where all sterilized samples had the same elution time as the non-sterilized control (8.25 min), indicating no significant changes in molecular weight due to sterilization.

This result is consistent with previous findings for high-performance polymers like PEEK, which are known for their stability under a variety of sterilization conditions. No degradation or reduction in molecular weight was observed.

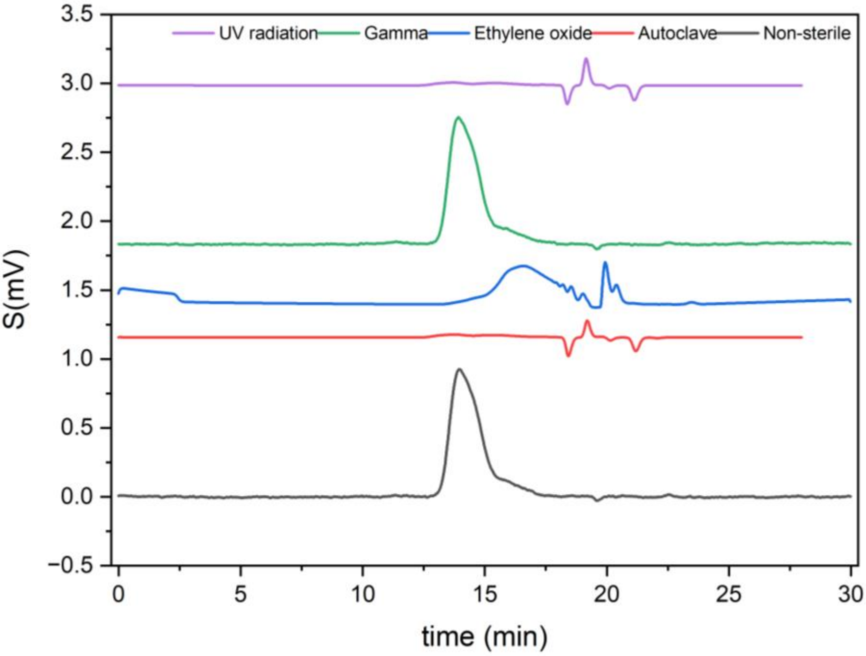
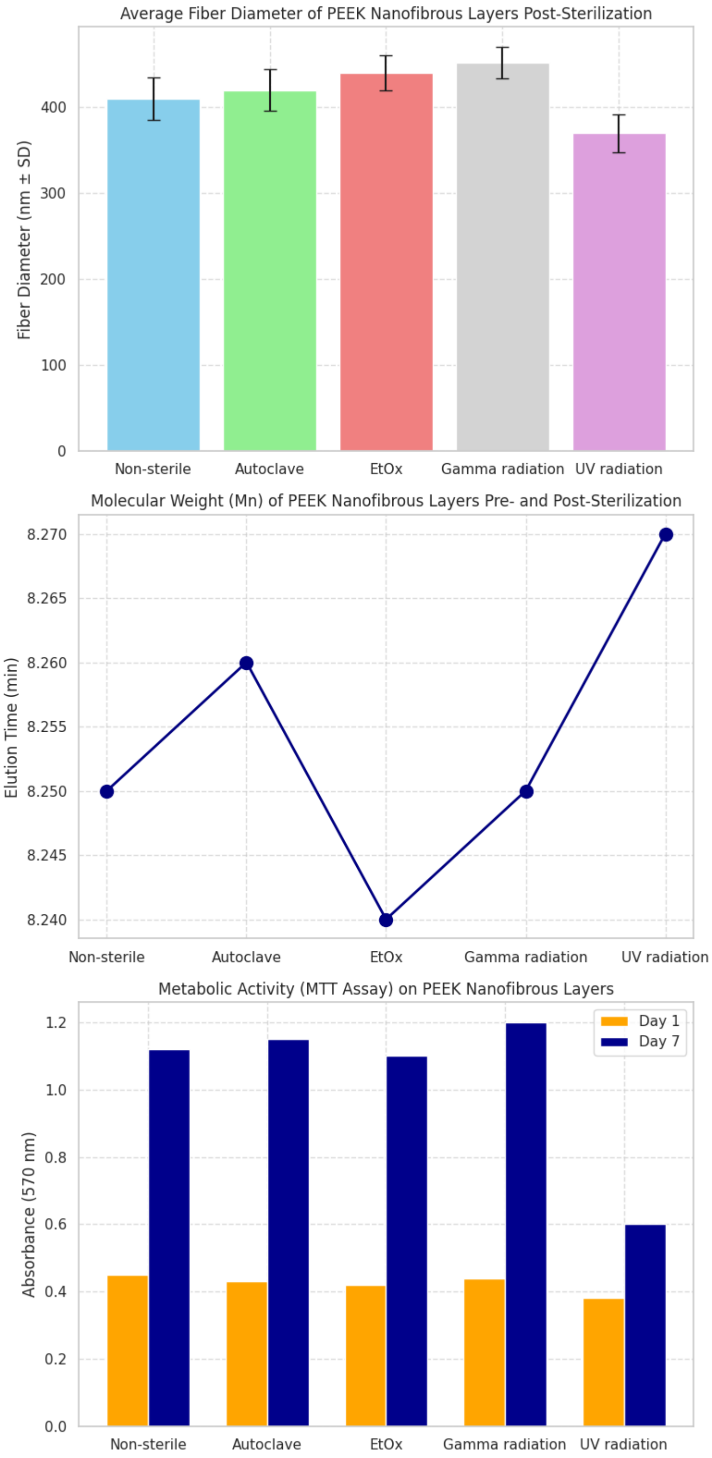


Fig. 3. UV absorption rate of material under consideration

3.3 In Vitro Study

The results of cellular adhesion and proliferation, as evaluated by the MTT assay, are presented in Figure 3. After 1 day of incubation, all sterilized PEEK samples, except the UV-treated ones, supported cell adhesion comparably to the non-sterilized control. However, the UV-sterilized samples showed a slight decrease in cell adhesion on day 1.

By day 7, cell proliferation increased on all sterilized PEEK samples except for the UV-treated ones, which exhibited a significantly lower level of metabolic activity. The gamma radiation-sterilized samples supported the highest level of cell proliferation, followed closely by the ethylene oxide and autoclave-treated samples. This result suggests that UV radiation may induce changes in the surface properties of PEEK nanofibers, potentially reducing their biocompatibility, as reported in similar studies on other polymers. The reduction in cell viability on the UV-treated samples might be explained by the surface modifications induced by UV sterilization, which can alter the fiber's surface wettability and roughness, thus affecting cellular adhesion and proliferation. These findings are consistent with earlier reports on other polymer-based materials, where plasma and UV sterilization have been shown to induce changes that negatively impact biocompatibility .



Average Fiber Diameter of PEEK Nanofibrous Layers Post-Sterilization

Molecular Weight (Mn) of PEEK Nanofibrous Layers Pre- and Post-Sterilization

Metabolic Activity (MTT Assay) on PEEK Nanofibrous Layers

Fig. 4. Complete Analysis of Results

4 Conclusion

In conclusion, this research sought to determine the impact of different sterilization methods on PEEK nanofiber coatings in terms of the layers' structure and chemical composition. All three sterilization techniques – gamma radiation, ethylene oxide and autoclaving – were observed to not alter the molecular weight and structural conformation of the PEEK nanofibers.However, ultraviolet (UV) light was seen to change the biocompatibility in that surface changes affect the cellular adhesion and proliferation.ical integrity. The PEEK nanofibers maintained their molecular weight and structural geometry following sterilization using gamma radiation, ethylene oxide (EtOx), and autoclaving. However, ultraviolet (UV) light was found to be alter the biocompatibility, due to surface modifications that inhibit cellular adhesion and proliferation. Thus, these results highlighted the need for proper choice of suitable sterilization techniques for PEEK nanofibers in the biomedical context.In particular, autoclaving, gamma radiation, and EtOx come into the fore as the most appropriate procedures since they do not compromise the nanofibrous architecture and material properties of fabricated PEEK layers together with the biofunctionality of the scaffolds.their molecular weight and structural geometry following sterilization using gamma radiation, ethylene oxide (EtOx), and autoclaving. However, ultraviolet (UV) light was found to be alter the biocompatibility, due to surface modifications that inhibit cellular adhesion and proliferation. Therefore, these findings suggested importance of selecting appropriate sterilization methods for PEEK nanofibers in biomedical applications. Specifically, autoclaving, gamma radiation, and EtOx emerge as the most suitable methods, as they preserve the nanofibrous structure and biofunctionality of PEEK layers as compared to UV sterilization.

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