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TiO$_2$-based nanomaterials assisted photocatalytic treatment for virus inactivation: perspectives and applications
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The COVID-19 pandemic has demonstrated the need for urgent access to measures to contain the spread of the virus and bacteria. In this frame, the use of photocatalytic nanomaterials can be a valuable alternative to chemical disinfectants without the limitation of generating polluting by-products and with the advantage of re-usability in time. Here, on the basis of up-to-date literature reports, the use of TiO$_2$-based photocatalytic nanomaterials in disinfection will be overviewed, considering the peculiar nanocatalysts assisted inactivation mechanisms. The potential of this class of photocatalysts for air, surface and water disinfection will be highlighted, critically revising the recent achievements in view of their potential in real application.

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Introduction
Among microbial contaminants, pathogenic viruses seriously threaten human health, being resistant to environmental inactivation and presenting high risks of illness, like the emergence of the recent severe acute respiratory syndrome-coronavirus (SARS-CoV-2) that has caused the COVID-19 pandemic with millions of deaths all over the world.

Viruses are known to spread in air through respiratory droplets over a short distance, by means of person-to-person contact, but also via contaminating surfaces, with different, but anyway not negligible viability on different materials. In addition, also faecal-oral routes have been reported, thus envisioning its persistence of virus in aquatic environments and wastewater treatment plants, though in concentration and viability which remain to be confirmed [1].

Effective outbreak control measures have been urged to contain pandemic and protect public health, and effective strategies to disinfect surface of a wide range of materials as well as water and air, have become crucial, given the possible impact of the different routes of virus transmission.

Achieving efficient disinfection of pathogens with minimized harmful by-products based on a facile, cost-effective, and environmentally friendly technologies has become imperative to cope with the enormous risks brought by microbial contaminants.

Many different virus inactivation methods have been proposed [2], and recently inorganic and hybrid nanomaterial-based solutions have been shown effective, thanks to the peculiar size dependent characteristics of nanoparticles (NPs) and nanostructured materials.

Significant efforts have been made to employ photocatalytic nanomaterials to achieve effective microbial inactivation avoiding the use of chemical disinfectants. Although photocatalysts has been extensively studied for their antibacterial function, photocatalytic disinfection of viruses still has to be fully explored, considering the quite different composition, structure, and resistance to oxidative stress compared to bacteria. Moreover, the mechanisms underlying the nanomaterial assisted photocatalytic inactivation of viruses are not fully understood, being, also, difficult to disentangle the photocatalytic effect from other viral inactivation non-photocatalytic paths taking place in presence of nanostructured materials.

Here, we intend to provide an overview and summary of the current knowledge on the use of photocatalytic nanomaterials and, in particular of TiO$_2$-based nanomaterials, in viral inactivation, on the basis of up-to-date literature.
reports, highlighting the potential of such class of methods in applications that may support the fight against SARS-CoV-2 as well as other possible emerging pathogens.

**Nanomaterials against virus**

Since last decades, nanomaterials have been intensively investigated to achieve alternative antimicrobial solutions, as attested by the large, and increasing, number of relevant reports aiming at addressing antimicrobial applications of nanomaterials [3]. Many, among these publications, dealt with nanomaterials assisted viral inactivation, highlighting their potential broad-spectrum antiviral capabilities [4]. Lower is the number of reports on nanostructured heterogeneous photocatalysts assisted virus inactivation, that, however, is increasing in the last years (Figure 1), pointing out the rising popularity of the photocatalytic based approaches, and, in particular of those involving use of TiO₂, that, on the other hand, is a comprehensively investigated material in a variety of fields [5–7].

So far, the effectiveness of photocatalytic nanomaterials, including TiO₂-based nanostructures, in disinfection of a range of viruses, such as human adenovirus GB, Influenza A and B, Hepatitis B, avian influenza virus (A/H5N2) and Norovirus has been reported, thus indicating the great promise of this class of materials in the fight against the risks deriving from these pathogens [8–11].

However, the research needs to be expanded in order to assess the viability of the proposed solutions as a function of the nature of the microorganism, and elucidate the potential of photocatalytic nanomaterials-based approaches in the various environments, and, hence, for different possible applications.

**Virus structure and nanomaterial-driven inactivation mechanism**

In order to understand the potential of nanomaterials in viral inactivation, it may be useful to look at typical structures of viruses and their infection mechanism. A virus is a small biological entity (0.01–0.03 μ), not-independent organism, as needs active cells to proliferate and to reproduce. Viruses transmit viral genome and, hence, infection from cell to cell. A large variety of viruses exists, which differ in terms of structure, shape and sizes (Figure 2a). Two types of virus structure are identified. Non-enveloped viruses, in their virion form are constituted by nucleic acid (DNA or RNA, single-stranded or double-stranded), and a protein shell, the capsid, formed by identical protein subunits, called capsomeres, surrounding and protecting the genetic material. Enveloped viruses (i.e. Influenza virus, Herpex simplex (HSV), Zaire ebolavirus, Human immunodeficiency virus (HIV) and SARS-CoV-2) instead, present an additional outermost lipid layer [12]. For instance, SARS-CoV-2 virus is a *Coronaviridae* belonging to *Nidovirales* family, deriving the name from their crown-like structures, visible under electron microscope investigation, and features a single-stranded positive-sense RNA virus and an outer envelope (Figure 2b) [13]. The coronavirus genome encodes for four to five structural proteins: spike (S), membrane (M), envelope (E), nucleocapsid (N), and hemagglutinin-esterase (HE) proteins. SARS-CoV-2 and SARS-CoV-1 spikes, structurally similar glycosylated homotrimers, are able to bind to the angiotensin-converting enzyme 2 (ACE2) receptor present on the cell surface.

Typically, the infection is guided by the specific viral attachment of viral protein (key), expressed on the virion, to the receptor (lock) on the host cell surface. In detail, the spike (S) protein of SARS-CoV-2 virus uses the human angiotensin-converting enzyme receptor (ACE2) for entry and the serine protease TMPRSS2 for S protein priming, and promote viral uptake and fusion at the cellular or endosomal membrane (Figure 2c) [13–15].

Many types of inorganic nanomaterials with different compositions have been proved effective for inactivating virus, though the mechanism behind their behaviour is not unique and unambiguously defined. The virus infection takes place in different stages, therefore the antiviral capacity of NPs can target attachment, penetration, replication and/or budding of viruses. In general, multiple, and sometime interconnected, mechanisms have been reported to account for disinfectant function of nanomaterials, however, four main paths can be recognized: (i) physical damage of viruses induced by puncture and consequent rupture of virus, (ii) toxicity of metal ions released from metal containing NPs, (iii) NP-virus interaction, as adhered NPs hinder cell attachment, inhibit cell entry and prevent genome injection and (iv) photocatalytic inactivation (Figure 3a) [8,16,17].

When photocatalytic NPs are considered, an additional active phase occurs: photoactivated NPs locally produce reactive species, that induce protein oxidation, degrading the capsid, causing a distortion of the structure and damage of the encapsulated genome, resulting in viral inactivation [18,19]. Indeed, the degradation of protein corona results in loss of recognition ability, while the degradation of protein envelope turns in the capsid breakdown. Afterwards, the nucleic acid can be attacked and destroyed. Also, NPs that can be photothermally activated, such as plasmonic NPs, have been found to induce a protein genome damage (Figure 3b).

Alternative disinfection mechanisms relying on uptake by cells, that occurs in certain bacteria strain and is able to induce mutagenicity for instance, does not exist for virus, as their size are too small to take up NPs.

Therefore, since nanomaterials may play their disinfection function via possible concomitant mechanisms, such
as free metal ions release, physical rupture due to the specific shape of the nanostructure, or other synergistic effects, the photocatalytic effect cannot be easily singled out from other possible paths.

**Photocatalytically assisted disinfection**
The antimicrobial activity of the semiconductor based nanostructured photocatalysts is primarily attributed to the photogeneration, under band-gap irradiation, of generate reactive oxygen species (ROS), such as hydroxyl radicals (OH–) and superoxide(O2•−), with high oxidative potentials, resulting in effective and long-lasting capabilities (Figure 4). Since the process does not rely on the release of metal ions, it is not limited by the consumption of the metal NPs, and can, in principle, possibly perform also a non-contact biocidal action though when
photocatalyst surface and virus come into contact, an increase in photocatalytic efficiency is accounted [20,21].

Virus inactivation kinetics cannot be characterized by a single kinetic model, even for the same virus. In general, viral disinfection kinetics are more complex than the first-order profiles usually found for chemical pollutant degradation by photocatalysis, due to the unclear relationship between viral chemical structure after oxidation and viral viability, as well as the complicated and still unknown viral repair mechanisms. Therefore, the photocatalytic activity for viral disinfection processes cannot be simply extrapolated from organic degradation. In addition, analogy in the inactivation kinetics of bacteria and viruses cannot be assumed, as were also observed by Cho et al. [22]. For instance, MS2 bacteriophage was found more resistant than Escherichia coli, a bacterium, to the oxidative attack of ROS generated from photocatalysts, due to the different surface structure of bacterial cells and viral particles. In fact, E. coli presents a complex surface structure of lipopolysaccharides, thin peptidoglycans, and lipid bilayers and even a slight damage to the bacterial surface can destroy key metabolic systems such as respiration. While, MS2 is a nanometer size icosahedral shaped virus with a simple surface structure of capsid proteins that forms a rigid shell, requiring a strong oxidant to be denatured.

Kim et al. reported that the conventional limitation of TiO₂ disinfection of inefficiency in the dark, could be overcome by applying prepared TiO₂ nanowire (TNW) films to bacterial and viral disinfection. TNW exhibited much higher antibacterial efficiencies against E. coli under dark and UV illumination conditions compared to TiO₂ NPs, found, instead, almost inactive in the dark. The report highlights the additional contribution due to the physical interaction between nanowire and membrane of a variety of microorganisms including...
Staphylococcus aureus and MS2, being such antibacterial activity related to the nanowire geometry such as diameter, length, and density leading to sort of physical puncture [23,24].

Photocatalytic surface disinfection

Although the role played by surface transmission is still to be thoroughly elucidated, viruses as SARS CoV-2 have been found viable on solid objects for a period of times ranging hours to days, and exposure to these solids may be responsible of superspreading the infection, thus making essential the development of biocidal coatings [25,26].

Many photocatalytic nanomaterial-based coatings have been reported to reduce the lifetime of viruses on the surfaces and thus resulting potential candidates for treatments able to limit contamination risk and spreading of virus, on a variety of objects, including surface in public transportations, schools, bars, hospitals, passing through a series of objects present all over, as doorknobs, countertops, and electronic equipment (Figure 4).

Khaiboullina et al. investigated the possibility to enhance the deactivation of Human coronavirus with UV light irradiation of TiO2 NPs coatings. Commercially available TiO2 P25-based coatings were found to inactivate HCoV-NL63, both in wet and dried environment, under UV light exposure [27].

Hamza et al. evaluated the antiviral activity of hydrothermal synthesized TiO2 nanotubes against SARS-CoV-2, demonstrating its efficacy at low concentrations. Based on these results the nanomaterial immobilization owes proposed to achieve antiviral surfaces to obtain antiviral surfaces [28].

Yoshizawa et al. obtained a TiO2-based coating spraying a commercial product composed of a peroxotitanium acid (70%) and a peroxy-modified anatase (30%) solution, that, in contact with an aliquot of bovine coronavirus suspension and irradiated with visible light for 4 hour according to ISO 18071, resulted in a decrease of viral titer of 2.4–2.8 log TCID50/0.1 mL, when compared to the uncoated substrate. The inactivation mechanism was thought to be based on the formation of a peroxotitanium complex, able to cause, concomitantly, physical damaging of viruses, metal ions induced toxicity and photogenerated ROS assisted photocatalytic oxidation. Interestingly, the effectiveness demonstrated also using a luminous flux comparable to that achievable in common indoor environments, makes the proposed coating an interesting candidate for surface sanitization [29].

Moon et al. synthesized by means of a sol gel process a Cu/TiO2-based coating that was deposited onto a polyethylene terephthalate (PET) non-woven membrane 20 μm thick. The Cu/TiO2 non-woven fabric (NWF) presented

Figure 3

(a) Scheme of the three main mechanisms of viral disinfection induced by catalysis; (b) sketch of the photoactivation of NPs, producing reactive species. Photocatalytic degradation of virus, resulting in viral inactivation.
an efficient disinfecting effect on HuNoV GI/4, upon prolonged (48 min) exposure to UVA light emitted by a LED source [30**].

**Photocatalytic air disinfection**

Powerful, green and sustainable technologies for airborne microbial decontamination represent another critical need, considering the risk of airborne transmission of many types of viruses, including coronavirus [19]. In particular, a significant attention is paid to limit indoor air contamination in different environments, including buildings, vehicles, factories, hospitals, and so on [31]. Many photocatalytic air treatment devices have been recently developed for microbial removal, also technologically advanced, like the very recent work by Zacarfas *et al.* that described an annular reactor for indoor air disinfection, consisting of a glass cylinder, packed with TiO$_2$ P25 glass rings, a set of internal and external UV tubular black-light fluorescent lamps and an air recirculation system, that has been, however, successfully tested only against vegetative cells and spores of *Bacillus subtilis* (complete removal of the bacterial spores from the air stream in 1 hour, and a 68% and 99% reduction of retained spores and vegetative cells, respectively, after 8 hour irradiation) [32*].

In fact, only few recent reports deal with air disinfection systems actually tested against viruses.

Shiraki *et al.* designed an air cleaner for indoor environment installing a TiO$_2$-coated aluminium plate and black light to the UV-LED array, downstream from an HEPA filter [33*]. An aerosol containing influenza virus A/PR/8/1934 (H1N1) was nebulized in a confined space. Without the photocatalytic air cleaner, traces of virus and RNA genome were found still detectable after 28 min, whereas when the system was installed a complete elimination took place after 7 min.

Doss *et al.* reported TiO$_2$/β-SiC solid alveolar foams for purifying air from airborne T2 bacteriophage viruses under UV LED irradiation (392 nm). A high photocatalytic filtration efficiency was observed with 56 LEDs and a logarithmic abatement of 3 was achieved for 60 min of run time [24].

Interestingly, the latter proposed devices represents a valuable integration for upgrading existing technologies [33*].

Kim *et al.* presented a vacuum UV air reactor containing a Pd-TiO$_2$ catalyst layer prepared in different shapes, that
upon spraying of a MS2 bacteriophage aerosol, was tested with an air flow rate of 33 L/min, and hence short air irradiation times (0.004–0.125 s). Starting from a 2.3 × 10^8 PFU/mL MS2 aerosol a 99% reduction was achieved in the best performing set-up. In addition, a reduction of amount of ozone produced by the UV light was observed, thus making the system especially appealing for its indoor use, as ozone formation should be specifically avoided [34].

Matsuura et al. studied the antiviral activity of TiO2 against SARS-CoV-2 when exposed to LED light (405 nm, 10 mW). The inactivation was firstly tested in a liquid and, subsequently, on an aerosol of the virus suspension, produced to have droplets of similar size of the respiratory droplets, and introduced in a box containing air cleaner coated with a TiO2 layer and a LED lamp, sampling at different times (0–20 min). A complete inactivation was observed in less than 20 min exposure [35**].

Poormohammadi et al. offered an extensive evaluation on the efficiency of photocatalysis processes for airborne viruses sanitation, investigating several parameters, as the composition of the photocatalysts (TiO2, Ag-TiO2 and Cu-TiO2), environmental factors, such as air flow rate, humidity, reactor temperature and type of light sources, and describing also the inactivation mechanisms [36].

Personal protection equipment
The use of personal protective equipment against airborne microbial contamination has become more and more important. Facial masks, lab or medical aprons and others equipment could be modified using nanengineered materials to provide new functions, for instance, hydrophobicity and antimicrobial activity without altering too much texture or breathability of fabrics [37].

Miyahuci et al. produced a nanocomposite based on copper oxide and TiO2 (CuO/TiO2) to be active under white light and in the dark thanks to the presence of copper, thus being suitable for indoor environment with its light/dark activity during the day/night cycles. This material on surfaces was found to reduce viral contamination. In addition, the nanocomposite proved to be efficient in QB bacteriophage inactivation also once deposited on a textile exposed to the aerosolized viral suspension. The performance of the nanocomposite on textile makes it suitable for possible application in protective equipment [38].

Monmaturapoj et al. synthetized TiO2/hydroxyapatite (HA), to be used as antiviral filtration applications (i.e. face masks). In the proposed system, the virus inhibition was achieved by a combination of the photocatalytic behaviour of TiO2 NPs, induced by UV-light irradiation and the adsorption capability of HA, that is able to trap H1N1 Influenza A Virus [39].

Also, it is worth mentioning the report of Horváth et al. on photocatalytic TiO2 nanowires-based air filter for protective face mask tested not only for the degradation of biological target molecules, namely commercial DNA pUC19, but also for inactivation of E. coli, that resulted completely inactivated after 10 min irradiation. The TiO2-based material, though not tested against viruses, looks to be promising for real scale application as produced in Kg-scale and applied to modify filters of facial masks, envisaging their possible re-use up to 1000 times, thanks to their photocatalytic regeneration, showing an interesting potential also in view of antiviral treatment [40].

Photocatalytic water disinfection
Viruses have been reported to survive and remain infective for up to 130 days in seawater, or up to 120 days in freshwater, and for up to 100 days in soil at 20–30°C [41]. On the other hand, Gundy et al. investigated the survival of coronaviruses (Human coronavirus 229E; HCoV) in water and wastewater demonstrating 99.9% reduction of HCoV population in wastewater in 2–3 days [42]. Similar results were observed in the case SARS-CoV in 2005 [43]. Despite the relatively low concentration of viruses in water, these microorganisms have very low infectious doses (10–100 virions) [41]. In this perspective, wastewater treatment center workers may be vulnerable to diseases caused by viruses [44].

Moreover, not waterborne viruses can be easily found in the water bodies as a result of human contamination in a specific location, being this effect also at the base of the wastewater-based epidemiology (WBE) approach, also proposed for the COVID-19 pandemic [45].

Among waterborne pathogens, viruses are, unfortunately, difficult to be physically removed due to their small sizes and unique surface properties, for example, granular activated carbon adsorption as a common barrier in water treatment was reported to effectively remove bacteria, but not for viruses [46]. In addition, some viruses that are often quite resistant to common disinfection techniques as UV exposure [21].

Therefore, photocatalytic water treatments may represent a green, sustainable, efficient and low cost disinfection technique, able to overcome limitations of conventional disinfection methods, thanks to its powerful oxidative capability, promising potential in solar energy utilization and concomitant absence of potentially mutagenic and carcinogenic by-products deriving from use of chemical oxidants [24,47].

However, the practical application of photocatalytic processes is hampered by the technological issues of
photocatalyst NPs separation from the treated and catalysts aggregation phenomena taking place mainly at high NPs concentration. In this perspective, effective immobilization procedures of photocatalyst NPs to form a coating, have been established to circumvent catalyst recovery procedures [5]. Indeed, only a few examples TiO₂-driven virus degradation have been reported in literature in the last two years.

Zheng et al. reported Cu-TiO₂ nanofibers obtained by electrospinning were tested in for f2 bacteriophage photocatalytic removal from water, under visible light. Complete inactivation was observed after 4 hour irradiation while undoped TiO₂ fibers were found not effective [48]. Recently the attention has been focused on the effective use of 2-D materials, including g-C₃N₄ and O-doped g-C₃N₄, for photocatalytic water disinfection [49,50].

Conclusions and perspectives
Semiconductor-based photocatalysis holds a great promise in terms of technological development of new and/or improved systems and devices, able to efficiently perform sustainable and clean photocatalytic disinfection of waterborne and airborne viruses, thus addressing new challenges roused by viral epidemic and pandemic cases.

Different disinfection paths have been identified as a function of photocatalysts composition and architecture and have been suitably applied exploited for removal of water and air contaminants and for self-cleaning surfaces. However, more efficient photocatalysts are still needed to be investigated for viral disinfections in the different applications.

Also, a proper testing of disinfection performance of any photocatalytic material should be carried out as a function of the envisaged applications. For instance, antimicrobial activity of photocatalytic nanomaterials for surface treatment or for personal protective equipment should be tested under visible radiation, with a proper light intensity. On the other hand, UV activated photocatalysts should be integrated in devices able to maximize the benefits of such costly high-energy lamps.

Moreover, virus inactivation ability of investigated materials should be ultimately assessed on virus, rather than on other type of microorganisms, still complying the safety measures required to handle high risk biological material, like viruses [51].

Finally, any possible release into the environment of potentially toxic NPs—with adverse effects on human health and natural environment—should be prevented, fostering the development of firmly immobilized photocatalysts based systems, so to achieve environmentally safe and sustainable solutions to fight spreading of virus and, hence, their associated diseases.

Conflict of interest statement
Nothing declared.

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