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Surface properties and in-vitro bioactivity studies of TiO_2 nanowire doped transition metal (M=Fe, Co, and Mn)

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Abstract

This study investigates the influence of transition metal (Fe, Co, Mn) doping on the surface properties and in-vitro bioactivity of TiO_2 nanowires. It aims to elucidate how transition-metal doping alters the surface behavior and biological response of TiO_2 nanowires, enabling their potential use in biocompatible and magnetically responsive materials. Magnetic TiO_2 nanowires doped with transition metals (M^{x+}/TiO_2) were successfully prepared by a hydrothermal method using titanium dioxide in alkaline solution. Cations were added with Ti/M^{x+} molar ratios of 5 to produce Fe/TNW, Co/TNW, and Mn/TNW. Characterization using SEM and XRD determine their surface properties. In-vitro bioactivity tests were conducted by observing the response of C_2C_{12} cells. A cytotoxicity assay determined the effect of TiO_2 Nanowires (5 $mg{\cdot}mL^{-1}$) on C_2C_{12} cell viability at 48 h and 72 h. The results showed that metal-doped TiO_2 nanowires did not significantly affect cell activity, and C_2C_{12} cell differentiation remained. In conclusion, transition metal-doped TiO_2 nanowires do not affect C_2C_{12} cell activity at certain doses. The magnetic properties of doped TiO_2 nanowires open new opportunities for controlled drug delivery using external magnets.

1. Introduction

Titanium dioxide (TiO_2) nanowires have attracted much attention in recent years due to their unique properties that combine semi-conductor and photocatalytic properties with broad potential applications, such as catalysts, sensors, biomaterials, cosmetics, food and solar cells [1]. Meanwhile, its photocatalytic properties trigger chemical reactions on the surface that initiate various applications in self-cleaning, where TiO_2 nanowire can break down organic pollutants under sunlight, ideal for applications such as surface coatings and antibacterials. Titanium dioxide (TiO_2) in the form of nanowire has attracted the attention of scientists and industrialists due to its unique one-dimensional structure as well as its extraordinary properties and potential in various applications [2].

The most important stage in producing the above material is the TiO_2 nanowire synthesis method. This stage is interesting and important to develop to produce materials with the desired properties. Several TiO_2 nanowire synthesis methods that are commonly used and reported by several researchers are the Hydrothermal synthesis method, Sol-gel, Electrochemical Evaporation and Template method [3,4]. The hydrothermal method is considered the most effective due to its simplicity, structural control, and scalabilit [5]. These advantages make it a popular and preferred choice by researchers and industry for TiO_2 nanowire synthesis.

Despite their remarkable properties and promising applications, TiO_2 nanowires face certain limitations that hinder their widespread adoption and performance enhancement. A primary constraint is their restricted photocatalytic activity under visible light irradiation, which significantly limits their potential for harnessing solar energy and driving various photocatalytic processes. Metal doping, particularly with elements like Fe, Co, and Mn, emerges as a promising strategy to address this limitation and unlock the full potential of TiO_2 nanowires [6]. By introducing new energy levels within the bandgap of TiO_2 nanowires, metal doping enhances their ability to absorb visible light and generate more charge carriers, leading to a significant improvement in photocatalytic activity. This enhanced photocatalytic activity expands the applicability of TiO_2 nanowires to various photocatalytic processes, including water purification, pollutant degradation, and hydrogen generation. Beyond enhancing photocatalytic activity, metal doping can impart magnetic properties to TiO_2 nanowires, opening up new opportunities for applications in magnetic separation, targeted drug delivery, and cancer therapy [7,8]. The introduction of magnetic properties enables the manipulation of TiO_2 nanowires using external magnetic fields, facilitating their separation from reaction mixtures and enhancing their targeted delivery to specific sites within the body. Additionally, metal doping can modify the optical properties of TiO_2 nanowires, extending their light absorption range to visible and near-infrared regions. This expanded light absorption range further enhances their photocatalytic efficiency under a broader spectrum of light, making them more versatile and effective for various photocatalytic applications.

Nanoparticles have emerged as indispensable tools in various fields of biotechnology and pharmacology, offering unique properties

and functionalities that have revolutionized research and applications. Among these nanoparticles, titanium dioxide (TiO_2) nanoparticles have gained significant attention due to their exceptional stability, biocompatibility, and diverse applications. Oral and intraperitoneal administration of TiO_2 nanoparticles has demonstrated their distribution to the liver, spleen, kidneys, and adipose tissues of mice. Systemic absorption of TiO_2 nanoparticles leads to elevated zinc levels in the liver, adipose tissue, and pancreas [9,10]. However, the cytotoxicity of these nanoparticles and their interactions with biological systems remain incompletely understood.

To address this gap in knowledge, this study investigates the in vitro effects of TiO_2 nanowires on C_2C_{12} cells. C_2C_{12} cells are mouse myoblast cells derived from C_3H mice and serve as a valuable tool for studying the expression of various proteins and exploring mechanistic pathways. Additionally, they are employed to investigate myoblast and osteoblast differentiation [11]. Previous studies have reported the toxicity of TiO_2 nanoparticles on antioxidant enzyme activity and mRNA expression in cells [12]. However, no investigations have explored the impact of transition metal-doped TiO_2 nanowires on the differentiation properties of C_2C_{12} cells. This research fills this critical gap and provides valuable insights into the cytotoxic effects of TiO_2 nanoparticles on C_2C_{12} cells. To comprehensively elucidate the effects of doping TiO_2 nanowires with different transition metals, this study explores the impact of doping with iron (Fe), cobalt (Co), and manganese (Mn) on the surface structure and biological activity of TiO_2 nanowires. The selection of these transition metals is motivated by their diverse properties, including abundance, magnetic characteristics, corrosion resistance, and catalytic performance. By examining the influence of each dopant, we aim to gain a comprehensive understanding of the structure-property relationships and potential applications of these metal-doped TiO_2 nanowires.

Furthermore, to assess the biocompatibility and potential biomedical applications of these nanowires, we employ in vitro assays using C_2C_{12} stem cells. C_2C_{12} cells are a well-established model system for investigating cellular responses to various materials and treatments. By evaluating the effects of Fe–, Co–, and Mn-doped TiO_2 nanowires on C_2C_{12} cell viability, proliferation, and differentiation, we can gain valuable insights into their potential for regenerative medicine and tissue engineering applications.

In summary, this study delves into the intricate relationship between doping and the surface structure and biological activity of TiO_2 nanowires. By investigating the effects of Fe, Co, and Mn doping on TiO_2 nanowires, we aim to expand our understanding of their structure-property relationships and identify promising candidates for biomedical applications. Additionally, in vitro assays using C_2C_{12} stem cells provide a platform to assess the biocompatibility and potential therapeutic efficacy of these nanowires.

2. Experimental

2.1 Materials

Titanium dioxide (TiO_2) nanowires were synthesized using P25 TiO_2 powder in an alkaline NaOH pellet solution via a hydrothermal method. Transition metal doping was achieved using $FeCl_3$, $MnCl_2$, and $CoCl_2$, followed by washing with ultrapure water to achieve neutral pH.

For cell culture experiments, Dulbecco's Modified Eagle Medium (DMEM), antibiotics, fetal bovine serum (FBS), and trypsin-EDTA were obtained from Gibco BRL, USA. Cell culture flasks and other plasticware used in the study were purchased from Nunc, Denmark. Milli Q water (double distilled, deionized water) was used in all experiments.

2.2 Instrumentation

To comprehensively characterize the synthesized samples, a suite of analytical techniques was employed. Scanning electron microscopy (SEM) was utilized to examine the surface morphology of the materials, providing insights into their microstructure and potential for surface interactions. X-ray diffraction (XRD) analysis was performed to assess the crystallinity of the materials, elucidating their crystal structure and phase composition.

2.3 Procedure

2.3.1 Synthesis of sodium titanate nanowires

Sodium titanate nanowires were prepared using a hydrothermal method as previously reported [13]. Briefly, 0.125 g of TiO_2 nanoparticles were mixed with 10 M alkali solution and placed on a magnetic stirrer for 2 h, followed by ultrasonic sonication for 15 min to form a milky white suspension. The suspension was then transferred to a Teflon-lined autoclave and heated at 240°C for 72 h. The sample was collected after cooling to room temperature and subsequently washed repeatedly with distilled water until neutral pH was achieved to obtain sodium titanate nanowires.

2.3.2 Synthesis of m-doped TiO_2 nanowires

Metal-doped TiO_2 nanowires were prepared by introducing Fe^{3+}, Co^{2+}, or Mn^{2+} salts into the previously obtained sodium titanate nanowire suspension. The metal precursors were added to achieve Ti/M ratios of 2.5:1, 3.3:1, 4.6:1, and 10:1. After the addition of metal ions, the pH of the mixture was maintained at pH 11 to pH 12 due to the NaOH medium. The suspension was stirred for 2 h and subsequently sonicated for 15 min to ensure homogeneity.

The resulting mixture was transferred into a Teflon-lined autoclave and subjected to hydrothermal treatment at 240°C for 72 h, identical to the undoped synthesis conditions. After cooling, the product was washed repeatedly with ultrapure water until reaching neutral pH, yielding Fe/TNW, Co/TNW, and Mn/TNW nanowires.

2.3.3 Cell culture

For proliferation studies, C_2C_{12} myoblasts were seeded at a density of 10,000 cells·cm^{-2}. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies) supplemented with 10% fetal bovine serum (FBS, Life Technologies) and 0.1 mg·mL^{-1} gentamicin (Life Technologies). The medium was changed every two days.

For differentiation studies, C_2C_{12} myoblasts were seeded at a density of 30,000 cells·cm^{-2}. Cell adhesion was allowed for 24 h after seeding, and then myoblast differentiation into myotubes was induced by providing DMEM medium supplemented with 1% FBS, 1% ITS (insulin, transferrin, sodium selenite, and ethanolamine, Sigma), and 0.1 mg·mL^{-1} gentamicin to confluent cells. The medium was changed daily for three days after differentiation induction. In both studies, cells were maintained at 37°C in a humidified atmosphere with 5% CO_2.

3. Results and discussion

3.1 Hydrothermal synthesis TiO_2 nanowire

The hydrothermal method produces TiO_2 nanowires with a controlled structure and uniform morphology. A synthesis method that involves the use of water and high temperatures to dissolve the TiO_2 precursor and crystallize it into nanowires. Among the various TiO_2 nanowire synthesis methods, the hydrothermal method is often referred to as the best choice for several reasons: Simplicity and Convenience, The hydrothermal method is relatively simple and easy to carry out. The process simply involves dissolving the TiO_2 precursor in water and then heating it at high temperature and pressure. This makes it suitable for laboratory and industrial scales. Structure and Morphology Control: The hydrothermal method offers good control over the structure and morphology of TiO_2 nanowires. Parameters such as temperature, pressure, and reaction time can be changed to produce TiO_2 nanowires with the desired size, shape, and crystal structure [13,14]. The hydrothermal method allows the production of TiO_2 nanowires in large quantities at relatively low cost. This makes it an attractive option for industrial applications that require large amounts of TiO_2 nanowire material. TiO_2 nanowires produced by the hydrothermal method are generally of high quality with good crystallinity and minimal defects. This is important to ensure optimal performance in applications that require the specific properties of TiO_2 nanowires. Compared to several other methods, the hydrothermal method is considered environmentally friendly. This process produces little hazardous waste and can be carried out using non-toxic solvents [15]. These advantages make the hydrothermal method a popular and preferred choice for researchers and industry for the synthesis of TiO_2 nanowires.

Doping modification with metals such as Fe, Co, and Mn is a promising strategy to improve their photocatalytic activity. Metal doping can change the electronic, magnetic, and optical properties of TiO_2 nanowire, opening new opportunities for various applications. Here are some reasons why Fe, Co, and Mn doping modifications on TiO_2 nanowire are necessary: Increasing photocatalytic activity: Fe, Co, and Mn doping can increase the photocatalytic activity of TiO_2 nanowire by expanding the light absorption range and extending the lifetime of charge carriers. This can improve the efficiency of TiO_2 nanowire in various applications, such as pollutant decomposition, hydrogen production, and disinfection [16,17]. Adding magnetic properties: Co and Mn doping can provide magnetic properties to TiO_2 nanowire. This opens up opportunities for magnetic applications such as data storage and magnetic sensors. Improving optical properties: Fe, and Co doping can change the optical properties of TiO_2 nanowire, making it more effective at absorbing light over a broader spectrum. Fe– and Co–doped nanowires exhibit weak ferromagnetism, enabling their manipulation under low-intensity magnetic fields (≤50 mT). Although external magnetic stimulation was not applied in this study, prior reports indicate that magnetically responsive nanowires can enhance myotube alignment and migration under cyclic field exposure without inducing cytotoxicity. However, high magnetic field strengths (>150 mT) may generate localized heating and mechanical stress, which could reduce viability. Future work will include magnetically assisted cell culture experiments to quantify this effect. This is useful for applications such as solar cells and optoelectronics. Improved Mechanical Properties: Metal doping can increase the strength and resistance of TiO_2 nanowire to cracking. This is important for applications where TiO_2 nanowire needs to withstand high mechanical stress [18].

3.2 SEM analysis

Nanowire morphology analysis was carried out using a very powerful and detailed Scanning Electron Microscope (SEM) and produced high resolution images of the sample surface. In this research, SEM was used to observe the shape and size of microfibers, especially TiO_2 microfibers and TiO_2 microfibers that have been doped with transition metal ions. The research results show that there are very significant differences in surface morphology after transition metal doping to form Fe/TNW, Co/TNW and Mn/TNW nanowires. This material is obtained by stirring TiO_2 powder in a sodium hydroxide solution and then heating it using the hydrothermal method. The significant difference is very clearly visible in the titanate nanowire, a wire length of 20 μm to 40 μm was obtained (average size 30 μm) and after doping a wire aggregate was obtained measuring 50 μm to 100 μm (average size 50 μm) as seen in Figure 1(a) and Figure 1(c). Quantitative SEM analysis showed that the average nanowire diameter was 85 ± 10 nm for undoped TiO_2 nanowires, increasing slightly to 95 nm to 110 nm after metal doping due to ion incorporation. The corresponding aspect ratios ranged from 250 to 450 for titanate nanowires and 450 to 700 for doped nanowires. The alkaline hydrothermal process turns TiO_2 nanoparticles into long nanowires like palm tree leaves. The presence of cations causes the fibers to be squeezed tightly in the middle with the other sides spread out. The addition of cations in solution produces new forms of Fe/TNW, Co/TNW and Mn/TNW nanowires (Figures 1(b-d)).

The difference in the molar ratio of Ti/M^{x+} causes non-uniform shape and length. The high amount of cation doping results in the formation of more aggregates and causes the wire length to become longer. On the other hand, small amounts of cations may not result in the formation of aggregates resulting in the formation of individual microfibers and longer wire lengths. Doping metal ions (M^{x+}) on TiO_2 is an effective strategy to increase its ability to absorb visible light. M^{x+} doping can create new energy bands in the TiO_2 band gap. This d band comes from the d orbitals of M^{x+} ions and allows TiO_2 to absorb visible light with lower energy than the conduction band energy of TiO_2. Several studies have shown that Mn^{2+} doping can significantly increase the visible light absorption of TiO_2 as reported in previous study [19].

One of the main advantages of TiO_2 nanowires is their large surface area. The nanowire structure significantly increases the surface area compared to bulk TiO_2 powder, thereby allowing greater interaction

with other substances. This makes them very effective as catalysts, speeding up the rate of chemical reactions and increasing the efficiency of various processes.

3.3 XRD analysis

Transition metal doped TiO_2 nanowires displayed an X-ray diffraction (XRD) pattern as shown in Figure 2.

The pattern consists of three characteristic peaks corresponding to the anatase phase of TiO_2 at $2\theta = 25°, 37°$, and $48°$, indexed to the (101), (103), and (004) planes (JCPDS 21-1276) [20]. M^{x+} doping does not significantly change the structure of pure TiO_2. The change is shown a new peak appears at $2\theta=20°$ with strong peak around $2\theta=20.9°$ indicated metallic ion which is the most stable form at room temperature and pressure. This is due to the change in d-spacing as an indication of the change in the distance between the crystal lattice. M^{x+} doping can cause a slight shift in the positions of the original TiO_2 peaks. This shift is due to the change in the lattice parameters of the crystal caused by the incorporation of metal ions. For XRD results, the crystallite size was estimated using the Scherrer equation:

$$D = K\lambda/(\beta\cos\theta)$$

The calculated crystallite sizes were 18.4 nm (TiO_2), 19.1 nm (Fe/TNW), 20.3 nm (Co/TNW), and 21.6 nm (Mn/TNW), indicating slight lattice expansion upon doping.

The peak shift in Figure 2(b) occurs because the Mn ion has a different size and electronic configuration compared to the titanium (Ti) ion. When manganese replaces Ti in the crystal lattice, Mn slightly distorts the arrangement of the surrounding atoms, causing a small shift in peak positions [21]. At higher Mn doping concentrations, the strains and distortions in the lattice can become more significant. In some cases, this can lead to the formation of new crystalline phases, such as MnO_2 or mixed Mn-Ti oxide compounds. These new phases will have their own different atomic arrangements, which is reflected in the appearance of new peaks in the XRD pattern. The shift in the XRD pattern reflects changes that occur in the TiO_2 crystal structure due to the presence of Mn ions [22]. This provides valuable information about the successful incorporation of Mn and other metal ions and their potential impact on material properties.

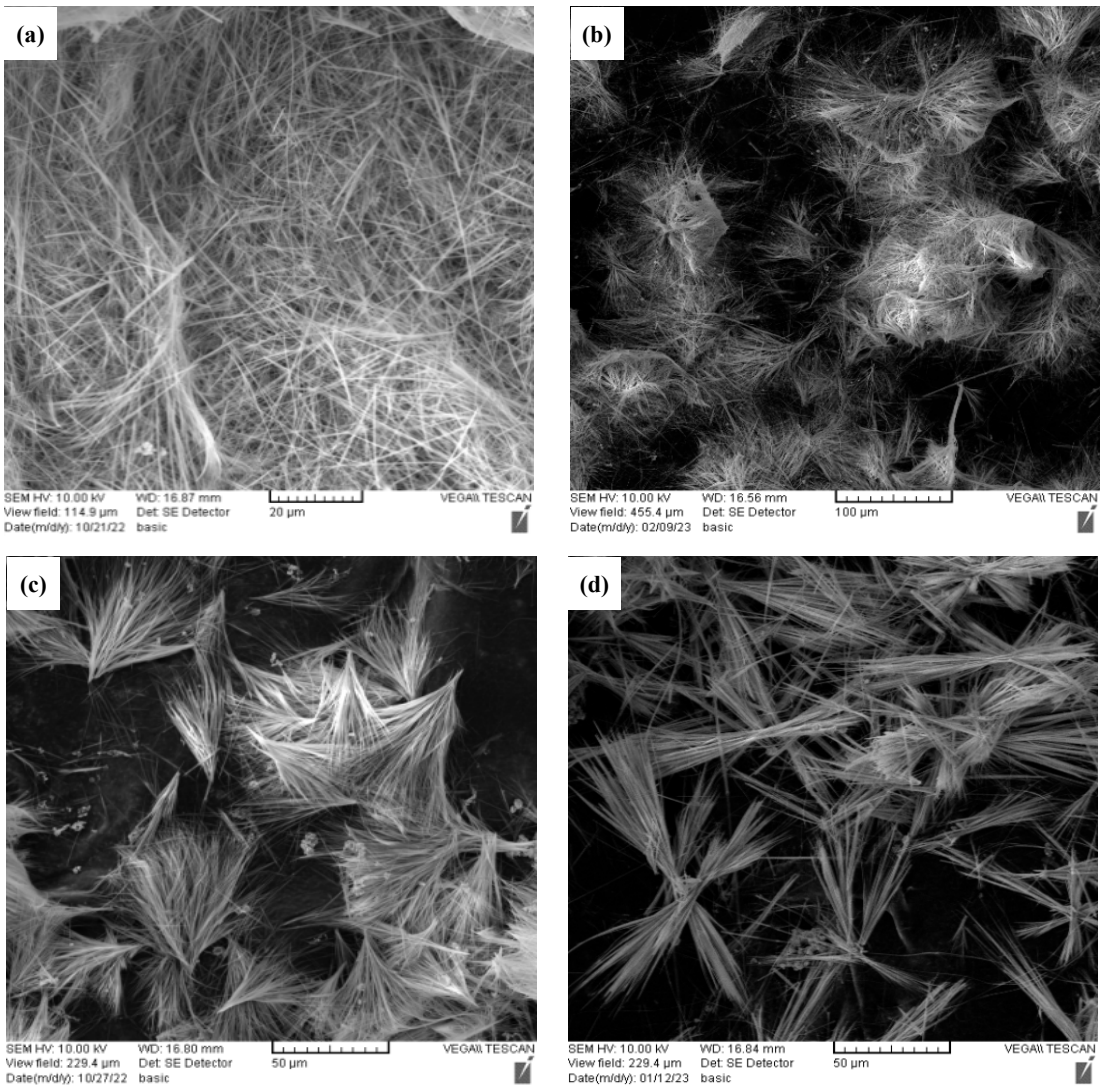

Figure 1. SEM image of (a) titanate nanowire, (b) Fe doped TiO_2NW, (c) Co doped TiO_2NW, and (d) Mn doped TiO_2NW.

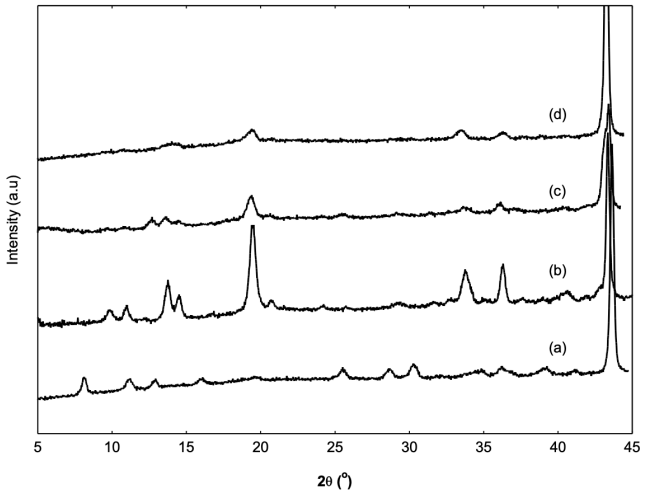

Figure 2. X-Ray diffraction pattern of microwire (a) TiO_2, (b) Fe/TNW, (c) Co/TNW, and (d) Mn/TNW nanowires.

With further research, TiO_2 nanowires doped with Fe, Co, and Mn can become important materials for the development of new technologies in various fields.

3.4 In-vitro bioactivity tests

Here, we investigate the effects of TiO_2 nanowire structures on skeletal muscle cell behavior considering the potential use of such arrangements as interfacial responses for muscle cell growth and stimulation. Titanium dioxide arrays can be easily fabricated with customized features (i.e., different wire diameters, lengths, thicknesses, etc.), and their bulk properties can be easily varied, for example, by thermal treatment and transition metal doping to exhibit enhanced magnetic properties. Additionally, their surface chemistry can be efficiently modified to improve functional properties and biocompatibility. Nevertheless, several studies in the literature show the synergistic effect of the diameter and coating of nanomaterials on the differentiation of Mesenchymal Stem cells (MSCs), for example, enhancing osteogenic differentiation upon nanowire treatment [23]. In this work, this study also proposed the modification of TiO_2 nanowires with transition metal doping to evaluate the potential synergistic effects of different surface nanotopography and surface chemistry on the proliferation and differentiation behavior of C_2C_{12} myoblasts. presence of a nanomaterial in the basal membrane of skeletal muscle that performs an important functional role, titanium dioxide is known to exert pro-adhesive and pro-differentiative effects on skeletal myoblasts as an in vitro bioactivity assay [24,25].

The results of treatment of Fe-doped titanium dioxide nanowires in stem cells support high viability and influence cell behavior. Our C_2C_{12} proliferation results show dependence on the type of nanowire dopant, both in the presence and absence of metformin as the model drug to be delivered. The highest increase in C_2C_{12} cell differentiation was observed in TiO_2 nanowires doped with cobalt ions and followed by manganese ions. Therefore, further research is needed to explain why C_2C_{12} myoblasts are affected by transition metal-doped TiO_2 nanowires. The surface charge (zeta potential) of TiO_2 nanowires is strongly pH-dependent. At physiological pH (7.2 to 7.4), Fe–, Co–, and Mn-doped nanowires exhibit a moderately negative surface charge (−18 mV to −28 mV), which promotes electrostatic attraction with positively charged regions of the cell membrane. This enhanced adhesion correlates with the increased differentiation efficiency observed in doped samples. At lower pH values (5 to 6), closer to the isoelectric point, the reduced magnitude of surface charge may weaken cell adhesion and reduce viability.

These images show that cell differentiation remains viable even in the presence of nanomaterials. However, on the other hand, TiO_2 nanomaterials can cause toxic effects on C_2C_{12} cells, potentially inducing oxidative stress, DNA damage, or apoptosis [26]. Signs of toxicity may manifest as morphological changes, decreased cell viability, or increased expression of stress proteins in response to a foreign body. Although TiO_2 is generally considered safe, it can exhibit cytotoxicity under certain conditions.

Investigation of the type of TiO_2, particle size, concentration, and duration of treatment is very important to determine the toxicity of TiO_2. Further investigations should also be carried out with different cell types and culture conditions to obtain a definitive indication of the potential of transition metal-doped TiO_2 nanowires for tissue engineering purposes.

As a preliminary study, our results indicate that Mn ion-doped TiO_2 nanowire structures are the most promising and may represent useful interfacial properties for interactions with skeletal muscle cells. The highest C_2C_{12} differentiation was observed in Co-doped TiO_2 nanowires, followed by Mn-doped samples, indicating a dopant-dependent enhancement in myogenic behavio.

3.5 Impact of nanomaterials on C_2C_{12} cell differentiation

Using the C_2C_{12} myoblast model, nanomaterials particularly nanostructures based on titanium dioxide (TiO_2) have been extensively investigated for their effects on skeletal muscle differentiation [27,28]. The physicochemical characteristics of TiO_2 nanowires, including surface charge, crystallinity, and the ability to generate reactive oxygen species (ROS), can be tailored by surface modifications such as transition metal doping (Fe, Co, or Mn) [27,29]. These dopant-induced modifications can influence cell–surface interactions, thereby altering adhesion and intracellular signal transduction pathways that are essential for myogenesis [30,31]. Nevertheless, research shows that moderate doping concentrations have no discernible effect on cell viability or the capacity of C_2C_{12} cells to differentiate into mature myotubes, despite these compositional differences. This suggests that structural or chemical changes largely affect the rate of differentiation rather than its outcome (Yin *et al.,*The physicochemical characteristics of TiO_2 nanowires, such as surface charge, crystallinity, and the potential for the generation of reactive oxygen species (ROS), can be changed by surface modifications like metal doping (Fe, Co, or Mn) [27]. Li *et al.* (2020) claim that by altering cell–surface interactions, these surface modifications can change adhesion and intracellular signal transduction pathways that are essential for myogenesis. Integrin-linked signaling cascades and the activation of the MAPK/ERK and PI3K/Akt pathways, which are essential for C_2C_{12} differentiation, are the primary mechanisms underlying these effects [30].

The morphology of C_2C_{12} cells derived from Muscle-Derived Stem Cells (MDSCs) in the images provides valuable insight into their differentiation status, morphology, and viability. C_2C_{12} cells

are myoblast cells originating from mouse embryos and are widely used in bio-logical research to study the differentiation of myoblasts into skeletal muscle cells [32]. Muscle Derived Stem Cells (MSCs) are a type of adult stem cell that can differentiate into various types of muscle cells, including skeletal muscle cells.

The morphology of C_2C_{12} cells from MSCs in Figure 3(a) depicts undifferentiated cells, showing a fusiform shape and random arrangement. Figure 3(b), Figure 2(c-d) show cells that differentiate into skeletal muscle cells from MSCs. These cells display an elongated shape with multiple nuclei, and their arrangement is consistent with the typical myotube pattern.

Increased cell proliferation and differentiation were observed on TiO_2 nanowires not only at 24 h but also at 72 h, indicating that the initial cell behavior was accelerated and developed. Increased cell differentiation was associated with increased vinculin expression, providing molecular evidence explaining how muscle cell interfacial interactions on the TiO_2 nanowire surface are increased [25]. Vinculin is a cellular protein like a bridge that connects the internal scaffolding (cytoskeleton) to structures on the cell surface (adhesion receptors) and helps manage the mechanical forces between them[33].

Not only cell-based vinculin expression but also cell-based area increased, indicating that the increased expression was not due to cell enlargement but due to substantial down-regulation of protein expression. Vinculin is involved in the connection between cell adhesion membrane molecules, integrins, and actin filaments, and plays an important role in initiating and establishing cell attachment, adhesion, cell shape formation, and cytoskeletal development. Actin, a versatile protein found in cells, is like tiny building blocks that come together to form microscopic fibers. It plays a crucial role in various cellular activities, from movement to transporting materials within the cell [34]. In addition, the number of cells showed higher on the surface treated with TiO_2. This suggests that cellular adhesion and retention are enhanced on the substrate, which is assumed to be due to the collective effect of increased vinculin and actin expression. Cell functional phenotypes, such as collagen production, also increased significantly on TiO_2 treated surfaces. The factors mentioned above partly explain the effect of TiO_2 nanowire treatment on the differentiation process of C_2C_{12} cells.

Several key parameters are used to quantitatively evaluate the biological impact of such nanomaterials: Fusion index measurements quantify the efficiency of myotube formation and are widely used as a benchmark for myogenic differentiation efficiency [31,35]. Myogenic gene expression, particularly MyoD and Myogenin, is evaluated using the $\Delta\Delta C_t$ method in RT-qPCR to assess transcriptional regulation during muscle cell maturation [36,37]. Cell viability assays, such as MTT or WST-1, are employed to determine the cytocompatibility of nanomaterials and to confirm that cell proliferation and metabolism remain within a physiological range [38,39].

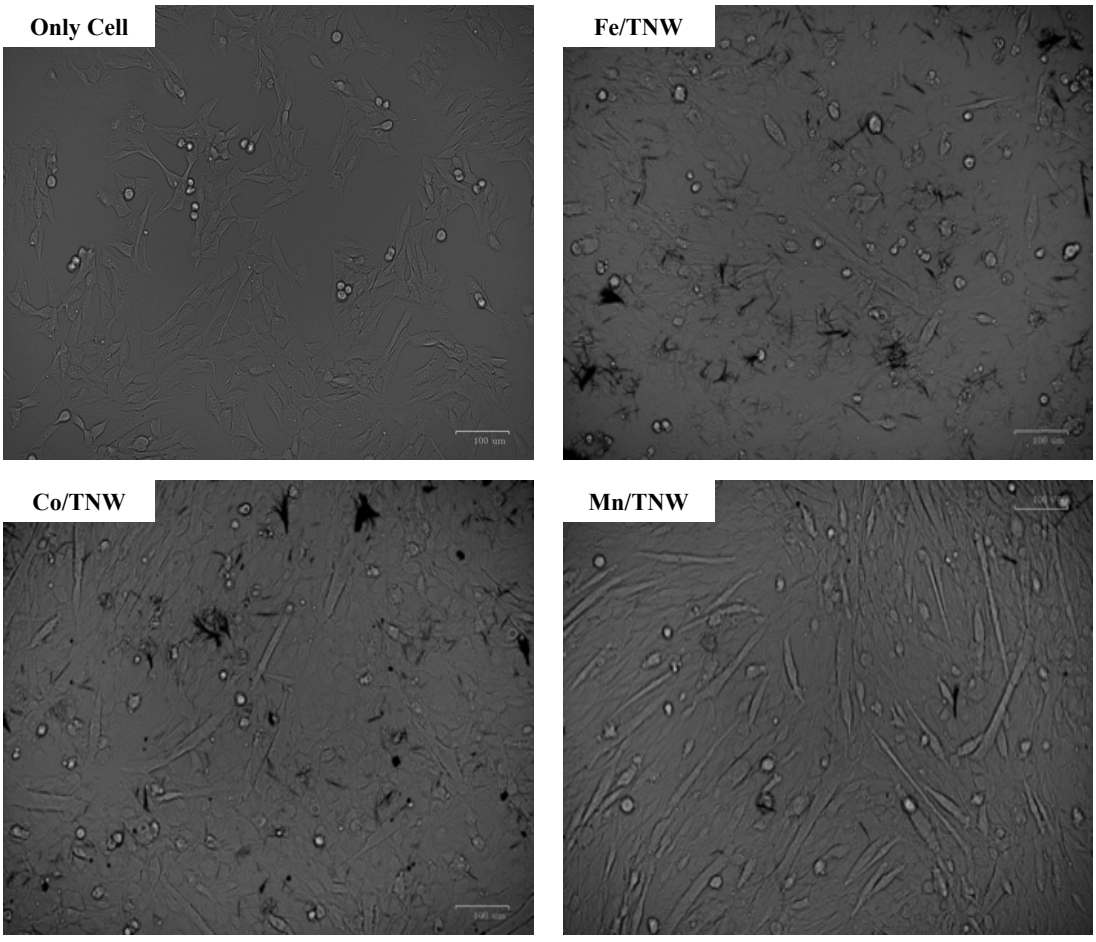

Figure 3. C2C12 cell morphology from Muscle derived stem cells (MDSCs) after differentiation with nanomaterial treatment a) only cell, b) cell with Fe/TNW, c) cell with Co/TNW, and d) Mn/TNW.

A number of analytical formulas were used to provide a more lucid and quantifiable interpretation of differentiation outcomes in order to further quantify the biological response of C_2C_{12} cells [36]. The relative gene expression levels of MyoD and Myogenin, two important transcription factors controlling myogenic differentiation, were determined using the $\Delta\Delta C_t$ (Delta Delta Ct) method [36]. Data reliability is ensured by normalizing gene expression against a house-keeping gene, like GAPDH, using the equation $\Delta\Delta C_t = (C_t,\text{target} - C_t, \text{reference})$ sample $- (C_t, \text{target} - C_t, \text{reference})$ control [37].

The degree of myotube formation, which reflects the effectiveness of myoblast fusion during differentiation, was assessed using the Fusion Index (FI), which is defined as FI = (Number of nuclei in myotubes/Total number of nuclei) × 100% [31,35].

In the meantime, the percentage of viable cells relative to controls was used to measure cell viability, frequently using the formula Viability (%) = (Absorbance_sample/Absorbance_control) × 100%, which provides an indication of the cytotoxic effect of nanomaterial exposure [38,39].

Beyond the tested dimensions (20 μm to 40 μm for titanate nanowires and 50 μm to 100 μm for doped nanowire bundles), previous studies show that excessively long nanowires (>150 μm) may limit cell spreading due to increased rigidity, whereas very short nanowires (<5 μm) reduce topographical guidance cues. Similarly, nanowire diameters above 200 nm can reduce C_2C_{12} differentiation efficiency by limiting focal adhesion maturation, while ultrathin nanowires (<50 nm) promote excessive membrane wrapping and reduce proliferation. Thus, an optimal balance between diameter (80 nm to 120 nm) and length (20 μm to 80 μm) is important for promoting myogenic differentiation.

4. Conclusions

The interaction of skeletal muscle cells with transition metal-doped titanium dioxide nanowires was investigated. Enhanced cell adhesion and differentiation were observed on Mn-ion-doped structures with distinctive extracellular matrix. These findings provide promising evidence of the strong interplay between material nanotopography and surface chemistry in influencing cell behavior. Further research is clearly warranted to further elucidate the differentiation of skeletal muscle cells on titanate nanowire array structures, as well as the functional behavior in light of the material's magnetic properties.

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