

Article

Immune Gene Expression Modulation and In Vitro Inhibitory Effect of TiO₂ Nanoparticles Under UV Irradiation on Viral Necrosis Virus

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Abstract

This study examines the potential in vitro application of different concentrations of titanium dioxide (TiO₂) nanoparticles (NPs) irradiated with UV light for the sanitation of recirculating aquaculture systems (RASs) and their antiviral activity. The diverse effects of Nodavirus on immune gene expression (i.e., pro-inflammatory and anti-inflammatory genes, cellular response genes, humoral response genes, and stress genes) were studied using RT-qPCR (Reverse Transcription Quantitative Polymerase Chain Reaction). In addition, the viability and cytopathic effect in E-11 fish cells were also investigated. The results obtained did not show a clear cytopathic effect under the reversed-phase microscope observation at different TiO₂ concentrations. A significant decrease in viral coat protein gene expression was observed when using 2.5 and 1.25 g/L TiO₂ suspensions under UV irradiation. TiO₂ at 1.25 g/L induced an inflammatory response to Nodavirus by increasing the expression of all target genes. Thus, this work suggests that TiO₂ NPs can strengthen the immune system of fish to fight virus infection and make aquaculture a greener and more sustainable activity.

Keywords: TiO₂ nanoparticles; antiviral activity; UV light; cell viability; immune system; recirculating aquaculture system (RAS)



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1. Introduction

Many fish farms use open systems, where water is taken from a natural pond and used in tanks before being returned to the main body of water. A large amount of water is consumed each day, and inorganic substances and organic nutrients are released, polluting the aquatic environment and affecting the fish health status [1,2].

In recent years, photocatalysts have been widely studied in water treatment [3], including in recirculating aquaculture systems (RASs) for the off-flavors [4] or ammonia

removal [5–8], with positive effects on water quality in terms of decreased ammonia and nitrogen compounds concentrations and increased animal health.

The photocatalytic treatment offers great potential as an industrial technology for the detoxification or remediation of wastewater due to several factors: the process uses natural oxygen and light, possibly also sunlight; thus, it is effective under ambient conditions. In recent years, novel insights into the photocatalytic properties of TiO_2 and related nanomaterials have expanded their potential in biomedical and environmental applications, with several studies reporting enhanced antiviral and antimicrobial activities under optimized conditions [9,10].

Among various photocatalyst nanomaterials being used as antibacterial or antiviral materials, titanium dioxide (TiO_2) is one of the best photocatalysts; it is well known, low-cost, readily available, non-toxic, and chemically and mechanically stable and has a high turnover [11–18]. Moreover, it can be prepared in different forms for different applications [19–22]. Under UV irradiation reactive oxygen species, namely hydroxyl radicals ($\bullet\text{OH}^-$) and superoxide anions ($\bullet\text{O}_2^-$), are produced on TiO_2 [19].

The industrial research on TiO_2 photocatalysis is mainly aimed at using its oxidation properties for sterilization, sanitation, and anti-pollution applications in the aqueous phase [23]. Nowadays, the photocatalytic destruction of organic matter is also exploited in photocatalytic antimicrobial coatings, typically thin films applied to furniture in hospitals and other surfaces susceptible to contamination with microorganisms [24].

In the case of viruses, these reactive radicals can degrade the capsid and envelope proteins and phospholipids of non-enveloped and enveloped viruses, respectively, leading to leakage and subsequent degradation of genetic information [17,25–27].

For instance, silver nanoparticles (AgNPs) inhibit hepatitis B virus (HBV), herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus 1 (HIV-1) [28,29] in vertebrates, as well as WSSV, *Vibrio cholerae*, *V. harveyi*, and *V. parahaemolyticus* infection in shrimp [30–33]. Gold nanoparticles (AuNPs) reduce the replication of foot-and-mouth disease virus (FMDV) in cloven-hoofed animals [34] and *V. parahaemolyticus* as shrimp infection [35]. Moreover, titanium dioxide can deactivate rotavirus, astrovirus, and feline calicivirus (FCV) [34]. In aquaculture, numerous viral organisms affect a significant portion of global fish production, resulting in significant economic impacts on aquaculture [36]. In particular, RNA viruses cause the most severe fish diseases, such as nervous necrosis virus, which is responsible for encephalopathy and retinopathy (VER) [37].

Fish nodavirus is a non-enveloped icosahedral virion containing two positive-sense single-stranded genomic RNAs and is classified as a betanodavirus of the Nodaviridae. Preventing VNN in Mediterranean aquaculture requires a comprehensive approach that combines measures aimed at reducing the introduction of betanodaviruses into production systems with strategies that strengthen fish resilience against infection and its consequences. In this framework, TiO_2 nanoparticles emerge as a promising tool for marine hatcheries, since under UV activation they generate reactive oxygen species capable of disrupting viral capsid proteins and RNA, resulting in effective viral inactivation. Their application in water treatment systems could significantly reduce viral contamination in rearing tanks, limit the spread of nodaviruses, and ultimately protect the most vulnerable early life stages of cultured fish.

In this study, the effect of TiO_2 , at different concentrations, was evaluated on Nodavirus in the dark and under UV irradiation to determine TiO_2 mechanisms of virus inactivation and of the cytopathic effect on fish cells, and immune system interactions of TiO_2 NPs at different concentrations, activated by UV irradiation.

2. Materials and Methods

2.1. TiO₂ Powder Characterization

Commercial Aeroxide TiO₂ P25 powder was purchased from Evonik (Essen, Germany) and used as received. Scanning electron microscopy (SEM) images for morphology investigation were acquired with a Zeiss (Oberkochen, Germany) SIGMA field emission instrument. The X-ray diffraction (XRD) pattern for crystal phase determination was recorded on a Philips (Amsterdam, Netherlands) PW3020 powder diffractometer, using the Cu K α radiation ($\lambda = 1.54056 \text{ \AA}$). The Rietveld refinement method was performed for quantitative phase analysis, using the “Quanto” software. The average crystallite size was calculated by means of the Scherrer equation applied to the main reflections centered at $2\theta = 25.425^\circ$ for anatase and $2\theta = 27.575^\circ$ for rutile. The BET specific surface area (SSA) and BJH mesopore volume and size were measured by N₂ adsorption/desorption at liquid nitrogen temperature (77 K) in a Micromeritics (Nocross, GA, USA) ASAP 2020 apparatus, after out-gassing the sample at 300 °C in vacuum for 1 h. The diffuse reflectance (R) spectrum was measured on a Jasco (Halifax, NS, Canada) V-670 spectrophotometer equipped with a PIN-757 integrating sphere, using barium sulfate as a reference, and then converted into a Taucplot, $(KM \text{ } h\nu)^{1/2}$ vs. $h\nu$, where KM is the Kubelka–Munk transform of the reflectance R, with $KM = (1 - R)^2/2R$, for bandgap energy determination.

2.2. Preparation of TiO₂ Suspensions

The P25 powder was dispersed in aqueous suspensions as follows: a suspension containing 5 g/L of TiO₂ was prepared first, which was diluted with distilled water, to obtain suspensions containing 2.5, 2.0, 1.25, 0.75, and 0.3 mg/mL of TiO₂.

2.3. Cells and Virus

The cell line (E-11) derived from the SSN-1 line of striped snakehead fish *Ophiocephalus striatus* [38] was maintained in L15 medium (Leibovitz L-15, Eurobio, Milan, Italy) supplemented with 10% fetal calf serum (Gibco) and 10% antibiotic (10,000 units/mL penicillin and 10,000 g/mL streptomycin, Gibco, Life Technologies, Waltham, MA, USA). The E-11 cells used in this study were kindly provided by Dr. Anna Toffan, head of the WOAHP Reference Laboratory for Viral Encephalopathy and Retinopathy (IZSVE, Legnaro of Padua, Italy). Betanodavirus isolate (PG105/D19) was obtained from clinically infected specimens of *Dicentrarchus labrax* from the isolate collection of the Virology Laboratory of the National Institute of Marine Sciences and Technologies (INSTM), Tunisia.

2.4. Investigation of TiO₂ Cytotoxic Properties on the E-11 Cell Line

The MTT assay was performed to measure the cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes that reduce MTT to formazan. The insoluble formazan crystals are dissolved with a solubilizing solution, and the resulting-colored solution is analyzed by measuring the absorbance at 500–600 nm with a multiwell spectrophotometer. In this study, the protocol reported in ref. [39] was adopted with a small change in the incubation temperature (25 °C instead of 37 °C). The absorbance was measured at 590 nm. The viability threshold (%) was calculated as follows: Viability threshold (%) = (absorbance of different TiO₂ suspensions / absorbance of the control) $\times$ 100.

2.5. TiO₂ Inhibitory Properties on Nodaviruses Replication

An equal volume (50 µL) of Nodavirus solution and TiO₂ suspension was mixed and used for the described treatments. Six TiO₂ concentrations were tested (5.0, 2.5, 2.0, 1.25, 0.75, and 0.3 mg/mL). The UV irradiation was performed by exposing VNNV-TiO₂ mix Eppendorf tubes, at a distance of 20 cm, to a clamped UV- source (15 W) with a peak at 253.7 nm. The irradiated mixtures were retrieved at 5 different time intervals (5 min, 15 min, 30 min, 1 h, and 16 h) and then inoculated on E-11 cells. The mock and positive controls were always added.

A total volume of the irradiated mixtures (100 µL) was withdrawn at 5 different time intervals. Simultaneously, the same assay was performed in the dark by mixing an equal volume of Nodavirus and TiO₂ (5 mg/mL) and incubating for 1 h at 25 °C prior to cell inoculation. The negative and positive controls were always added. Five 96-well plates with confluent E-11 cells (7×10^4 cells/well) were freshly prepared 24 h before the start of the experiment. Each plate corresponds to a sample interval. The assay was created in duplicate.

2.6. RNA Extraction and Real-Time qRT-PCR Analysis

Total RNA was isolated from cell supernatants using the GF-1 kit (VIVANTIS) according to the manufacturer's instructions. Quantitative reverse transcription qRT-PCR was performed using Luna One-Step Universal Probe RT-qPCR kit (New England Biolabs, Ipswich, MA, USA) and with gene-specific primers related to Nodavirus and various immune genes, such as antiviral protein genes (IFN-I, Mx); pro-inflammatory genes (IL-1β, IL-8); anti-inflammatory genes (TNF-α, TGF-β); genes involved in the cellular response (CD4, CMH1-β); genes involved in the humoral response (IgM); and a stress gene (Hsp30) (Table 1). The reaction was processed in the 7500 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) from ThermoFisher (Waltham, MA, USA). The β-actin gene was selected as the housekeeping gene. The ΔΔCT method was used to calculate relative gene expression [40]. Validation of amplification efficiency was performed for each gene to use the amplification protocols in the current study.

Table 1. Primers used for relative quantification RT-PCR using the ΔΔCT method with β-actin as the housekeeping gene.

Gene	Forward	Reverse	Reference
Nodavirus	CAACTGACARCGAHCACAC	CCCACCAYTTGGCVAC	[41]
β-actin	GCC TTC CTT CCT TGG TAT GG	GTG TTG GCG TAC AGG TCC TT	[42]
IFN-I	GGCTCTACTGGATACGATGG	CT GCGTCCAAAGCATCAGCT	[43]
Mx	ATTCTGAGTTCTTGCTGAAGG	CCTCTAGAACTCCACCAGG	[44]
IL-1β	CAGGACTCCGGTTTGAACAT	GTCCATTCAAAGGGGACAA	[44]
IL-8	GTGCTCCTGGCGTTC	CTTCACCCAGGGAGC	[45]
TNF-α	AGA CAA GGT GGA GTG GAA GA	CCT GGC TGT AGA CGA AGT AGA	[46]
TGF-β	GACCTGGGATGGAAGTGG	CAGCTGCTCCACCTTGTG	[43]
IgM	GAGCTGCAGAAGGACAGTG	TCAGACTGGCCTCACAGCT	[47]
CD4	GTGATAACGCTGAAGATCGAGCC	GAGGTGTGTCATCTTCCGTTG	[43]
CMH1-β	CAGAGACGGACAGGAAG	CAAGATCAGACCCAGGA	[48]
Hsp30	CAG GTG GGC AGG AAG CTG	ACC CCT TCA GGC AGA TCA AAC TC	[46]

2.7. Statistical Analysis

All experiments were performed at least in duplicate, and results are presented as mean ± SD. Differences in the response between infected and mock groups were represented as means ± SD. Data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences Software (SPSS) version 20.0 (SPSS Inc., Chicago, IL,

USA), and when statistically significant differences were observed ($P < 0.05$), a comparison of mean test was applied.

3. Results

3.1. TiO_2 Powder Characterization

The SEM micrograph (Figure 1a) reveals that commercial P25 TiO_2 consists of micro-aggregates of irregularly shaped, ca. 10 to 50 nm, NPs. According to the Rietveld refinement of the XRD pattern (Figure 1b), it is composed of 82% anatase and 18% rutile with no trace of brookite. The Scherrer average crystallite size is 21 nm for anatase and 26 nm for rutile. The N_2 adsorption/desorption isotherm at 77 K (Figure 1c) shows a relatively narrow H1-type hysteresis loop typical of mesoporous materials with a BJH desorption cumulative volume of pores of $0.357 \text{ cm}^3/\text{g}$ and average pore width of $170 \text{ \AA}$.

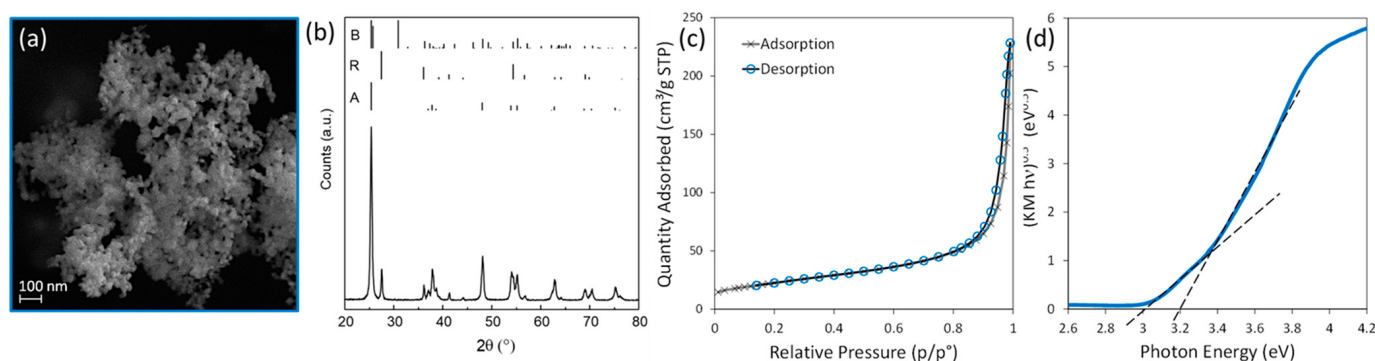


Figure 1. TiO_2 P25 powder characterization: (a) SEM image; (b) XRD diffractogram (the reflection positions and relative intensities of Anatase (A), Brookite (B), and Rutile (R) phases are reported at the top, for comparison); (c) N_2 adsorption/desorption isotherm at 77 K; and (d) Tauc plot of the diffuse reflectance spectrum.

The measured BET specific surface area (SSA_{BET}) is $48 \text{ m}^2/\text{g}$. If a spherical shape of the particles were assumed, the average particle diameter D_m can be calculated from the SSA as follows:

$$D_m = 6000 / (\text{SSA}_{\text{BET}} \times \rho_{\text{TiO}_2}), \quad (1)$$

with SSA_{BET} in m^2/g , ρ_{TiO_2} , the TiO_2 density, in g/cm^3 and D_m in nm. If $\rho_{\text{TiO}_2} = 4 \text{ g}/\text{cm}^3$ is used (the mean value between the densities of anatase and rutile), then $D_m = 30 \text{ nm}$, in line with the SEM micrograph and the Scherrer average crystallite size. Finally, the Tauc plot of the diffuse reflectance spectrum (Figure 1d) clearly shows the presence of two slopes. The extrapolation of these two linear regions to the abscissa yields an energy of the optical bandgap of 3.0 eV, characteristic of the rutile phase, and of 3.2 eV, typical of the anatase phase.

3.2. Investigation of TiO_2 Cytotoxic Properties on the E-11 Cell Line

Based on the MTT measurements, the wells treated with TiO_2 (5 mg/mL) showed a higher viability threshold compared to those treated with the other TiO_2 concentrations (Figure 2). In fact, TiO_2 concentrations ranging from 2.5 to 0.3 mg/mL significantly reduced the viability of E-11 cells.

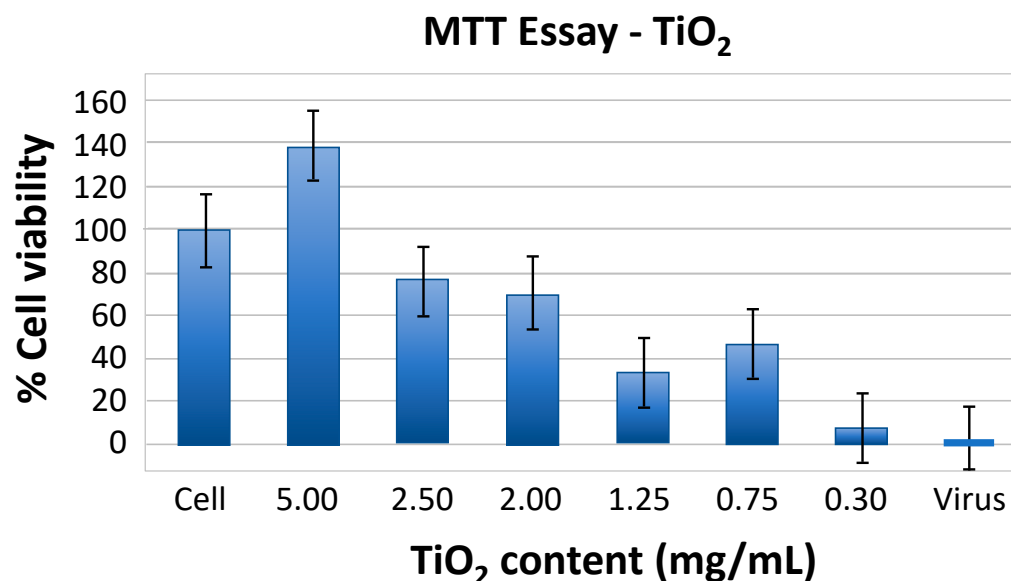
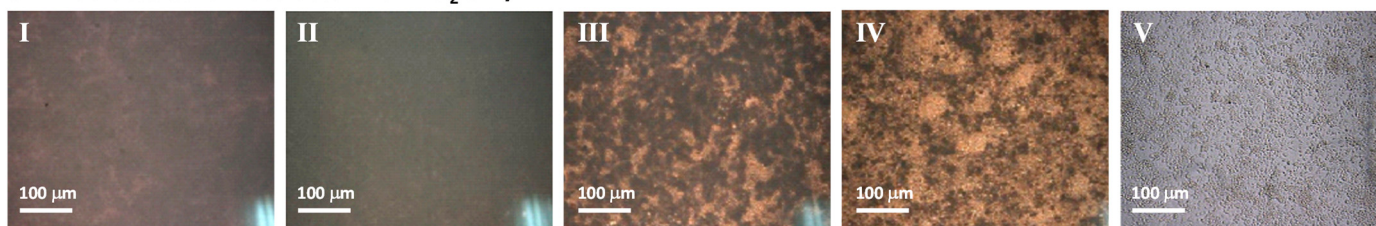


Figure 2. Determination of cell viability (%) for different concentrations of TiO₂.

3.3. *In Vitro Investigation of the Inhibitory Properties of TiO₂ on Nodavirus Replication*

Daily observations were performed to check the cellular cytopathic effect (CPE) after different treatment conditions (see Figure 3).

UV Irradiated cells inoculated with TiO₂ only



UV Irradiated cells co-infected with TiO₂ and VNNV

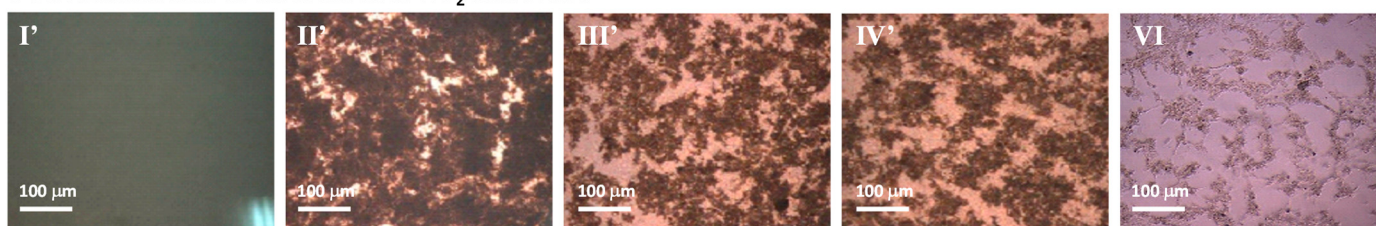


Figure 3. Cytopathic effect of different concentrations of TiO₂ after 60 min UV irradiation, without (I–IV) or with (I'–IV') Nodavirus infection. TiO₂ concentration: (I,I') 5 mg/mL; (II,II') 2.5 mg/mL; (III,III'): 1.25 mg/mL; (IV,IV') 0.3 mg/mL; compared to (V) water only and (VI) Nodavirus only. Scale bar = 100 μ m.

The cytopathic effect (CPE) of nodavirus on E-11 cells, and on wells containing Nodavirus exposed to different concentrations of TiO₂ suspension, was assessed daily based on cell morphology (rounding, detachment, and lysis), the percentage of affected cells compared to controls, and the time of onset and progression of visible changes. However, the cell supernatants were very turbid, and morphological changes were not visible under the reversed-phase microscope. Therefore, the well content, which was already irradiated by UV at different time points after UV irradiation (5 min, 15 min, 30 min, 1 h, and 16 h), was collected regardless of the occurrence of CPE. It is worth noting that treatment of cells with a TiO₂–Nodavirus mixture incubated in the dark (i.e., non-irradiated) did not signifi-

cantly inhibit Nodavirus replication on E-11. This result highlights the synergistic effect of combined UV-irradiation and TiO₂ NPs inoculation. Continuous CPE was observed daily. The results obtained in darkness were then omitted from the rest of this study.

3.4. Relative Expression of the Viral Capsid Gene (CP) in E-11 Cells

E-11 cells were inoculated with several UV-irradiated TiO₂–Nodavirus mixtures. No clear dose-dependent inhibitory effect was obtained. However, a significant decrease in viral CP gene expression was observed when suspensions containing 2.5 mg/mL and 1.25 mg/mL of TiO₂ were used (Figure 4). Interestingly, the inhibition of viral gene expression lasted up to 16 h after infection.

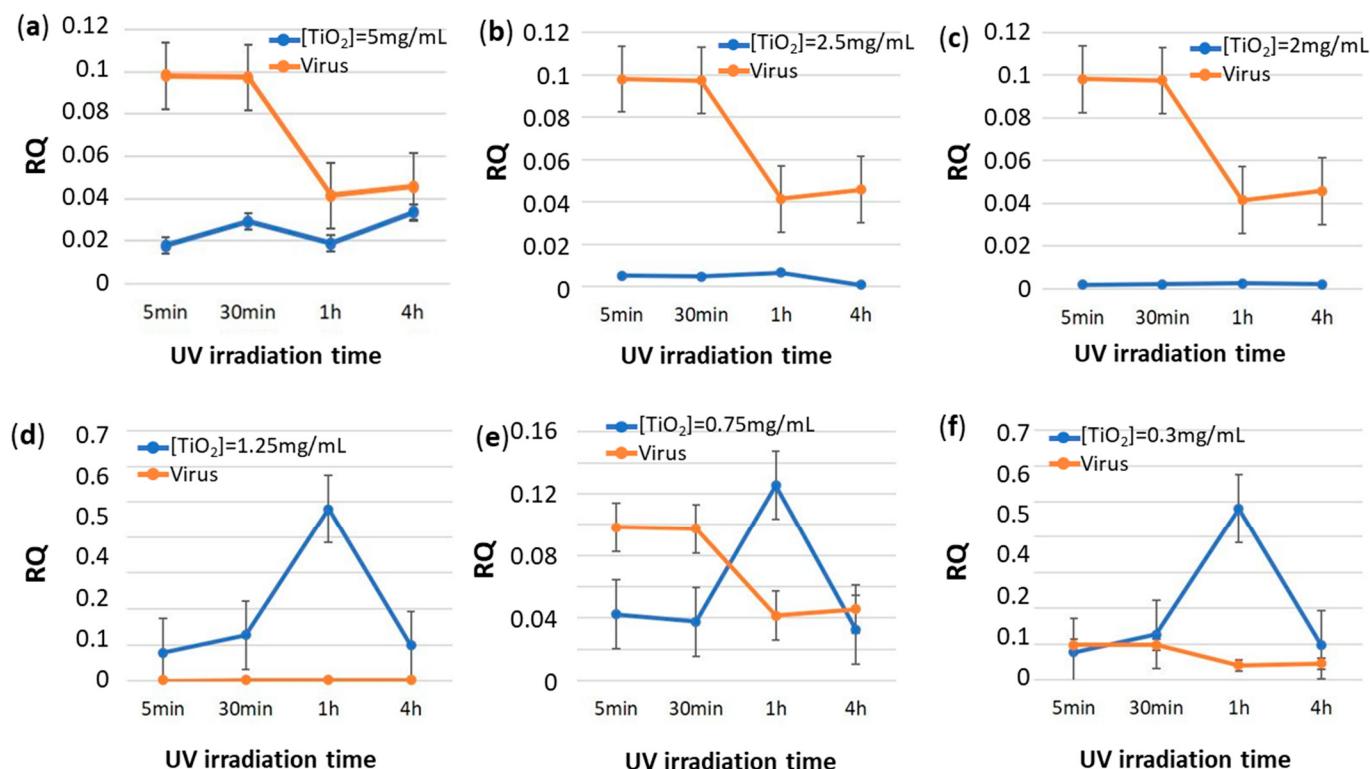


Figure 4. Effect of UV-irradiation time on the relative expression of the viral gene CP after inoculation with different concentrations of TiO₂: (a) 5 mg/mL; (b) 2.5 mg/mL; (c) 2 mg/mL; (d): 1.25 mg/mL; (e): 0.75 mg/mL; and (f) 0.3 mg/mL.

3.5. Immune Genes Expression

Immunogenes from E-11 fish cells exposed to TiO₂ co-incubated with Nodavirus showed dose-dependent up-regulation at 1 h. Cells exposed to 1.25 mg/mL of TiO₂ showed the highest level with increased expression of TGF- α , TGF- β , and Hsp30 genes compared to virus control. In addition, there was an increase in the expression of IFN-1, Mx, Hsp30, Il-1 β , CD4, and Il-8 genes for cells exposed to 2.0 and 2.5 mg/mL TiO₂ concentrations. With a TiO₂ concentration range of 2.0 to 0.3g/L, MHC1- β gene expression increased, while cells exposed to low TiO₂ concentrations (0.75 and 0.3 mg/mL) showed an increase in IgM gene expression (Figure 5).

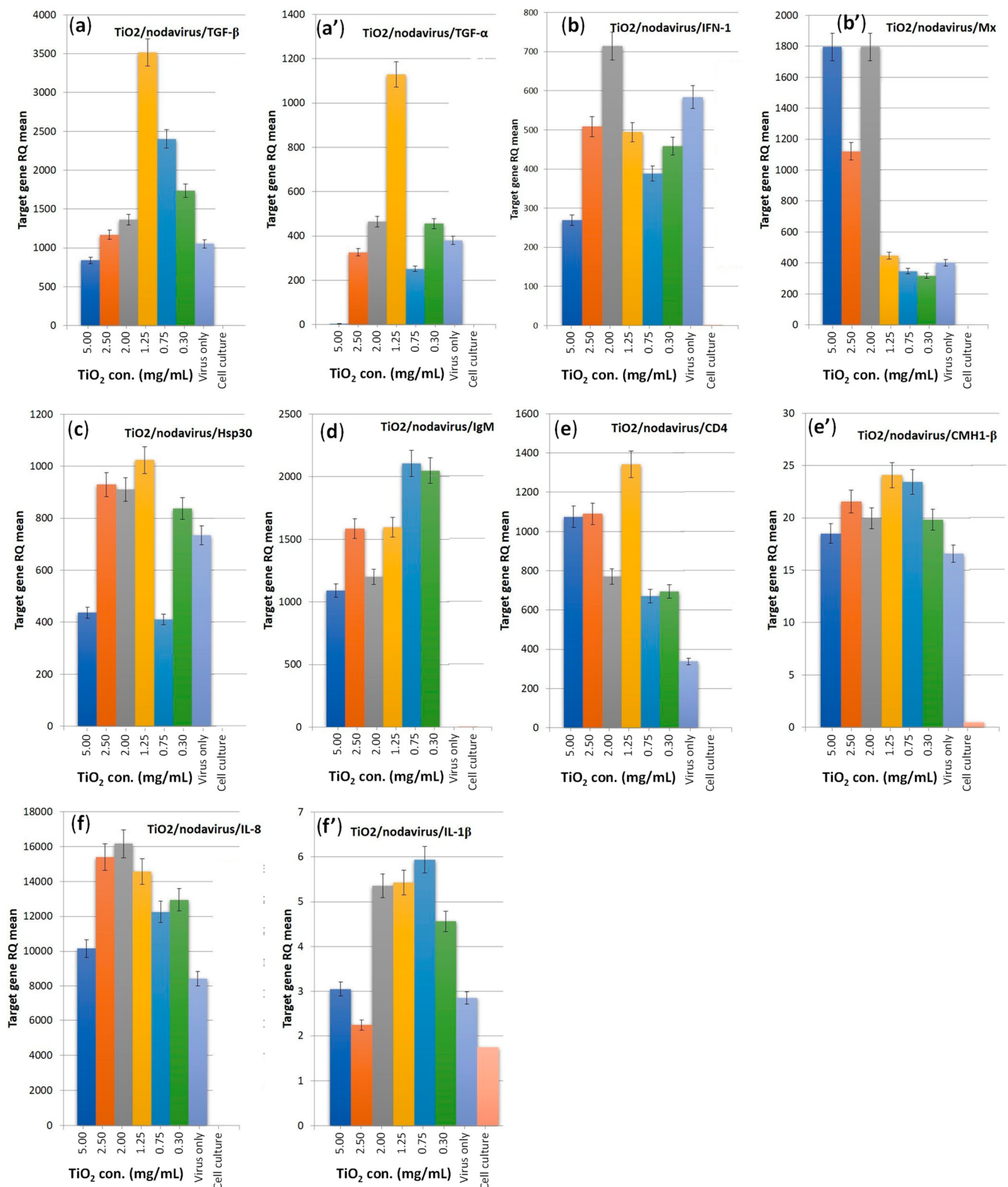


Figure 5. Effect of the TiO₂ content (from 5.0 to 0.3 mg/mL) co-incubated with Nodavirus and under UV irradiation on immune genes expression profiles, compared to virus only and cell culture. (a,a') Anti-inflammatory gene; (b,b') antiviral protein genes; (c) stress gene; (d) gene involved in humoral response; (e,e') genes involved in cellular response; and (f,f') pro-inflammatory genes.

4. Discussion

Numerous viral organisms affect a significant portion of global fish production, resulting in significant economic impacts on aquaculture. In particular, RNA viruses cause

the most serious fish diseases like the neural necrosis virus: betanodavirus responsible for encephalopathy and retinopathy [49,50]. The current study is an in vitro evaluation of the application of TiO₂ powders as an antiviral treatment in aquaculture and TiO₂ powder characterization.

A comparative study of the effect of TiO₂ at different concentrations on Nodavirus in the dark and under UV irradiation was performed to determine the mechanisms of the inactivating effect of TiO₂. The investigation of the cytopathic effect was also carried out. The MTT assay shows that the cell viability decreases after the addition of TiO₂ at different concentrations, especially in the 2.5–0.3 mg/mL range. These results are consistent with those of previous studies [51], showing a significant reduction in cell viability upon co-exposure of the BF2 fish cell line to TiO₂ at 2.5 and 1.25 g/L. Despite the fact that the MTT assay either overestimates the cell viability or fails to detect a reduction in cell number and viability, this effect is most likely produced by the superoxide anions ($\bullet\text{O}_2^-$) generated by TiO₂ NPs [52].

No clear cytopathic effect was observed under the reversed-phase microscope at different TiO₂ concentrations. Consistent with present results, a comparative analysis between TiO₂ NPs and ZnO NPs has shown that TiO₂ NPs are less cytotoxic than ZnO NPs [53], meaning that TiO₂ NPs are safer than ZnO NPs with lower cytotoxicity. Another study showed that TiO₂ NPs, when coated with glycated polyethylene, exhibit stronger antiviral activity and lower cytotoxicity compared to bare TiO₂ NPs [16].

To further investigate the antiviral activity of TiO₂, the relative expression of the viral capsid gene (CP) in E-11 cells was examined. The results showed a significant decrease in viral CP gene expression using a TiO₂ suspension at a concentration of 2.5 g/L or 1.25 g/L (Figure 4). This result is in line with those reported by others [23], who showed that the UV-activated TiO₂ surface is able to degrade the capsid and envelope proteins as well as the phospholipids of the non-enveloped virus. Consistent with the present results, previous studies have shown that an astrovirus, a non-enveloped virus that causes diarrheal diseases, was inactivated, but in this case, TiO₂ was irradiated with visible light [36]. Future studies will further investigate the direct inactivation of free Nodavirus by TiO₂ nanoparticles under UV irradiation in cell-free conditions to better elucidate the underlying antiviral mechanisms.

Consistent results were obtained in previous studies on the impact of the use of different NPs on the immune system. Studies on *Mytilus galloprovincialis* showed that such particles induce pro-apoptotic processes. Their uptake by hemocytes was rapid and affected the phagocytic function [54–56]. Similarly, studies of earthworms exposed to TiO₂ revealed bioaccumulation and significantly reduced phagocytosis from 0.1 mg/mL of TiO₂ NPs. However, TiO₂ did not cause cytotoxicity on coelomocytes [54].

In the present study, co-incubation of Nodavirus with different concentrations of TiO₂ exposed to UV light for 1 h differentially regulated the relative expression of genes associated with immunity and stress in cells, such as antiviral protein genes (IFN-I, Mx); pro-inflammatory genes (IL-1, IL-8), anti-inflammatory genes (TGF-, TGF-), genes involved in the cellular response (CD4, CMH1-), a gene involved in the humoral response (IgM), and a stress gene (Hsp30). Remarkably, the TiO₂ concentration of 1.25 mg/mL increased the expression of all target genes and induced an inflammatory response to Nodavirus, which has never been reported in the literature. Studies have now been conducted to examine the interaction of TiO₂ NPs with the immune system: human macrophages were exposed in vitro to non-toxic concentrations of various nanoparticles, including TiO₂, which were shown [55] to significantly affect the expression of several Toll-Like Receptor (TLR) molecules. These latter receptors represent a critical class involved in the initiation of the inflammatory response and innate immune responses. Particularly intriguing is that

NPs like TiO₂ could increase the expression of TLR chains required for virus-dependent stimulation [55]. TLR7 is another receptor that is crucial for detecting viruses. It binds to viral ssRNA and activates the signaling pathway that depends on IRF-3 to produce IFN- α /b [56,57]. According to [55], macrophages exposed to nanoparticles such as TiO₂ have increased susceptibility to viral infections. Our in vitro research has shown that the different doses of TiO₂-NPs can possibly boost the fish's immune system to fight viral infections, although the doses are much higher compared to the estimated environmental concentration of 0.025 mg/mL [58,59].

Several research results suggest that nanoparticles can replace antibiotics in the treatment of infections. TiO₂ NPs proved to be the safest of all NPs, cheap and easy to manufacture, compared to other NPs.

5. Conclusions

This in vitro study shows that photocatalysis with TiO₂ NPs reduces viral infection, while boosting the fish's innate immunity markers, and might be able to suppress more pathogens, although further experiments, such as in vivo tests, are required. In particular, further investigations should clarify whether the inactivation of TiO₂ NPs depends on the virus titer, the incubation time, and the TiO₂ concentration. However, this work demonstrates the great potential of photocatalysis with TiO₂ NPs to control Nodaviruses in fish farming. Importantly, TiO₂ also aligns with the principles of green chemistry, offering a low-cost, non-toxic, and chemically stable alternative to harmful chemical disinfectants traditionally used in aquaculture. Future research should focus on optimizing photocatalytic efficiency under UV or natural light to lower energy demand, and on assessing TiO₂ recyclability to minimize waste, thereby promoting more sustainable aquaculture practices. By combining antiviral efficacy with eco-friendly features, TiO₂ NPs represent a promising avenue for greener aquatic disease management.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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