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ORIGINAL ARTICLE

Cytotoxic Evaluation of Ultra High Molecular Weight Polyethylene for Medical Device Applications

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Abstract

Objectives: This study evaluates the cytotoxicity of ultra-high molecular weight polyethylene (UHMWPE) using the Neutral Red Uptake (NRU) assay and examines whether any leachable substances affect cell health. The findings add to the evidence that UHMWPE is safe for long-term use in medical implants. **Method:** The Elution Method was used to evaluate the cytotoxic effects of UHMWPE extract by ISO 10993-5:2009 in Balb/c 3T3 using Neutral Red Uptake method. **Findings:** The extract of UHMWPE did not induce any cytotoxicity effects using the qualitative and quantitative methods. In the qualitative method the cultures treated at different concentrations (30% to 100%) appeared normal without any change in their morphology (grade 0). In the quantitative method, the viability was greater than 90 %, indicating no cytotoxicity. **Novelty:** This study evaluated the cytotoxicity of unmodified UHMWPE using ISO 10993-5/-12 compliant protocols under GLP conditions. By generating concentration-dependent viability data, it offers strong evidence for the baseline biocompatibility of UHMWPE, supporting its continued use in implantable medical devices.

Keywords: Ultrahigh molecular weight polyethylene; Polymer; Cytotoxicity; Elution Method; Neutral Red Uptake

1 Introduction

Ultra-high molecular weight polyethylene (UHMWPE) is widely employed in long-term orthopedic and surgical implants due to its superior mechanical strength, wear resistance, and chemical stability[(1)],[(2)]. These properties are largely attributed to its extremely long polymer chains that enhance Van der Waals interactions, leading to exceptional durability and low friction[(3)].

Owing to these advantages, UHMWPE has become the gold standard material for load-bearing components in orthopedic prosthetics, such as acetabular cup liners in total hip arthroplasty and tibial inserts in knee replacements[(4)],[(5)]. Its biomechanical stability, inert surface, and self-lubricating behavior allow it to perform reliably in high-friction environments[(6)].

However, newer formulations of UHMWPE—modified through cross-linking, vitamin E stabilization, or nanocomposite incorporation—have raised questions about

long-term safety. Sterilization techniques such as gamma or UV irradiation may cause surface oxidation, leading to oxidative degradation and release of potentially cytotoxic byproducts[7],[8].

Studies have reported mitochondrial dysfunction, dose-dependent cytotoxicity, or immune activation associated with aged, irradiated, or surface-modified UHMWPE[9],[10]. Furthermore, many published reports lack ISO 10993-5-compliant methodologies, limiting their regulatory relevance and reproducibility[11].

A critical gap remains few studies have evaluated the cytotoxicity of medical-grade, unmodified UHMWPE under standardized ISO protocols using fibroblasts—cells directly relevant to tissue-implant interactions[12]. This study addresses that gap by using the ISO 10993-5-compliant Neutral Red Uptake (NRU) assay in Balb/c 3T3 fibroblasts to evaluate extractables from UHMWPE. The objective is to establish a robust cytocompatibility profile and contribute regulatory-grade safety data to support its continued medical use.

2 Methodology

2.1 Study Design

This study was designed in accordance with ISO 10993-5:2009 to evaluate the in vitro cytotoxicity of ultra-high molecular weight polyethylene (UHMWPE) using the Neutral Red Uptake (NRU) assay using Balb/c 3T3.

2.2 Test Material

The test item UHMWPE-based components of an orthopedic implant were extracted at 0.2 g/mL in 1x DMEM medium supplemented with 5% heat-inactivated newborn calf serum and 1% penicillin/streptomycin solution. Additionally, sodium lauryl sulphate and high-density polyethylene (HDPE) strips were positive and negative controls.

2.3 Preparation of extract

A 3.7 g of UHMWPE test material was extracted in 18.5 mL of extraction medium under aseptic conditions. The extraction was done at a 0.2 g/mL ratio using 1× DMEM supplemented with 5% heat-inactivated newborn calf serum and 1% penicillin/streptomycin. The mixture was incubated at 37 °C for 72 hours. No filtration, centrifugation, or pH adjustment was performed during extraction. At the end of the extraction, the extract was clear, with no colour change, and no particulates were found. Hence, no additional processing was made, such as filtration, centrifugation, pH adjustments or other processing. Eight different concentrations (30, 40, 50, 60, 70, 80, 90 and 100%) of the test extract, positive control, and 3 cm^2/mL for the negative control.[13]

2.4 Experimental design

Mouse fibroblast cell line Balb/c 3T3 was employed as the biological model. Cells were cultured in complete growth medium (DMEM 10) supplemented with 10% heat-inactivated newborn calf serum and 1% penicillin/streptomycin. Cultures were maintained in a humidified incubator at 37 $\pm$ 1 °C, 5% CO_2, and >90% relative humidity. For extract preparation, DMEM 05 was used as the extraction medium, consisting of DMEM supplemented with 5% heat-inactivated newborn calf serum and 1% antibiotics.

Exponentially growing Balb/c 3T3 cells were harvested using trypsin-EDTA and counted with a hemocytometer using 0.4% trypan blue exclusion to ensure viability. A working suspension of 1×10^5 cells/mL was prepared by diluting 0.366 mL of a stock suspension (32.75×10^5 cells/mL) into 11.634 mL of complete medium. Cells were then seeded into 96-well culture plates at a density of 1×10^4 cells/well and incubated for 24 hours to allow adherence and monolayer formation. After confirming $\geq 70\%$ confluency and normal cell morphology under an inverted microscope, the culture medium was aspirated and replaced with six replicates of the test material extract at varying concentrations (30–100%), along with neat extracts of the positive and negative control materials.

Cells were incubated for an additional 24 hours, after which they were visually assessed for signs of cytotoxicity, including cell rounding, detachment, and lysis, as defined in the ISO 10993-5 grading criteria. For the quantitative assessment, wells were gently washed with 150 μL of phosphate-buffered saline (PBS), and 100 μL of Neutral Red (NR) medium was added to each well. Plates were incubated at 37 °C for 3 hours, allowing viable cells to incorporate and retain the neutral red dye within their lysosomes.

After incubation, the NR medium was discarded, and wells were briefly rinsed with PBS to remove excess dye. Subsequently, 150 μL of NR desorb solution, consisting of ethanol, glacial acetic acid, and distilled water (15:0.3:14.7 mL), was added to each

well. The plates were placed on a shaker to extract the dye from viable cells into the solution. The optical density (OD) of each well was measured at 546 nm using a Mindray MR-96A microplate reader.

Neutral Red uptake was quantified as OD_{546}, with background correction using blank wells. Cell viability was calculated relative to the negative control, which consisted of cells treated with the vehicle medium without any test material. Cell viability was calculated using the formula:

Viability (%) = 100 × (OD_{546} of sample) / (OD_{546} of negative control)

A reduction in viability to below 70% of the negative control was considered indicative of cytotoxic potential.

Grading of qualitative and quantitative analysis:

Table 1. Qualitative grading of cytotoxic effects

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Source: ISO 10993-5:2009.

Quantitative grading of cytotoxic effects:

Cell viability was calculated relative to the vehicle control. Viability <70% was considered cytotoxic, whereas viability $\geq 70\%$ indicated a non-cytotoxic response

3 Results and Discussion

3.1 Qualitative Analysis

Morphological assessment of Balb/c 3T3 was performed after 24-hour exposure to test and control extracts, as per ISO 10993-5:2009 guidelines. As shown in Figure 1, cells treated with the vehicle control (DMEM 05), and the negative control (HDPE extract) retained normal spindle-shaped morphology with uniform cell distribution and no observable cytotoxic effects. Both conditions were assigned a morphological reactivity grade of 0, indicating no reactivity.

In contrast, cultures exposed to the positive control (sodium lauryl sulfate) exhibited significant cytotoxic changes, including cell rounding, detachment, and membrane lysis. This condition was assigned a Grade 4, confirming the validity and sensitivity of the assay system

Cells treated with neat UHMWPE extract (100%) and serial dilutions (30%, 50%, 70%) displayed no morphological alterations compared to the negative control. The fibroblasts remained well spread, viable, and exhibited intact membranes with no visible detachment or structural damage. All concentrations of UHMWPE were graded as Grade 0 for cytotoxic.

3.2 Quantitative Analysis

Quantitative assessment of cell viability was carried out using the Neutral Red Uptake assay to evaluate the cytotoxic potential of the test and control extracts. The negative control exhibited a mean cell viability of 97.00%, confirming its non-cytotoxic profile in accordance with ISO 10993-5:2009, which designates materials as non-cytotoxic when viability remains above 70%. Conversely, positive control resulted in a marked decrease in cell viability to 13.80%, clearly indicating cytotoxicity and confirming the reliability and responsiveness of the assay system. Cells treated with the UHMWPE extract across all tested concentrations—30%, 50%, 70%, and 100% (neat)—demonstrated high viability values of 95.84%, 94.02%, 91.12%, and 93.26%, respectively (Table 1). These values were well above the cytotoxicity threshold, and no dose-dependent decrease in viability was observed.

Our results align with previous reports showing favorable cytocompatibility of native UHMWPE[14],[15]. Unlike studies using UV- or gamma-irradiated UHMWPE[7],[16], which reported reduced fibroblast viability or sub-lethal immune activation, our work demonstrates that non-irradiated, medical-grade UHMWPE extracts exhibit no cytotoxicity across all tested

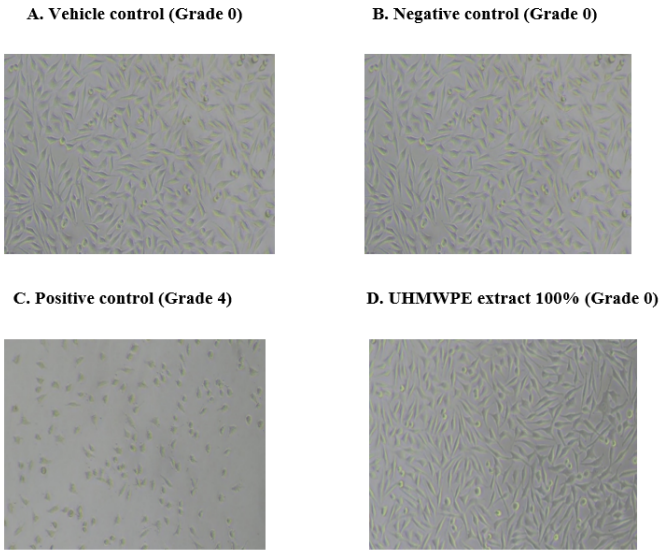

Fig 1. Representative images of Balb/c 3T3 fibroblast cells following 24h of exposure to extract of vehicle control, negative control, positive control and test material. (A) Vehicle control (medium), (B) Negative control (HDPE extract), (C) Positive control (sodium lauryl sulfate), and (D) UHMWPE extract (100%).

Table 2. Quantitative Analysis of cell viability and cytotoxicity in the elution assay

	Negative Control	Vehicle Control	UHMWPE extract concentrations (%)								Positive Control
			30	40	50	60	70	80	90	100	
Mean OD	0.512	0.529	0.507	0.499	0.497	0.495	0.494	0.489	0.482	0.483	0.073
SD (±)	0.007	0.008	0.005	0.006	0.009	0.009	0.007	0.011	0.006	0.004	0.007
Viability (%)	96.79	NA	95.84	94.33	93.95	93.57	93.38	92.44	91.12	91.3	13.8
Cytotoxicity (%)	3.21	NA	4.16	5.67	6.05	6.43	6.62	7.56	8.88	8.7	86.2

concentrations. This establishes a baseline cytocompatibility profile under ISO 10993-5 conditions, highlighting that observed cytotoxic effects in earlier studies are likely due to surface modification or sterilization rather than the material itself. These findings provide critical reference data for regulatory assessment and future development of UHMWPE-based implants.

Previous studies have reported challenges with reproducibility in 3D-printed UHMWPE and a decrease in cell viability when UHMWPE was reinforced with high filler concentrations [17], [18]. These findings emphasize that formulation and processing parameters can significantly influence cytocompatibility. In contrast, our study provides ISO 10993-5-compliant baseline data for unmodified, medical-grade UHMWPE, showing high fibroblast viability across all extract concentrations. This establishes a reliable reference for future modifications or processing variations.

Furthermore, while UHMWPE wear debris has been shown to induce osteolytic responses in macrophage-rich environments [19], and functionalized UHMWPE extracts demonstrated dose-dependent cytotoxicity [9], our results confirm that unmodified UHMWPE extracts do not elicit cytotoxic effects in fibroblasts. This highlights the importance of assessing the baseline material independently from surface modifications or wear products and provides critical data for designing safer UHMWPE-based implants.

In the present work, non-sterilized UHMWPE was evaluated to understand its baseline cytocompatibility. Since sterilization is mandatory for clinical use, future studies should assess whether commonly used sterilization methods (e.g., gamma irradiation, EtO) influence the release of cytotoxic extractables or alter the material's mechanical integrity. Identifying sterilization approaches that minimize oxidative degradation remains a critical step toward ensuring long-term implant safety.

In addition, studies reported improved wound healing with coated UHMWPE, showing that surface modification can enhance or impair biological response depending on the formulation [9]. While promising, such modifications require independent cytotoxicity assessments, as they may introduce variables not present in pure UHMWPE.

Table 3. Comparison with Previously Reported Studies

S. No.	Author(s) & Year	Focus of the study	Key Findings
1	Hasan R et al., 2024	Composites & Mechanical Enhancement	UHMWPE–MMT composites improved mechanical strength and demonstrated good biocompatibility.
2	Gopanna A et al., 2019	Composites & Mechanical Enhancement	Reviewed polyethylene/polypropylene composites for biomedical applications, focusing on reinforcement techniques and potential uses.
3	Hussain M et al., 2020	Reviews & General Applications	Comprehensive review of UHMWPE in biomedical devices, including processing methods, wear mechanisms, and challenges.
4	Singh DK & Verma RK, 2021	Surface Modification & Functionalization	Discussed UHMWPE functionalization for improved mechanical strength and wear resistance.
5	Oleiwi JK & Kadhim TR, 2023	Surface Modification & Functionalization	Showed that surface treatment enhances UHMWPE's biological properties for orthopedic applications.
6	Rodrigues MM et al., 2019	Surface Modification & Functionalization	Plasma-treated UHMWPE surfaces exhibited improved fibroblast adhesion.
7	Verma N et al., 2022	Surface Modification & Functionalization	UV-irradiated UHMWPE demonstrated reduced wear rate and excellent biocompatibility.
8	Nakanishi Y et al., 2025	Surface Modification & Functionalization	Gamma-irradiated vitamin E-stabilized UHMWPE reduced inflammatory cytokine production.
9	Singh DK & Verma RK, 2021	Cytotoxicity & Biocompatibility	Functionalized UHMWPE exhibited concentration-dependent cytotoxicity in vitro.
10	Catauro M et al., 2020	Cytotoxicity & Biocompatibility	UHMWPE nanocomposites displayed improved wear resistance, biocompatibility, and antibacterial activity.
11	Helmus MN et al., 2008	Reviews & General Applications	Emphasized biocompatibility as a critical requirement for next-generation medical devices.
12	Hassanein N et al., 2020	Cytotoxicity & Biocompatibility	Coated UHMWPE materials showed improved in vitro biocompatibility and reduced cytotoxicity.
13	ISO 10993-12:2021	Standards & Guidelines	Standard for sample preparation and reference materials in biological evaluation of medical devices.
14	Oleiwi JK & Kadhim TR, 2023 (duplicate)	Surface Modification & Functionalization	Same as Ref 5 — surface treatment improving biological properties of UHMWPE.
15	Wahed SB et al., 2022	Composites & Mechanical Enhancement	Developed functional UHMWPE composites for ligament reconstruction, suitable for load-bearing applications.
16	Nakanishi Y et al., 2025 (duplicate)	Surface Modification & Functionalization	Same as Ref 8 — vitamin E-stabilized UHMWPE reduced inflammatory cytokine production.
17	Yoo SL et al., 2024	Cytotoxicity & Biocompatibility	In vitro cytotoxicity study confirmed UHMWPE's safety and cell viability for 3D-printed artificial joints.
18	Duraccio D et al., 2019	Composites & Mechanical Enhancement	Alumina–zirconia-loaded UHMWPE showed improved biological and mechanical performance.
19	Gruisen JAE et al., 2025	In Vivo Biological Response	In vivo studies confirmed that UHMWPE wear debris can trigger immune activation and inflammatory responses.

Recent advancements in surface-engineered UHMWPE fibers, show mechanical and thermal improvements through polyimide coatings[12]. However, the cytotoxic impact of such surface treatments was not well explored—further emphasizing the value of baseline data like ours.

Collectively, these comparative findings highlight that the biocompatibility of UHMWPE is highly contingent on its surface chemistry, sterilization history, and formulation. Our study fills a crucial gap by evaluating unmodified UHMWPE under strict ISO 10993-5 conditions, providing foundational safety data that regulatory bodies and manufacturers can rely on.

This evidence supports UHMWPE's continued use in orthopedic implants and other load-bearing applications, and advocates for further cytotoxicity and immunogenicity testing on newer formulations to ensure patient safety.

4 Conclusion

Most earlier studies have focused on modified UHMWPE—such as composites, surface-treated, or functionalized forms—rather than its original material. In this work, the cytotoxicity of unmodified UHMWPE extracts was systematically assessed following ISO 10993-5 and ISO 10993-12 guidelines. Using a GLP-compliant method and testing multiple extract concentrations, this study provides a clear cytotoxicity profile. The observation that cell viability remained above the cytotoxicity threshold supports the biological safety of UHMWPE and reinforces its suitability for long-term implantable medical devices.

The cytotoxicity assessment of ultra-high molecular weight polyethylene (UHMWPE) extract using the Neutral Red Uptake (NRU) elution method demonstrated no adverse effects on cell viability or cellular morphology across all tested concentrations. These findings indicate that, under the specified ISO 10993-12 extraction conditions, UHMWPE does not release cytotoxic leachables and fully complies with the cytocompatibility requirements defined by ISO 10993-5.

Given the consistent non-cytotoxic response observed in Balb/c 3T3 fibroblasts, UHMWPE can be confidently considered safe for use in medical device applications, particularly for orthopedic implants where long-term tissue contact is required.

Direct assays such as cell adhesion and morphology analysis via SEM could provide deeper insight into cell–material interactions. Additionally, chemical characterization of extracts to detect potential leachables would strengthen understanding of UHMWPE's biological behavior. Such studies will help guide safer implant development and inform regulatory assessments.

Future prospects include extending this biocompatibility analysis to more advanced biological endpoints, such as genotoxicity, sensitisation, and in vivo implantation, to establish a comprehensive safety profile. Additionally, similar ISO-compliant studies should be encouraged for modified UHMWPE variants (e.g., cross-linked, antioxidant-infused, or surface-treated) to ensure their safe integration into next-generation biomedical devices.

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6 Contributory statement

Kalaivani conducted experimental work, data analysis, and initial manuscript drafting. **Dr. S.S. Murugan** supervised the study design, interpretation of results, and contributed to manuscript revisions. **Dr. T.S. Kumaravel** provided critical input on the methodology and final review of the manuscript. All authors read and approved the final version of the manuscript.

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