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ARTICLES

# Interaction of Titanium Dioxide Nanoparticles with Influenza Virus

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**Abstract**—Titanium dioxide ($TiO_2$) in a suspension or absorbed on a film possesses bactericidal and virucidal properties caused by photocatalytic reactions. Our electron microscopic examinations showed that titanium dioxide nanoparticles destroyed the influenza virus after 30 min incubation. Virological studies revealed the inactivation of the influenza virus by $TiO_2$, depending on the concentration of nanoparticles and the incubation period. The inactivation effect was observed when the incubation was performed in darkness, unlike the $TiO_2$ suspension. We propose that the virus-inactivation properties of $TiO_2$ are mainly based on the direct contact between nanoparticles and virus particles.

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## INTRODUCTION

Searching for substances and ways to inactivate pathogenic microorganisms that are safe for humans and the environment is an important for providing for the health and epidemic safety of the population. Sunlight and its ultraviolet component are powerful natural disinfectants. Titanium dioxide has photocatalytic properties and produces active oxygen-containing radicals under ultraviolet irradiation [1–3]. Bactericidal and virucidal features of titanium dioxide ($TiO_2$) in a suspension form or layered onto a film are described in many works where the destruction of viruses and bacteria under ultraviolet irradiation was presented [3–5]. Published investigations on the effect of a $TiO_2$ nanoform onto microorganisms are rare; the destruction of particles of the surface hepatitis B antigen (HBsAg) as a result of photocatalytic reaction developed on the surface of $TiO_2$ nanoparticles (anatase) under the action of UV and sunlight was shown [6]. The task of the present work is to study the effect of $TiO_2$ nanoparticles on the influenza virus.

## EXPERIMENTAL METHODS

### *Preparations of Titanium Dioxide*

$TiO_2$ nanoparticles ($TiO_2$-1) were obtained by the hydrolysis of $TiCl_4$ in a form of colloid solution (pH = 6.7) containing 2.5 wt % $TiO_2$ and less than 0.05 wt % of Na and Cl [7]. Titanium dioxide powder ($TiO_2$-2) was obtained by the exiccation of a colloid solution of $TiO_2$-1 in air with its subsequent calcination at 700°C for 3 h. The size of the nanoparticles ($TiO_2$-1) was about 4–10 nm according to electron microscopy data, with the predominance of particles being 4–5 nm in size; powder particles ($TiO_2$-2) were 500 nm and larger.

To perform virologic and ultrastructural investigations, suspensions of $TiO_2$-1 and $TiO_2$-2 preparations with a concentration of 7 mg/ml were used. The smallest nanoparticle concentration (0.2 mg/ml) was determined in preliminary experiments using the visible destruction of influenza virus particles according to electron microscopy data (the data are not presented). To evaluate the level that nanoparticles influence virions, large concentrations (10-fold and 35-fold, 2 and 7 mg/ml, respectively) were used. Concentrations larger than 7 mg/ml were not used due to the toxic action it caused to the MDCK cell culture used for assessing the amount of living virus.

### *Influenza Virus*

An A/Aichi/2/68(H3N2) virus strain grown on chicken embryos in a suspension form (virus–allantois liquid) with an infection titer of 9.5 lg $TCD_{50}$/ml was used to prepare working dilutions.

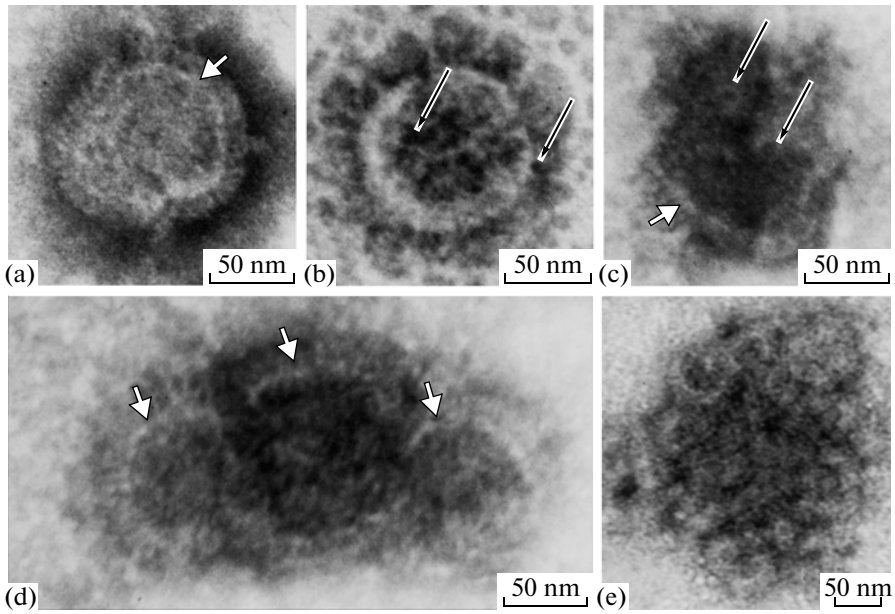


**Fig. 1.** Interaction between $TiO_2$-1 nanoparticles and particles of the A/Aichi/2/68 influenza virus (H3N2). White arrow indicates the virus envelope; black arrow indicates the nanoparticles: (a) intact influenza virus particle, (b) glued surface spinules and the adherence of particles onto virion surface (15 min of incubation), (c) the destruction of the virion envelope, and (d, e) the aggregation of virus particles entering the contrasting stain into the virion (3 and 5 h of incubation). Negative contrasting with phosphotungstic acid.

### *Inactivation of Influenza Virus with $TiO_2$ Preparations*

Equal volumes of the suspension of influenza virus and $TiO_2$ preparations (500 μl) were mixed and incubated at room temperature for 0.5, 1, 5, 14, and 24 h in wells with a 2 cm diameter of the shielded plastic plate. The incubation was performed in the dark or under irradiation by a fluorescent lamp (40-W power) located at a distance of 160 cm or with an ultraviolet-light source (30-W power) located at a distance of 25 cm.

The biological activity of the influenza virus treated with $TiO_2$ nanoparticles

| Concentration of $TiO_2$-1 nanoparticles (mg/ml) | Concentration of influenza virus (log $TCD_{50}$/ml) | Time of incubation of $TiO_2$-1 nanoparticles with influenza virus (h) | Virus production in MDCK cell culture (log $TCD_{50}$/ml) |
|---|---|---|---|
| 0.2 | 9.5 | 0.5 | $7.5 \pm 0.03$ ($n = 3$) |
| | | 1.0 | $5.5 \pm 0.03^{\bullet}$ ($n = 3$) |
| | | 5.0 | $4.5 \pm 0.03^{\clubsuit}$ ($n = 3$) |
| 2 | 9.5 | 0.5 | $4.5 \pm 0.06^{*}$ ($n = 3$) |
| | | 1.0 | $4.5 \pm 0.08^{\blacksquare}$ ($n = 3$) |
| | | 5.0 | $3.0 \pm 0.29^{\blacktriangledown}$ ($n = 3$) |
| Absence (control) | 9.5 | 0.5 | $8.5 \pm 0.29^{*}$ ($n = 3$) |
| | | 1.0 | $8.5 \pm 0.06^{\bullet\blacksquare}$ ($n = 3$) |
| | | 5.0 | $8.5 \pm 0.58^{\clubsuit\blacktriangledown}$ ($n = 3$) |
| 0.2 | 6.5 | 5 | $3.5 \pm 0.29$ ($n = 3$) |
| | | 14 | $2.5 \pm 0.29$ ($n = 3$) |
| | | 24 | $0^{\blacktriangleright}$ ($n = 3$) |
| 2 | 6.5 | 5 | $2.5 \pm 0.29$ ($n = 3$) |
| | | 14 | $0^{\spadesuit}$ ($n = 3$) |
| | | 24 | $0^{\blacktriangleright\!\!\blacktriangleright}$ ($n = 3$) |
| 7 mg/ml | 6.5 | 5 | $0.5 \pm 0.29^{\circ}$ ($n = 3$) |
| | | 14 | $0^{\blacklozenge}$ ($n = 3$) |
| | | 24 | $0^{\blacktriangleleft}$ ($n = 3$) |
| Absence (control) | 6.5 | 5 | $3.5 \pm 0.29^{\circ}$ ($n = 3$) |
| | | 14 | $3.5 \pm 0.58^{\spadesuit\blacklozenge}$ ($n = 3$) |
| | | 24 | $2.5 \pm 0.29^{\blacktriangleright\!\!\blacktriangleright\blacktriangleleft}$ ($n = 3$) |

Notes: *, °, •, ■, ♠, ♦, ♣, ♥, ▸, ♫, ◂—credible differences between two values.

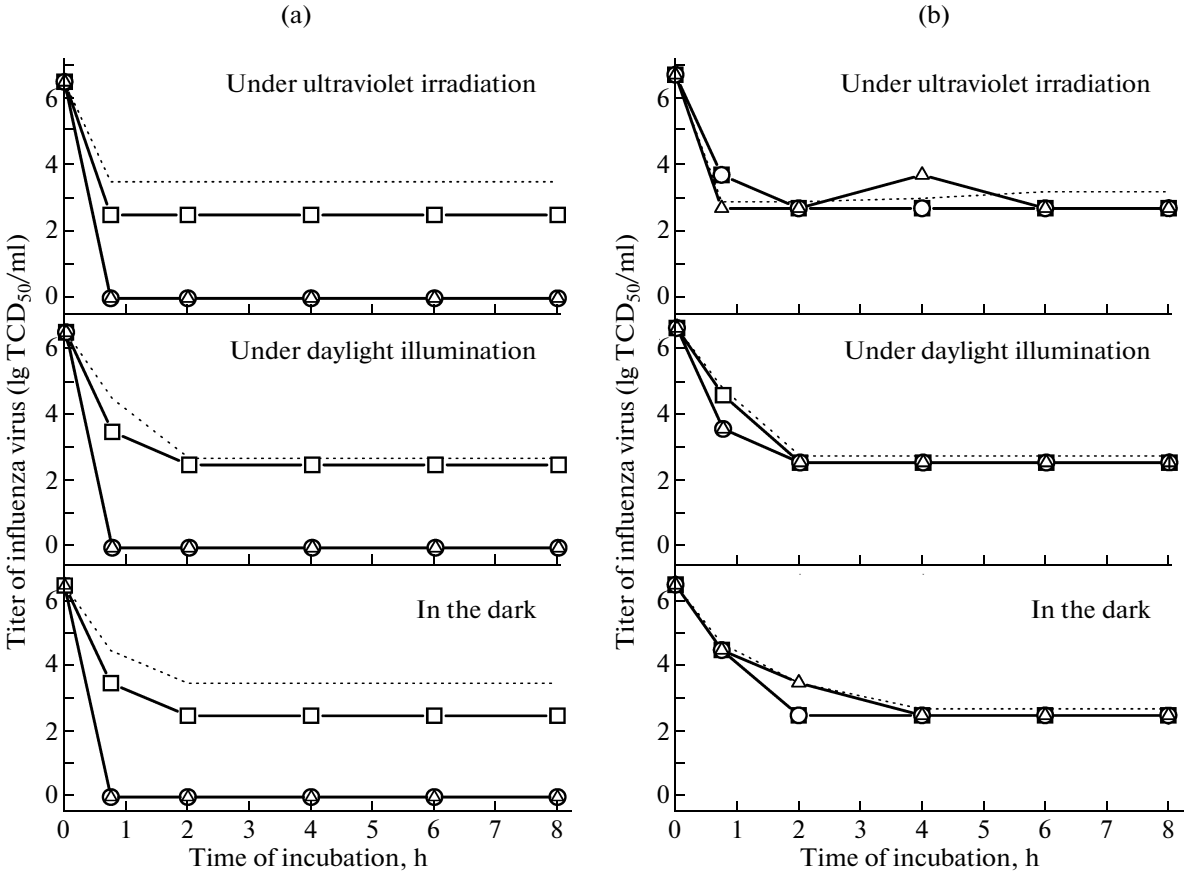


**Fig. 2.** Biological activity of influenza virus treated with nanoparticles and $TiO_2$ suspension (log$TCD_{50}$/ml): (a) $TiO_2$-1 sample and (b) $TiO_2$-2 sample. Concentration of $TiO_2$: (□) 0.2 mg/ml, (○) 2 mg/ml, and (△) 7 mg/ml. The dotted line shows the control influenza virus (without treatment with titanium dioxide).

### *Detection of the Infectious Activity of Influenza Virus*

Tenfold dilutions of the $TiO_2$-mix preparation and virus suspension were prepared; then each dilution was introduced into separate well with MDCK cell culture and incubated at 37°C for 20 h. The production of the infectious influenza virus was assessed in a reaction of hemagglutination with 1% chicken erythrocytes [8]. Samples of the virus suspension incubated similarly to a mix with $TiO_2$ were used as controls. All experiments were performed in triplicate.

*An electron microscopy investigation of the interaction between $TiO_2$-1 and the influenza virus* was done using the method of negative staining. A mix of the nanoparticle suspension with a concentration of 2 mg/ml and an influenza virus suspension with a particle concentration of $10^9$/ml was incubated at room temperature for 0, 1, 2, 3, and 5 h. A drop of the mix was coated onto a copper network with formvar support and incubated for 30 s; then the excess of liquid was eliminated with a filter paper and the networks were incubated in a drop of phosphotungstic acid for 30 s. The preparations were studied using a JEM 1400 electron transmission microscope (Jeol, Japan) at an accelerating voltage of 80 kV.

## RESULTS AND DISCUSSION

The direct action of $TiO_2$-1 nanoparticles on the influenza virus was studied using an electron microscope; 15–20 units of each network were browsed. Virus particles mixed with $TiO_2$-1 nanoparticles are destroyed (Fig. 1). After 15 min of incubation, nanoparticles adhered onto the external surface of the virus envelope, surface spinules of the virus were glued together, and the envelope was broken. After 30 min, the degree of destruction was increased; the virus envelope was fragmented; a contrasting substance was penetrated into the virus vesicle and the aggregation of the virus particles was detected. After 1–5 h of incubation, virus particles were destroyed; their fragments associated with nanoparticles were found on networks. It should be noted that, at all stages of incubation, there were separate virus particles that still had their normal structures and did not contact the nanoparticles.

The inactivating effect of $TiO_2$-1 nanoparticles, which depends on the incubation time, concentration

of the virus, and concentration of nanoparticles, was revealed during an investigation of $TiO_2$-1 action on the virus vitality under daylight illumination (table).

To reveal the mechanisms of the inactivating effect of the $TiO_2$ nanoform, a comparative investigation of $TiO_2$-1 and $TiO_2$-2 action on the influenza virus was performed in the dark, in ultraviolet irradiation, and during daylight illumination (Fig. 2).

The obtained results show that $TiO_2$-1 nanoparticles express inactivation action on influenza virus regardless of the presence of daylight illumination or ultraviolet illumination; this is probably not connected with photocatalytic effects described previously for $TiO_2$ suspensions [1–3]. It is very likely that virus inactivation occurs due to the destruction of the virus envelope by nanoparticles, which results in the destruction and disintegration of the virion entirely. The envelope of influenza virus particles is a lipoprotein membrane, and it has a structure similar to the biological membranes of eukaryotic cells [9]. The ability of $TiO_2$ nanoparticles to enter virus particles that was revealed in our work may suggest the necessity of a detailed investigation of this process, since it may be used for the delivery of biologically active compounds associated with nanoparticles to a cell. From the other side, membrane destruction by nanoparticles may result in cell death, which should be kept in mind during the development of preparations on their base. The inactivating action of $TiO_2$ nanoparticles toward the influenza virus may be used for the development of new compounds and ways for disinfections to inactivate the influenza virus.

## ACKNOWLEDGMENTS

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