BIOCOMPATIBILITY ASSESSMENT OF PHOTOCATALYTIC NANOPARTICLE-FUNCTIONALIZED POLYACRYLONITRILE NANOFIBERS FOR PROTECTIVE TEXTILES

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Abstract

Polyacrylonitrile (PAN) electrospun nanofibers are promising for use in protective clothing due to their non-toxic character, large surface area, high porosity, and chemical resistance. Incorporation of nanoparticle (NPs) into polymeric nanofibers can provide them with additional functionalities, such as antimicrobial, chemical or radiation protection. In this study, PAN nanofibers were functionalized with various photocatalytic NPs, namely with 15% TiO_2, CeO_2, Er_2O_3, or WO_3, to produce multifunctional hybrid materials. FTIR, SEM, and TGA were used to verify morphology and the presence of NPs within the fibers. Given their potential use in skin-contact textiles (e.g., masks, protective garments), we performed an *in vitro* biocompatibility evaluation. Murine 3T3 embryonic fibroblasts were exposed to 24-hour leachates obtained from nanofibers incubated in cell culture medium, and to controls (PAN and cell culture medium). After 24- and 72-hour incubation, cell viability, morphology, and metabolic activity were assessed by live/dead fluorescence staining, light microscopy, and MTS assay, respectively. All samples maintained > 70% cell viability compared to control cells, indicating the absence of cytotoxicity. Dynamic light scattering analysis did not show release of NPs from fibers into distilled water nor phosphate buffer saline. Altogether, these results demonstrate that hybrid PAN nanofibers with incorporated TiO_2, CeO_2, Er_2O_3, or WO_3 NPs are biocompatible and stable, supporting their potential for use in protective materials designed for direct skin contact.

Keywords: Hybrid Nanofibers, TiO_2, CeO_2, Er_2O_3, WO_3, Cytotoxicity, DLS

1. INTRODUCTION

Protective textiles represent a rapidly growing field of material science, driven by the demand for advanced clothing able to protect against physical, chemical, and biological hazards. Electrospun nanofibers are particularly promising for these applications due to their high surface-to-volume ratio, tunable porosity, and ability to incorporate a wide range of functional additives [1]. Polyacrylonitrile (PAN; $(C_3H_3N)_n$) is a synthetic organic polymer exhibiting various useful properties, such as excellent chemical resistivity, thermal stability, and biocompatibility; furthermore, PAN is not soluble in water, does not soften or melt upon pyrolysis, and is the most resilient polymer to degradation by ultraviolet rays of sunlight. Its accessibility and affordability allow for the large-scale production of nanofibers. PAN fibers are soft and resistant to breaking. Altogether, these properties make PAN nanofibers promising for applications for protective clothing [2].

To further enhance the protective performance of PAN nanofibers, surface functionalization with nanoparticles (NPs) has been extensively investigated [3]. Incorporation of inorganic NPs can provide the fibers with additional protective capabilities. Photocatalytic oxides, such as titanium dioxide (TiO_2), cerium oxide (CeO_2),

erbium oxide (Er_2O_3), and tungsten oxide (WO_3) are of particular interest, as they are known to exhibit light-driven antimicrobial effects [4], radiation protection [5], and self-cleaning properties [6]. Embedding such NPs into nanofibers can result in multifunctional hybrid materials with long-term stability and high efficiency.

In this study, we prepared electrospun PAN nanofibers functionalized with four different photocatalytic NPs (TiO_2, CeO_2, Er_2O_3, WO_3). Given their potential use in skin-contact textiles (e.g., masks, protective garments), we performed *in vitro* biocompatibility evaluation. By combining material characterization with biological testing, this work aims to provide insight into the safety and application potential of hybrid multifunctional PAN nanofibers in protective textiles.

2. METHODS

2.1 Preparation and characterization of the hybrid PAN nanofibers

PAN solutions were prepared by dissolving PAN fine powders (Goodfellow) in dimethylformamide (DMF) at a concentration of 15% (w/v). The mixtures were stirred magnetically at 500 rpm for up to 6 hours until complete dissolution and the formation of homogeneous polymer solutions. NPs (TiO_2, CeO_2, Er_2O_3, or WO_3) were first dispersed in DMF using IKA T-25 digital ULTRA-TURRAX homogenizer and subsequently incorporated into the PAN solution. Hybrid nanofibers were fabricated using an industrial-scale needleless electrospinning system equipped with blade spinnerets and a roll-to-roll deposition unit. The spinning chamber was maintained at 35% relative humidity and 22 °C. Nonwoven polypropylene (40 gsm) substrates were fed into the chamber for nanofiber collection. Electrospinning was performed under optimized parameters, including controlled solution feed rates, shuttle speeds, and applied high voltage, to ensure uniform nanofiber deposition. The composition of the hybrid nanofibers used in this study is summarized in Table 1.

Table 1 Composition of hybrid polymers

Polymer	NPs	Particle diameter (nm)	NP concentration (wt%)	NP supplier
PAN	-	-	-	-
PAN	TiO_2	200	15	Nanografi
PAN	CeO_2	8-28	15	Nanografi
PAN	Er_2O_3	8-90	15	Nanografi
PAN	WO_3	20-60	15	Nanografi

Fourier transform infrared spectroscopy (FT-IR) using a Thermo Scientific™ Nicolet iZ10 spectrometer was employed to determine chemical composition of the hybrid nanofibers. Thermogravimetric analysis (TGA) was performed to evaluate the thermal stability and degradation behavior utilizing a Q500, TA Instruments—Nicolet iS10 (Thermo Scientific) instrument over a temperature range from 25 to 700 °C, with a linear heating rate of 10 °C/min under a nitrogen flow. Morphology of the nanofibers was determined using scanning electron microscopy (SEM, JEOL JSM-IT100; Japan/Zeiss).

2.2 *In vitro* biocompatibility evaluation

Biocompatibility was evaluated in murine 3T3 embryonic fibroblasts as an *in vitro* model for dermal exposure. Samples were cut out as squares with an area equal to 25 wells of a 96-well plate by laser and sterilized with ethylene oxide at 37 °C. Leachates were obtained from nanofiber samples incubated for 24 hours in 6.25 ml complete cell culture medium (Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich), supplemented with 10% fetal bovine serum (Gibco), and 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco) at 37 °C, high humidity, and 10% CO_2. Cell culture medium incubated under the same conditions served as a control.

Cells were seeded at a density of 5,000 cells/well in a 96-well plate 24 hours before the addition of 200 µl of leachates. After 24- and 72-hour incubation, cell morphology, viability, and metabolic activity were assessed.

Cell morphology was observed by light microscopy (LM) using Olympus IX70 with Camera Olympus DP80 with 20× objective. Cell viability was detected using fluorescent live/dead staining. To stain live cells, 10 µM 2',7'-Bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM, Sigma-Aldrich) probe was incubated with cells for 30 min at 37°C in the dark). Propidium iodide (PI, Sigma-Aldrich) at a concentration of 5 µg/mL was added to cells for 10 min at 37°C in the dark to stain DNA of dead cells. Subsequently, samples were rinsed with DMEM and visualized using a fluorescence microscope Olympus IX70 with camera Olympus DP80 with 10× objective. MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay; Promega) measured absorbance from 6 replicates of cells incubated in 20 µL MTS solution and 100 µL of the fresh culture medium at 37°C for 2 hours. Mitochondrial enzymes metabolize the MTS substrate to violet formazan absorbing light at 490 nm. 100 µL of the solution was used to measure absorbance on microplate reader (Infinite M200 PRO, Tecan, Switzerland) at 490 nm with reference wavelength 690 nm. The absorbance of the background well was subtracted from the values of the corresponding group.

2.3 Dynamic light scattering (DLS) measurement

ZetaSizer NanoZS (Malvern) was used to determine the presence of released NPs in the phosphate saline buffer (PBS) and distilled water by DLS measurement. The leachates were prepared as described above; PBS and distilled water were used instead of cell culture medium to minimize background peaks (e.g. protein aggregates). To resuspend potential particle aggregates, the leachates were sonicated before the DLS measurements using 400 W, 20 kHz Branson Sonifier S-450 D (Branson Ultrasonics Corp.) equipped with a 13 mm disruptor horn. The 16 min sonication at 10% amplitude delivered acoustic energy of 7056 J. Ice-water bath prevented heating of the dispersion.

2.4 Statistical analysis

Statistical evaluation was performed using GraphPad Prism 8. Normality was tested by Shapiro-Wilk test, followed by one-way ANOVA with Dunnett's multiple comparisons test to compare the materials to the control. A p-value ≤ 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Characterization of the hybrid nanofibers

SEM images (**Figure 1**) confirmed the nanofiber structure in all samples as well as the presence of NPs within the fiber matrixes. Hybrid nanofibers exhibited thicknesses diameters ranging from 150 to 400 nm. SEM also confirmed uniform distribution of the fibers and absence of non-fibrous regions.

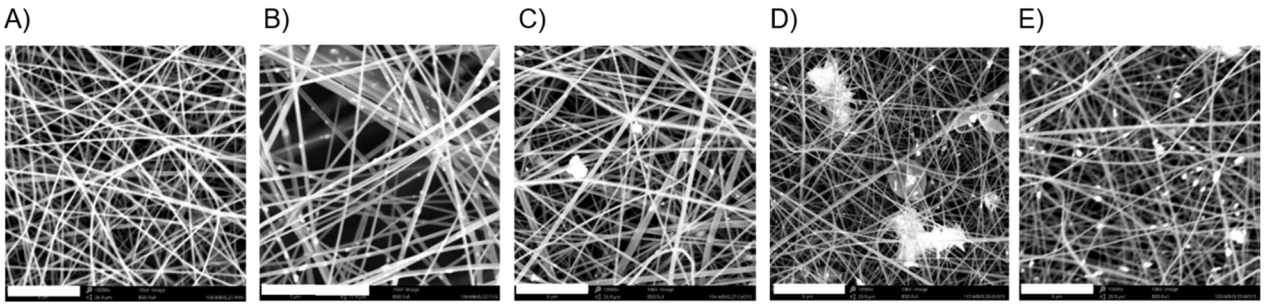

Figure 1 SEM micrograph showing nanofibers with contained spherical structures, indicating the presence of NPs: A) PAN; PAN with 15 % B) TiO_2 NPs; C) CeO_{2_3} NPs; D) Er_2O_3 NPs; E) WO_3 NPs. Scale bar = 8 µm.

The FTIR spectra (**Figure 2A**) exhibited characteristic absorption peaks at 2938 cm^{-1}, 2243 cm^{-1}, 1452 cm^{-1}, 1359 cm^{-1}, and 1253 cm^{-1}, corresponding to C–H stretching, C≡N stretching, and various aliphatic C–H vibrations, respectively. A distinct peak at 1666 cm^{-1} was associated with the C=O group, originating from residual DMF solvent. In the case of PAN hybrid nanofibers, the spectra revealed the typical PAN functional group peaks, along with additional signals at 1111 cm^{-1} and 618 cm^{-1}, which are attributed to NP and oxide vibrations.

The TGA analyses (**Figure 2B**) revealed roughly three distinct stages of degradation in both pristine PAN nanofibers and PAN–NP hybrid nanofibers. In an inert atmosphere, PAN typically exhibits an exothermic reaction between 200 and 350 °C, with the onset of degradation occurring at around 290 °C. This initial stage is associated with cyclization reactions between adjacent C–N groups, which contribute to improved thermal stability. The second stage corresponds to the decomposition of the remaining nanofiber structure. For the nanocomposites, the onset of degradation shifts upward by approximately 10–30 °C, depending on the NP content. Overall, these findings indicate that PAN–NP hybrid fibers decompose at higher temperatures than pure PAN nanofibers, reflecting the stabilizing influence of the NPs.

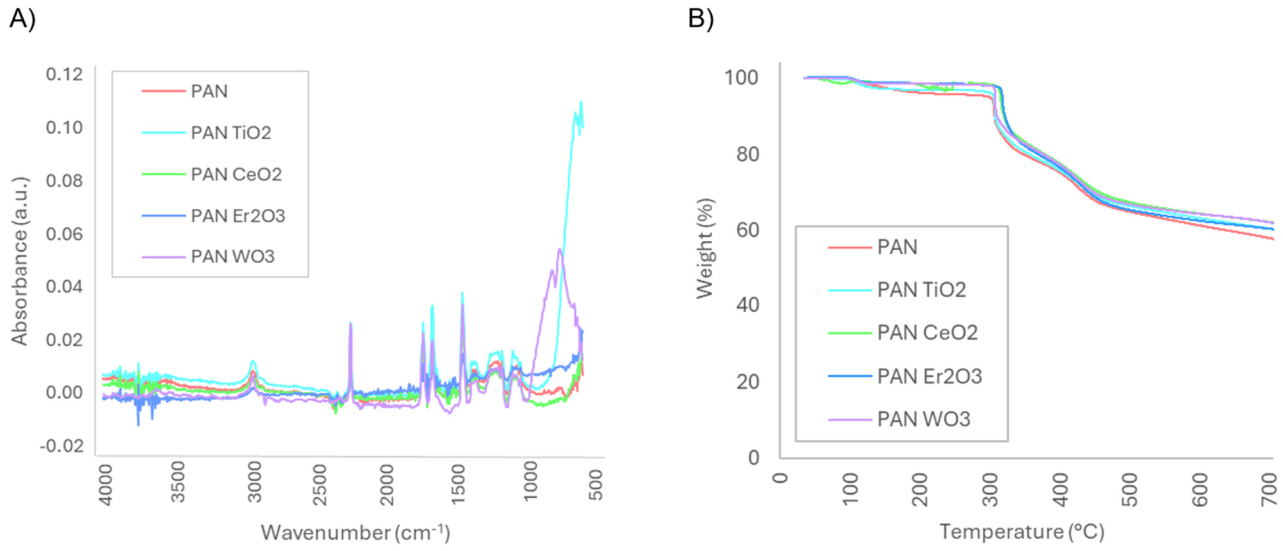

Figure 2 Physico-chemical characterization of the samples: A) Fourier transform infrared spectroscopy spectra, B) Thermogravimetric analysis (TGA) curves.

3.2 Cell viability

Light microcopy observation showed that the morphology of the cells exposed to nanofiber leachates corresponded to that of the control cells (**Figure 3**). No signs of cell detachment or disturbed morphology (such as cell rounding, shrinkage, membrane blebbing, cytoplasmic granularity or vacuolization) were present after 72-hour exposure.

Live/dead fluorescence staining (**Figure 4**) confirmed the healthy morphology and viability of the cells (green BCECF indicating active metabolic enzyme in live cells) and absence of dead or dying cells (red PI staining DNA in cells with disrupted cell membrane).

Viability of the cells was further confirmed by the MTS assay (**Figure 5**) that measures metabolic activity of the cells. Any of the tested samples did not decrease the viability below 70 %, a cytotoxicity threshold according to ISO 10993 [7]. PAN and PAN with TiO_2 NPs slightly, yet significantly ($p < 0.05$) decreased cell viability in comparison to control cells after 24-hour exposure. However, after 72-hour incubation, no significant difference remained, indicating that cell viability or proliferation were not affected by the leachates.

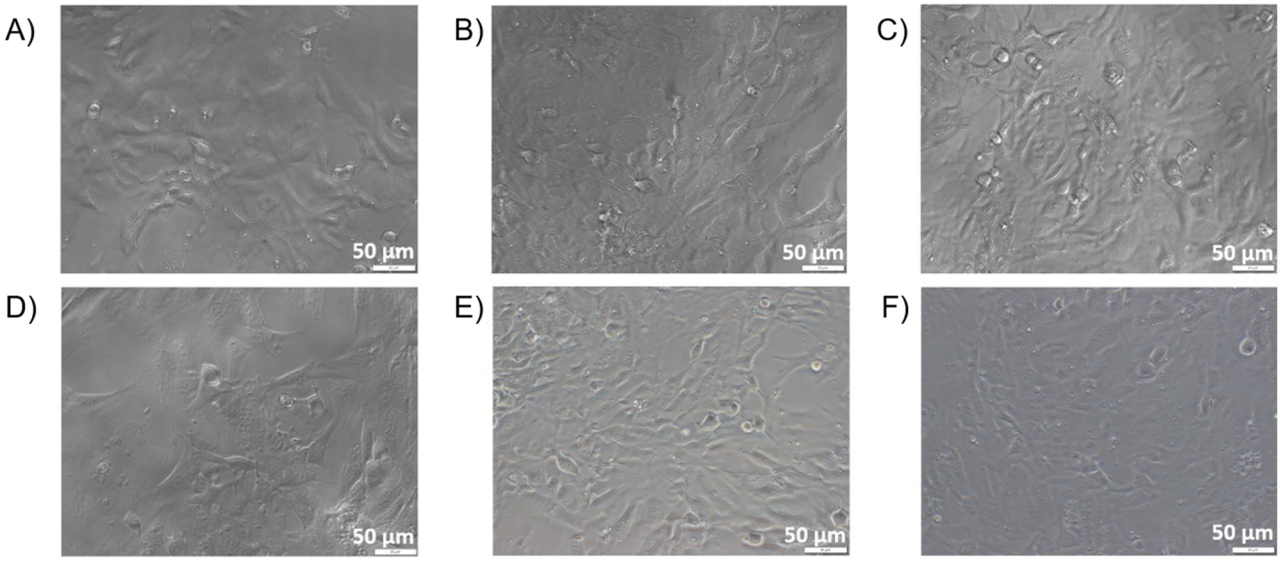

Figure 3 Morphology of 3T3 cells under light-inverted microscope. Cells were incubated for 72 hours with leachates of A) PAN; and PAN with 15 % B) TiO_2 NPs; C) Ce_2O_3 NPs; D) Er_2O_3 NPs; E) WO_3 NPs; and F) cell culture medium as a control. Scale bar = 50 µm.

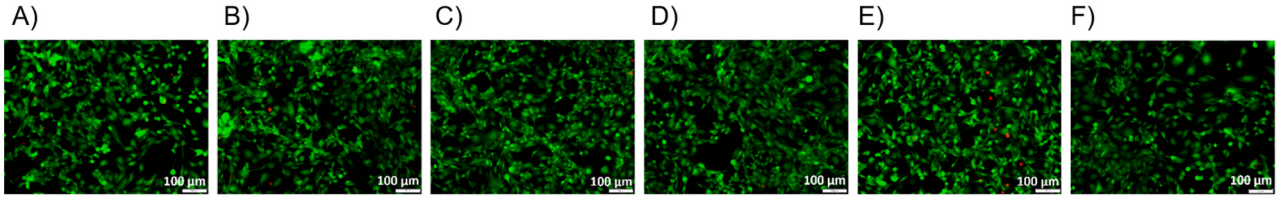

Figure 4 Live/dead staining images of 3T3 cells incubated for 72 hours with leachates of A) PAN; and PAN with 15 % B) TiO_2 NPs; C) CeO_2 NPs; D) Er_2O_3 NPs; E) WO_3 NPs; and F) cell culture medium as a control. Scale bar = 100 µm. Green – live cells stained by BCFEC, red – dead cells stained by Propidium Iodide.

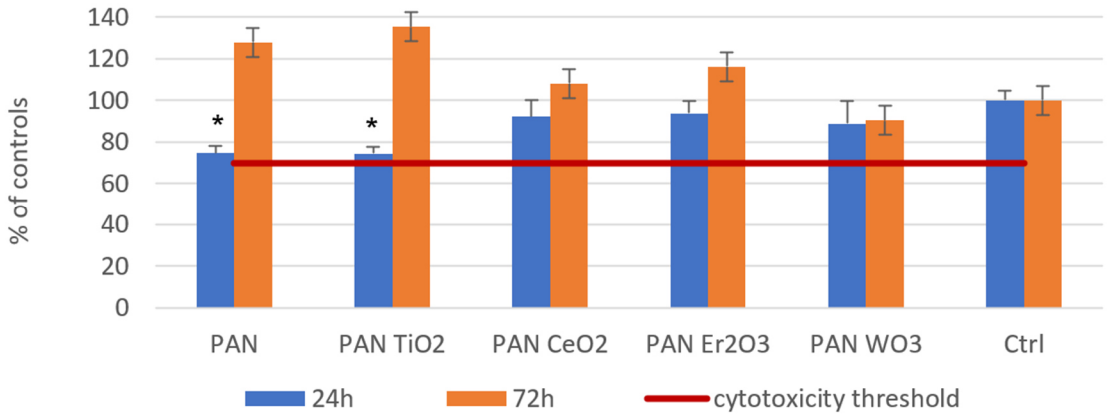

Figure 5 MTS assay results of 3T3 cells incubated for 24 and 72 hours with hybrid nanofibers and controls (cell culture medium). Results are expressed as mean percentage of cell viability ± standard deviation (n=6). Statistical significance is denoted * ($p < 0.05$).

3.3 Detection of the release of NPs by DLS

DLS measurement did not show any specific peak related to the released NPs or their aggregates and agglomerates, as the DLS intensity curves of the leachates corresponded to the background controls (sterilized

PBS and distilled water; data not shown). The lack of specific peaks in DLS indicates that no significant amount of NPs were released into the liquid environment.

4. CONCLUSION

This study demonstrated the successful fabrication of PAN electrospun nanofibers functionalized with various photocatalytic NPs (TiO_2, CeO_2, Er_2O_3, and WO_3). Material characterization (SEM, FTIR, TGA) confirmed the incorporation of NPs within the fibers, uniform nanofiber morphology, and enhanced thermal stability compared to pristine PAN. *In vitro* biocompatibility assays using murine 3T3 fibroblasts revealed no evidence of cytotoxicity. Furthermore, dynamic light scattering analysis showed no release of NPs into aqueous environments, confirming the stability of the hybrid materials.

Altogether, these results demonstrate that hybrid PAN nanofibers with incorporated photocatalytic NPs are biocompatible and stable, supporting their potential for use in protective materials designed for direct skin contact.

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