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Abstract

Nanomaterials are materials that have at least one dimension within the nanometric scale (10^{-9} m), in a range between 1 and 100 nm. For years, nanotechnology has been studying new antibacterial and antifungal agents. Nanomaterials can interact with microbial species thanks to their size and surface characteristics, which allow strong interactions with bacteria. In recent years, nanotechnology is also being studied to open new routes to fight and prevent viral diseases and viral spread.

The nanomaterials size could be in the same range in which the diameter of SARS-CoV2 and many viruses falls. The dimensions close to the virus size allow the interaction of the nanomaterials with the whole viral particle or with the surface proteins or other structural parts, to inhibit or inactivate the viral infectiousness.

This thesis concerns nanomaterials that have been studied in recent years against the spread of viruses, in particular against enveloped viruses that can infect animals and humans. These nanomaterials interact with viruses through various mechanisms depending on their chemical-physical properties. The reported interactions occur before the virus can infect a host cell.

The interaction mechanism certainly needs to be explored, but several studies have highlighted its non-specificity, for this reason these nanomaterials are being studied to obtain new antiviral solutions with a broad spectrum of action. The engineering of antiviral nanomaterials is exploited for various applications in the field of personal protective equipment (PPE), such as face mask or surface coatings. These new solutions could avoid a large viral spread of unknown and infectious pathogens, while the research of an adequate vaccination coverage goes on. In fact, the study and the development of a new vaccine take time and represent a very specific solution, valid only for a specific species.

This thesis is a review of the antiviral nanomaterials studied in the last decades, such as metal nanoparticles, metal nano-oxides and other nano-compounds. Their application requires further studies on their potential cytotoxicity and on their possible engineering methods.

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List of abbreviations

ACE2 angiotensin-converting enzyme 2

ADME adsorption, distribution, metabolism, and elimination

ADS asian dust storms

AgNMs silver nanomaterials

AgNPs silver nanoparticles

AOPs advanced oxidation processes

API air pollution index

AuNPs gold nanoparticles

BVDV bovine viral diarrhea virus

BZM-CDs carbon dots from benzoxazine monomers

CB conduction band

CBBA carboxyl phenylboronic acid

CCM-CDs cationic carbon dots with curcumin

CD cyclodextrin

CQdots or CDs carbon-based quantum dots

CTDs C-terminal domains

CuINPs copper iodide nanoparticles

ΔE energy difference

DNA deoxyribonucleic acid

FCoV feline coronavirus

FCV feline calicivirus

FIPV feline infectious peritonitis

GA gallic acid

GO graphene oxide

gp120 glycoprotein 120

gp41 glycoproteins 41

HA hemagglutinin

HA hydroxyapatite

HAADF high-angle annular dark-field

HBV hepatitis B virus

HBsAg hepatitis B virus antigen

HCV hepatitis C virus

HIV human immunodeficiency virus

HNMs hard nanomaterials

HS heparan sulfate

HSPG heparan sulphate proteoglycan

HSV-1 herpes simplex virus-1

HSV-2 herpes simplex virus-2

IFV influenza virus

LD₅₀ lethal dose 50

LPS lipopolysaccharide

MES mercaptoethane sulfonate

MERS middle east respiratory syndrome

MERS-CoV middle east respiratory syndrome coronavirus

MONPs metal oxide nanoparticles

MPV monkeypox virus

MUS-AuNPs mercapto undecane sulfonic acid gold nanoparticles

NA neuraminidase

NDV newcastle disease virus

nG-PGS-C_x, with **x = 3,6,9,12,18** nanographene functionalized with polyglycerol sulfate and alkyl chains of different lengths

PE polyethylene

PEDV porcine epidemic diarrhea virus

PM particulate matter

PM_{2.5} particulate matter smaller than 2.5 μm

PM₁₀ particulate matter smaller than 10 μm

PoGNPs porous gold nanoparticles

PP polypropylene

PPE personal protective equipment

PRRSV respiratory syndrome virus

PRV pseudorabies virus

PS porous silicon

PSiNPs porous silicon nanoparticles

PSiNWs porous silicon nanowires

PVP polyvinylpyrrolidone

REACH Registration, Evaluation, Authorization and Restriction of Chemicals

rGO reduced graphene oxide

RhE reconstructed human epidermis

RNA ribonucleic acid

ROS reactive radical species

RSV respiratory syncytial virus

SARS severe acute respiratory syndrome

SARS-CoV-1 severe acute respiratory syndrome-coronavirus-1

SARS-CoV-2 severe acute respiratory syndrome – coronavirus – 2

SEM scanning electron microscope

SiNPs silicon nanoparticles

TEM transmission electron microscopy

TGEV transmissible gastroenteritis virus

TRGO-dPG-S graphene oxide thermally reduced by graphite and functionalized with polyglycerol sulfate

Trp-Cu₂O tryptophan capped copper(I) oxide

Trp-CuO tryptophan capped copper(II) oxide

VB valence band

WHO world health organization

Introduction

For a year now we have been in a particular situation that is involving the whole world. The rapid spread of SARS-CoV-2 (Severe Acute Respiratory Syndrome – Coronavirus – 2) has brought several negative aspects to human health. Since the beginning of the epidemic, researchers have been called upon to search solutions that could stem the problem as soon as possible. The best solution against viral spread would be the creation of an active vaccine against the virus. However, the synthesis of a vaccine usually takes a long time and several studies [1]. To date, vaccine against SARS-CoV-2 virus has arrived earlier than expected, but the difficult organization of a global vaccination campaign is still an issue.

A reflection on the current situation led me to study additional possible approaches that could be applied in the future. In recent years in the biomedical and materials fields, nanotechnologies are increasingly being explored.

Originally, studies involving nanotechnology in the antimicrobial field involved experiments targeting bacteria. Subsequently to the latest outbreaks of avian influenza, SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome), such experiments have been expanded on viral species [2].

The study presented in this thesis aims to review the nanomaterials that to date have presented an interesting antiviral efficacy. These new antiviral solutions based on nanomaterials could be applied to prevent contagion and contamination. In fact, the next best solution after the vaccine is certainly to avoid the spread of the infection. This could imply the use of nanomaterials that can act directly on the virus, to inhibit its infectivity or destroy it [3].

There are several viral species in the world, those responsible for the past epidemics and the current one, all belong to species characterized by the presence of a lipid envelope. Antiviral nanomaterials could act on this structural feature; therefore, the same nanomaterial could be effective on different enveloped viruses. The vaccine and drugs, in general, represent very specific antiviral solutions, so they may not be effective against variants of the same viral species [3].

This thesis presents a survey on nanomaterials that have been shown to interact with the virus, although the mechanisms of this interaction are still to be explored. Therefore, the work carried out was born from a reflection on the virus responsible for today's pandemic but presents solutions applicable to various enveloped viruses.

Nanomaterials have at least one size between 1 and 100 nm and are classified into 0D, 1D, 2D, and 3D nanomaterials. This work took into consideration the most used classes in the antiviral studies: the nanoparticles (0D nanomaterials) and graphene-based nanomaterials (2D nanomaterials) [4].

In the first chapter of the thesis are presented the nanomaterials and their characteristics involved in research against viral infection. Chapters II-V concerns the studies dedicated to the interaction between enveloped viruses and different nanomaterials, in order: from metal nanoparticles to metal nano oxides, to other compounds (especially those on carbon-based, including 2D graphene-based nanomaterials), to metal oxides that exploit the photo-induced production of radical species capable of negatively intervening on viral particles. The mechanisms presented by the different nanomaterials mainly involve electrostatic and hydrophobic interactions.

The review presented from chapter II to chapter V analyses the interaction between viruses and nanomaterials. The reflection on this interaction has led the research to an in-depth analysis of a possible intervention of atmospheric particulate matter (PM) in the spread of different viruses [5]. Chapter VI presents the theses reported by various scientists on the potential interaction between PM and viral particles, especially with SARS-CoV-2. These assumptions still represent a controversial topic.

In conclusion, Chapter VII shows the future perspectives on the potential application of the antiviral nanomaterials in personal protective equipment (PPE), nanocoatings, and sanitizers. Such applications could represent good protection solutions from potential infections, helping to limit viral spreads. Urgent research for new antiviral solutions must not lead to underestimate the potential nanomaterials toxicities [6]. The last chapter takes into consideration their nanosafety and environmental impact.

Chapter I

Nanomaterials against viral spread

1.1 Nanomaterials

Nanomaterials are materials that have at least one dimension within the nanometric scale (10^{-9} m), in a range between 1 and 100 nm [7]. The nanometric size allows the material to have a very high surface area formed by surface atoms, characterized by high energy and reactivity due to their instability. In the last decades, their use and study have increased explosively, due to the improvement and availability of synthesis methods, and characterization techniques. The world of nanomaterials differs from that of bulk materials for their unique physical and chemical properties. They have properties different from those of the single atom or molecule and from those of bulk matter with the same chemical composition. This uniqueness is due to the structures, shapes, phase changes energetic and electronic structures, chemical reactivity, and catalytic characteristics of these large finite systems [7].

The potential applications of nanomaterials are in nanodevices, nanoelectronics, computer technology, medicine, and healthcare [7].

Nanomaterials are widely studied in order to be used in medicine and pharmaceuticals, due to their efficiency and convenience compared to other traditional materials [8]. Their specific optical properties and their nanometric size allow the wide use in imaging techniques and in diagnostic, thanks to their sensitivity in the detection of key biological molecules [9]. Other medical applications involve drug delivery and new therapies for the treatment of cancer, diabetes, infections, and others [9]. Nanomaterials used against infections perform an active action against microbes and pathogens [8]. For years, nanotechnology has been studying new antibacterial and antifungal agents. Nanomaterials can interact with microbial species thanks to their size and surface characteristics, that allow for strong interactions with bacteria [8].

In recent years, nanotechnology is also being studied to open new routes to fight and prevent viral diseases and viral spread [8].

1.2 Viruses and nanomaterials

Nowadays discussions about viruses and pandemics are a very topical issue.

On December 31, 2019 pneumonia of unknown cause has been reported to the WHO's office for the first time in China, it was spreading very quickly starting from the city of Wuhan. On 11 March 2020, the epidemic was declared by the WHO as a public health emergency of international interest, to which was assigned the name 'Covid-19' [10]. This infection can vary from manifesting itself more mildly not presenting any symptoms, to provoking more serious dysfunctions, leading the patient to hospitalization. Older individuals and those with severe underlying medical conditions are more exposed to the riskier aspects of the disease [11]. The cause of the pandemic has been identified in the spread of the SARS-CoV2 virus (Severe Acute Respiratory Syndrome – Coronavirus – 2), part of the genus Betacoronavirus of the Coronaviridae family, it is the seventh coronavirus identified by researchers capable of infecting the human being [11].

Epidemics and pandemics caused by new viruses have characterized human and animal history. The last decades have seen the spread of several pandemic viruses such as H2N2 (1956–1958) and H3N3 (1968) influenza viruses, HIV (Human Immunodeficiency Virus) (peak reached between 2005 and 2012), and SARS-CoV-1 (Severe Acute Respiratory Syndrome-Coronavirus-1) (2009), while others such as MERS-CoV (Middle East respiratory syndrome coronavirus) (2012 to now) and Ebola (1975 to now) viruses are considered still in a pre-pandemic phase [8]. The spread is favoured when initially the pathogens are unknown and especially when they are very infectious. To stop a new infection, it is necessary to provide adequate vaccination coverage and new syntheses of drugs that can kill the virus. The study and development of a new vaccine take time and represents a very specific solution, valid only for a specific species [12].

The virus undergoes constant mutations, and therefore we need materials that have an antiviral property with the most varied applications [13]. For example, influenza A virus has several subtypes, and they change very fast, so that potentially a sudden pandemic caused by a new type of influenza virus could always be possible [12]. These viruses change constantly the surface proteins using two main mechanisms: antigenic drift, that is, gradual modification of the sequence of amino acids that constitute the proteins that stimulate an immune response, and antigenic shift, which leads to the appearance of a new viral strain due to the presence of new surface glycoproteins not belonging to the subtypes already circulating in man [14]. The sources of these new subtypes are usually animal viruses [12]. This is the reason why it is recognized that for good prevention, such as to reduce a large contagion, it is very important to develop new solutions [2].

These solutions could be represented by materials that incorporate nanoparticles capable of degrading or inhibiting the infectiousness of a virus [3]. Mainly these nanomaterials are applied either on synthetic and natural fibers, which make up clothes and medical protective devices (masks, gowns, and gloves), or come in the form of sprays, or coatings or composites, or filters for the air. Thanks to their small size, nanomaterials have a high specific area that confers an increase or a new development of chemical and physical properties [3].

Coatings or medical protective devices are useful because contagion between individuals occurs mainly through the contact of surfaces contaminated with droplets containing the virus, landed on them, or directly through the respiratory secretions of others [3]. Several studies show that the virus maintains its vitality on the surfaces present in the environment for several days and can be transferred from the hands to the surface and vice versa. To contain and/or block the viral spreading, several materials for preventive virus inhibition have been listed [3].

Many scientific articles, which develop the new solutions given by nanomaterials, introduce their research by naming the spread of diseases such as SARS (Severe Acute Respiratory Syndrome), avian influenza, swine flu, MERS (Middle East respiratory syndrome), which have occurred in past years, to make clear the urgency and necessity of these studies. Probably the social and technological progress due to the high globalization, which has brought with it an ease and a greater speed for the movements and the possibility to participate in numerous overcrowded events, has contributed to the spread of the respective viruses, preventing their circumscription that perhaps occurred in past centuries [8]. Today more precautions and methodologies are needed to stop or slow down a contagion [8].

This thesis analyses and finally compares the experiments of different nanomaterials tested on viruses, which have structural characteristics common to SARS-CoV-2, such as the envelope.

The SARS-CoV-2 single viral infectious particle has a diameter ranging from 80 to 160 nm, it belongs to group IV of the Baltimore classification: single stranded RNA (RiboNucleic Acid) viruses with positive polarity, (+)ssRNA, all the Coronaviridae viruses belongs to this group [11] [15]. Genomes are inside the nucleocapsid, that is covered by the so-called envelope, a phospholipid bilayer derived from host cell membrane [15]. The presence of this membrane differentiates the enveloped viruses from the naked viruses [15]. Proteins of the envelope are indispensable for the replication of the enveloped virus and its infectivity: the virus activities are carried out thanks to the collaboration between spike proteins, nucleocapsid proteins, membrane proteins, and envelope proteins, so their persistence in the environment is different from those of non-enveloped viruses, for example, enveloped viruses are generally more susceptible to oxidant disinfectants [11]. The structure described is visible in figure 1.1. The antiviral mechanism of different nanomaterials against these enveloped viruses mainly involves the lipid layer, therefore, in this thesis, are considered nanomaterials active against enveloped viruses, although not belonging to the same family of Coronaviridae, such as the different species of influenza A viruses [8]. The papers considered analyse animal enveloped viruses that are comparable to the study of nanomaterials activity against coronavirus [16].

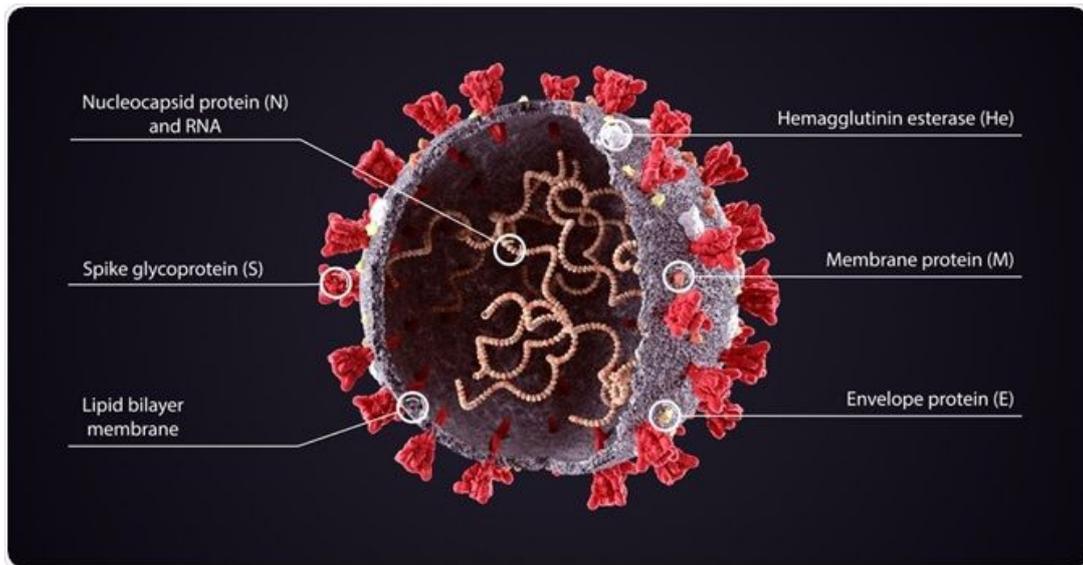


Figure 2.1: SARS-CoV2 virus structure [17]

1.3 Chemistry and properties of nanomaterials towards viruses

It is possible to summarize the phases that describe the extracellular interaction of a virus with a cellular system in 3 phases: the phase before the infection, the penetration of virus in the host cell and the immune response of the host cellular system [8]. The most common procedure to block or contain the virus spreading is to act before the infection, when the pathogen has not yet interacted with the host cell. To avoid the first phase of the viruses' attachment, the phase identified by the letter 'i' in figure 1.2b, antiviral nanomaterials intervene in 3 different ways [8].

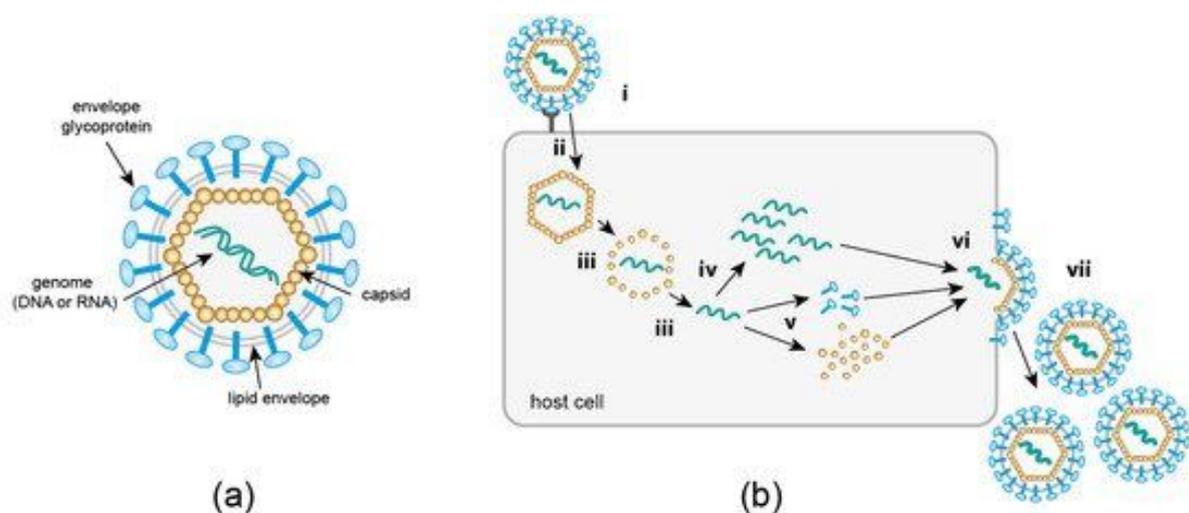


Figure 1.2: a) enveloped virus B) virus replication cycle in a host cell: i) virus attachment mediated by cell receptors; ii) cytosolic entry; iii) viral genomic release; iv) viral genomic replication; v) newly synthesized virion become progeny particle; vi) release of progeny virus [18]

These 3 mechanisms depend on the chemical characteristics, size, and shape of the nanomaterials. Moreover, the nanomaterials can undergo additional surface treatments to confer further antiviral properties or improve those they already possess. Therefore, once the virucidal agent has been synthesized, before the actual infection occurs it can:

- provoke the proteins' oxidation that makes up the virion envelope (denaturation of the external coating), mechanism shown in the left imagine of figure 3;

- mimic the surface of the host cell, to compete with the latter in the interaction with the virus, mechanism shown in the central imagine of figure 3;

- cause a mechanical braking of the virus, mechanism shown in the right imagine of figure 1.3. [8]

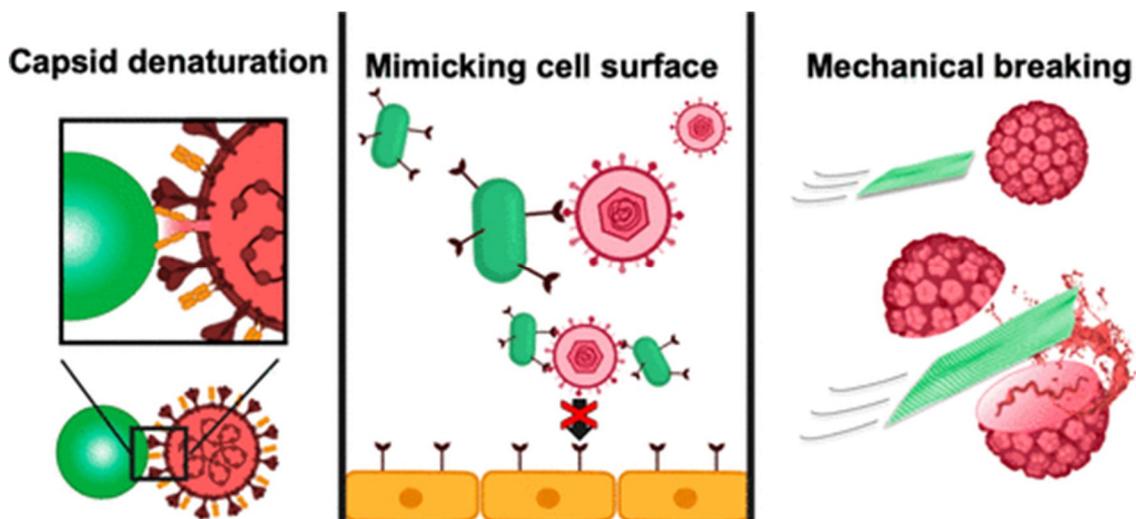


Figure 1.3: the main blocking virus entry mechanisms by hard nanomaterials (HNMs) [8]

The characteristics of the nanomaterials necessary in this field of application strongly depend on the mode of interaction between the virus and nanoparticles. Since viruses have nanometric dimensions, scientists began to consider nanomaterials, those that, based on their surface chemistry, can give a positive response in a general antiviral action [8].

1.3.1 Nanomaterials classification

Nanomaterials involve a large field of materials that can be classified on different criteria [4]. Based on the dimensionality and the general structure, it is possible to divide the nanomaterials in four classes:

- 0D, zero-dimensional nanomaterials have all the three dimensions in the nanoscale;
- 1D, one-dimensional nanomaterials have two dimensions in the nanoscale;
- 2D, two-dimensional nanomaterials have only one dimension in the nanoscale;

- 3D, three-dimensional materials have various dimensions in the nanoscale and are formed by multiple combination of nanocrystals in different directions [4].

The antiviral agents most studied are in 0D class, due to the chemical composition of the nanomaterials which belong to this group. Zero-dimensional class includes all the so called ‘nanoparticles’, which could assume different shapes and structures: spherical and hollow sphere, core-shell quantum dots, cubical, polygonal and nanorods. Metals and metallic oxides have a potential antiviral activity and they are synthesized mainly in the form of nanoparticles [4].

2D nanomaterials are also studied in the fight against the viral spread, graphene is the major exponent of this class [4].

1.3.2 Chemistry and properties of nanoparticles towards viruses

1.3.2.1 Nanoparticles size

Today the research for the vaccine for Covid-19 continues and numerous drugs are being analysed and tested, but, as reported above and as Sportelli et al. [3] also underline, to limit the spread of viruses it is important to increase the research in the use of nanotechnologies for prevention techniques, rather than for treatments and diagnoses, which in any case are deepening more and more. In fact, scientists report that the improvement of personal protective equipment (PPE) and the development of new antiviral coatings represent an important help, both for the current situation and for any future pandemics. In the past, it was said that the spread of new viruses was always possible: SARS-CoV-2 is not the first and will not be the last. Therefore, unlike antiviral treatments consisting of vaccines and drugs, antiviral nanomaterials would represent expendable solutions for a future global health emergency [3].

The fact that nanomaterials are active against multiple viral species is primarily due to their characteristic size [19]. Nanomaterials, in general, are structures that have at least one size within the nanometric range, the same range in which the diameter of SARS-CoV-2 and many viruses falls. The dimensions close to that of the virus, often even smaller (Vazquez-Munoz et al. [19] suggest a range between 1 and 50 nm), allow the interaction of the nanomaterial with the whole viral particle or with the surface proteins or other structural parts, so to inhibit or inactivate the viral power of the pathogen [19]. Figure 1.4 shows the size comparison between the SARS-CoV-2 virus and a nanoparticle with a size of 20 nm.

To get a clearer idea of the interaction between viruses and nanoparticles it is necessary to report some range of sizes treated in this review:

- Nanoparticle range size is about 1-100 nm.
- Viral range size is about 8-160 nm (for SARS-CoV-2) [15].
- Glycoprotein range size is about 20 nm (for SARS-CoV-2 spikes) [20].

- Bacterial range size is about 1.0-2.0 μm for the length, with radius about 0.5 μm (for *Escherichia coli*) [21].

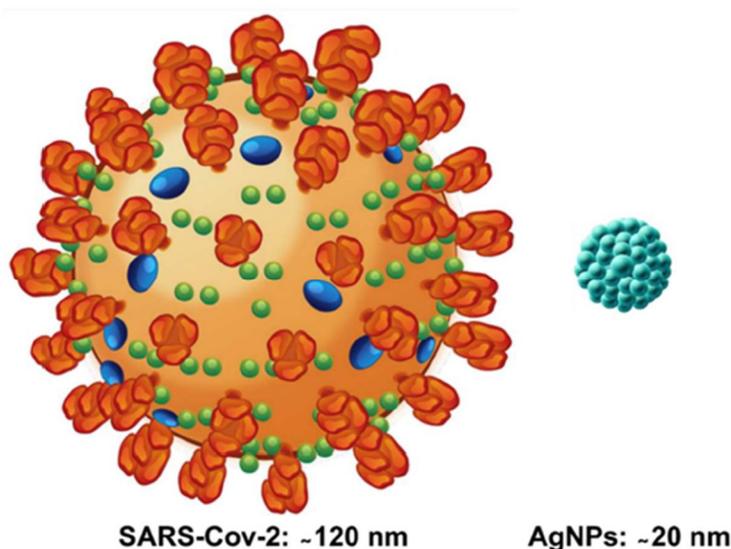


Figure 1.4: size of the virus SARS-CoV2 and size of AgNPs (silver nanoparticles) [19]

One of the most present and observed mechanisms of interaction is that involving the proteins of the viruses' surface envelope. The nanoscale materials therefore seem to respond to the two main tasks of the antiviral solutions studied in this work: they intervene on the viral particle before infection occurs and they are active against enveloped viruses [19].

The reduced size of nanomaterials, in addition to favouring interaction with the virus for reasons of dimensional comparability, leads to a high surface area, which increases as the size decreases [19]. As the surface area increases, the percentage of surface atoms increases, creating unsaturated bonds due to the lack of nearby atoms. The higher percentage of unstable atoms allows to have a high surface energy and important physical-chemical properties [22].

The size is often smaller than that of the virus diameter, since the nanomaterials mainly intervene on some of its structural elements, such as in the case study in which metal nanoparticles of Ag or Au cause the denaturation of the surface hemagglutinin (HA) protein of influenza A virus, due to the breaking of disulphide bonds [8], or in the case study in which Ag nanoparticles bind with the gp120 glycoprotein knobs on the lipid membrane of the HIV-1 virus, thus preventing the binding of these glycoproteins with the host cell receptors [23]. Examples of surface proteins are visible in the stylized drawing of figure 1.4. Size reduction is the most developed method to increase the surface area and improve such inhibition mechanisms. The surface area could be increased by the creation of porous nanomaterials [24].

However, the nanometric size also has a lower dimensional limit, above which it is necessary to remain to obtain a good antiviral action [25]. In fact, it is necessary to consider the consequences of the size reduction, the limits and, according to the properties to be obtained, the optimal size range. Papp et al. [25] performed a study in which they compared the antiviral

action of sialic-acid-coated 14 nm gold Particles with a diameter of 2 nm and 14 nm on influenza A virus. The larger size nanoparticles had a stronger bond with virions than the 2nm ones, so the former inhibit the virus more. The 2 nm nanoparticles for the size too small present a weak bond with the virions, because they cannot bind multiple HA trimers simultaneously as those with a diameter of 14 nm. Therefore, it is true that decreasing the size of the antiviral nanomaterials increases the inhibition capacity against the virus, but beyond a certain level it could weaken or cancel the mechanisms of interaction between the nanomaterial and the pathogen [25].

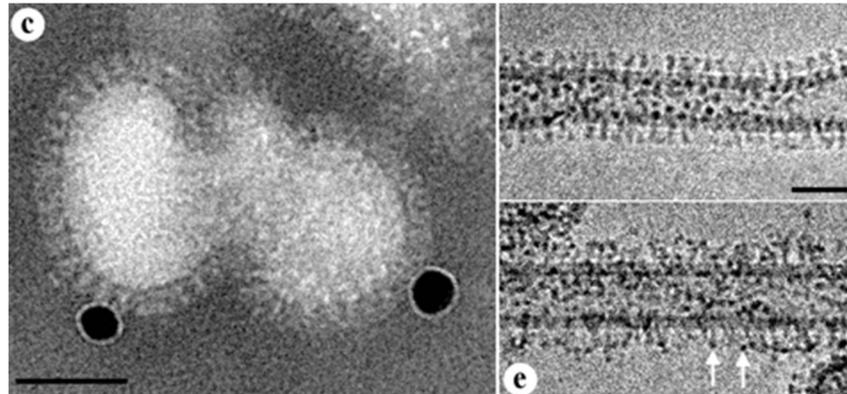


Figure 1.5: on the left there is a Cryo-TEM preparation of influenza A virions with AuNPs of 14 nm size, where the multiple attachment of particles to virions is visible, scale bar is 50 nm. On the right there are Cryo-TEM preparations of influenza A virions before (top) and after (bottom) 60 min incubation with AuNPs of 2 nm size. Arrows indicate positions of high density, which might indicate the binding of particles [25].

With the reduction in size it must also be considered the potential toxicity of nanomaterials on human cells, thinking about their potential engineering application on various protective devices, on respirators, on protective coatings and disinfectants. According to their application, in fact, these can be inhaled or have lasting contact with the skin. For this there are cytotoxicity tests [25]. Nanomaterials can be functionalized easily, examples have already been mentioned above, in which metal nanoparticles have been treated with specific ligands, this property can also be exploited to reduce excessive cytotoxicity of nanomaterials, for example in the case of polyvinylpyrrolidone (PVP)-coated AgNPs, tested on the Transmissible Gastroenteritis Virus (TGEV), belonging to the coronavirus family [26].

The functionalization of the surface is facilitated in nanomaterials of reduced size and is often applied to increase the antiviral action [8]. In fact, the higher surface area leads to a greater number of reaction point and adsorption sites. The search for the optimal size range is also valid for this, since by decreasing the size of the material it is possible to increase the concentration of ligands on the surface, but it must not be too low for reasons of steric hindrance. It is necessary to consider the properties and dimensions of the ligands and the dimensions of the nanomaterials on which they are applied, to guarantee a good functionalization [8].

The nanometric size of metal nanomaterials is responsible for a further mechanism of inhibition of the viral activity: they act as reserves of ions for their continuous and controlled release, ions aimed at producing radical species capable of destroying the viral lipid envelope [3]. This and the other modes of interaction mentioned previously will be analysed specifically in the next chapters.

These considerations about the size of the nanomaterials are valid for the activity against the virus before the infection, in the subsequent phases of the pathogen attachment they may not be the same [8].

1.3.2.2 Nanoparticles shape

The shape in which nanomaterials are synthesized represents the second most important factor that can influence their antiviral capacity. The shape is often linked to the crystalline structure of the material [8]. The most studied nanomaterials in general are nanoparticles, especially in the field of nanobiotechnology. Previous studies, in fact, count numerous experiments on silver and gold metallic nanoparticles, since their antibacterial power has been known since time. Only in recently times they were introduced into the search for antiviral precautionary treatments and technologies [8].

The optimal shape for the nanomaterial in its action against the virus, before infection occurs, does not have a general rule, but depends on the material taken into consideration and consequently on the mechanism of interaction [8].

As for nanoparticles such as silver or gold, which do not change crystalline structure, the virus inhibition mechanism occurs through the interaction of the metal with the surface proteins. The optimal shape has a high surface to volume ratio to increase the reactivity of the nanomaterials. Ag or Au nanoparticles exhibit greater antiviral activity than nanowires (with nanoparticles 0D and nanowires 1D) [26]. Lv et al. verified a greater reduction in the viral titre of the TGEV virus than Ag nanowires of different sizes, but also greater cytotoxicity [26].

Metal nanoparticles 0D such as AgNPS show different crystalline surfaces with different shapes: nanoplates have more {111} surfaces than nanocubes and the reactivity towards microbes could change on different surfaces [27].

A virus inhibition mechanism is the virion trapping on the surface of nanomaterials [28]. A factor that greatly affects the shape in this mechanism is the possible presence of a partial negative charge on the surface of the nanomaterial. This is important because the lipid membrane of enveloped viruses has a partial positive charge and the potential electrostatic attraction between the two opposite charges is exploited [28]. In the case of zinc(II) oxide ZnO structures, a partial negative charge is formed during the synthesis of the nanomaterials. The most favoured shape to grow are either one-dimensional canes or tetrapods due to the hexagonal crystal structure of wurtzite and the c-axis, the easiest axis for growth. The tetrapod shape of ZnO provides more arms and therefore more space to trap viruses via the partial surface

negative charge. This negative charge is due to the oxygen vacancy in the structure [28]. Figure 1.6 shows a scheme of ZnO tetrapod mechanism with and without the UV (ultraviolet) irradiation.

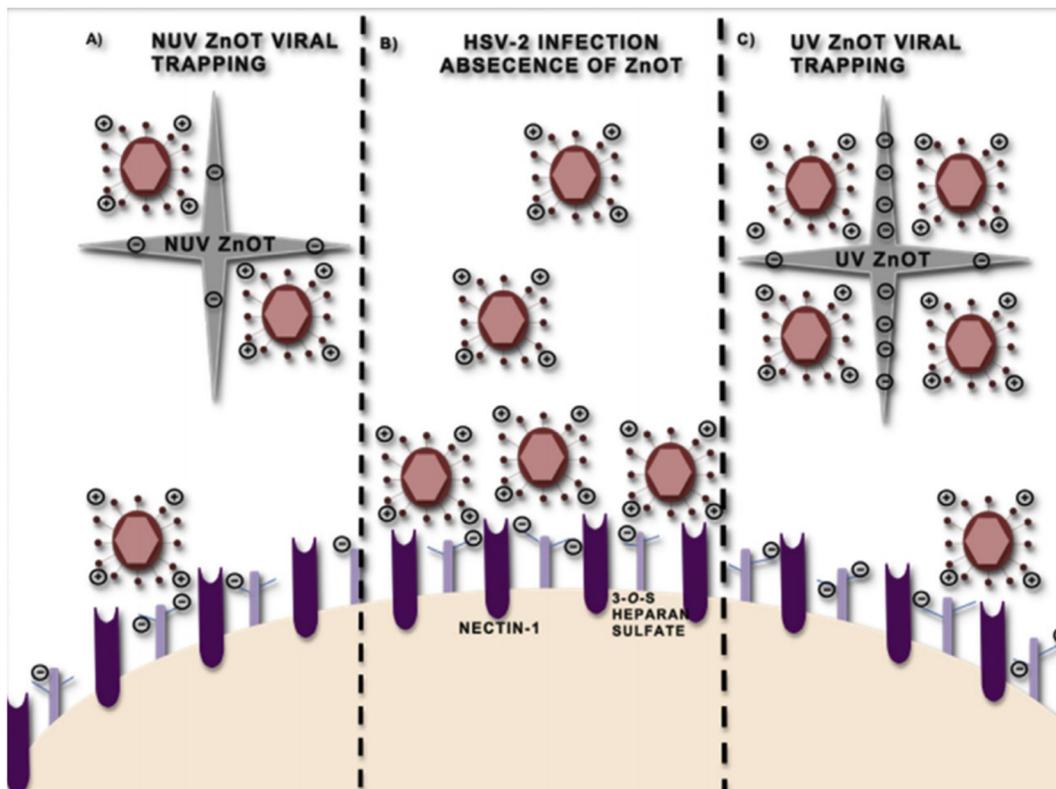


Figure 1.6: A) inhibition mechanism of tetrapod shape of ZnO structures with HSV-2 virions; B) HSV-2 virus attack without the ZnO tetrapods; C) improvement of the inhibition mechanism of ZnO tetrapods with HSV-2 (Herpes Simplex Virus-2) virions under a UV light treatment, which makes the nanomaterial more partially negative [28]

Therefore, the optimal shape of the nanomaterial depends on the type of material and therefore on the surface chemistry, but it is also necessary to consider that the possible shapes that can be applied may depend on the crystalline structure of the material [28].

In summary, the optimal shape that increases the antiviral power of the nanomaterial depends on the type of material, on its crystalline structure and eventually on its polymorphism [28]. Once these factors have been established, based on the mechanism by which the interaction between NMs and viruses occurs, the shape obtained after the synthesis must increase this mechanism [8].

1.3.2.3 Nanoparticles surface treatments

A nanomaterial is treated with surface treatments to obtain new properties or to increase or limit existing ones [8].

For the functions performed by antiviral nanomaterials, through the mechanisms previously mentioned, there are mainly two treatments used to increase antiviral activity:

- The chemical functionalization of the nanomaterial surface.
- A visible or UV lights treatment.

The first is related to the surface area characterized by high reactivity, due to the nanometric size of the nanomaterials. The instability of the surface atoms leads an easy functionalization of the surface through the implantation of a wide range of ligands. The chemical groups to be applied on the surface of the nanomaterial vary according to the desired function [8].

Functionalization can be used to increase the mechanism of interaction between the virus and the nanomaterial, to exploit the synergistic effect of multiple mechanisms of interaction with the virus or to inhibit the potential toxicity and environmental pollution by the naked nanomaterial [8]. In the latter case, it is possible to functionalize the nanomaterial by creating a protective layer that limits its cytotoxicity, for example the Ag nanoparticles can be covered by polyvinylpyrrolidone PVP [26]. The PVP coated AgNPs are less active against enveloped viruses: it is necessary to choose the balance between cytotoxicity and the most convenient antiviral activity [26].

Therefore, the chemical groups for functionalizing the surface derive from studies on the surface of the lipid coating of the virus and on the interaction mechanism of the naked material and the virus [8]. Further studies can be done on host cell surfaces when viral infection occurs. In this way the groups involved in the reception of the viral surface proteins are selected, then it is possible to apply them on the surface of the nanomaterials and thus simulate the receptors binding with the virus. Organic sulphate or sulfonate groups are selected in the case of enveloped viruses [8].

The visible / UV lights treatments allow to increase the surface negative charge by inducing additional oxygen vacancies in the previously mentioned tetrapod ZnO structure and consequently increase the antiviral power of these nanomaterials [28].

Most commonly these latter treatments lead to the development of reactive oxygen species (ROS) capable of attacking the enveloped virus. In this way they activate the inhibition or virucidal mechanism of the nanomaterial [8].

These treatments are very specific, based on the selected nanomaterial and the consequent inhibition / virucidal mechanism, therefore they will be further treated in the sections dedicated to each material.

Structure with multiple porous could increase virion-nanomaterial non-specific interactions. This is the case of porous silicon nanoparticles, that can block virions better than non-porous silicon surface, also because the surface proteins of an enveloped virus could get stuck in the nano-porous [29] [30].

1.3.3 Chemistry and properties of 2D nanomaterials towards viruses

Ultra-thin 2D nanomaterials are investigated since 2004, after the exfoliation of graphene from graphite [31].

Carbon is an allotropic material and it can assume different morphologies based on the different hybridization of the atoms. Graphene is the atomic single layer obtained from the exfoliation of the carbon allotropic state of graphite. Carbon sp^2 hybridized atoms create double C bonds and organize themselves to form hexagons. Graphene is made up of an atomic layer of these C hexagons [31]. Graphene oxide GO is often used in bio-nanotechnology for its two-dimensional crystal structure of nanometric thickness due to the monoatomic plane of sp^2 C and other functional groups [32]. This structure conducts the electrostatic interactions between the surface negative charge of the GO and the positive charge of the viral lipid membrane. But another factor that contributes to the antiviral action of these sheets is the presence of their sharp edges. In this case, therefore, the shape of the nanomaterial induces the mechanical breakage of the lipid coating through the edges of GO. In addition to the entrapment due to electrostatic interaction there is a virucidal action given by the mechanical breakage of the envelope [32].

To exploit the synergistic effect of two or more mechanisms, a composite can be created to improve a combination of two different shapes given by two different materials. For example, the shape of GO sheets is useful for having a good uniform distribution of Ag nanoparticles [33]. GO sheets attract the membrane of enveloped viruses and act mechanically to break the envelope, while the nanoparticles bind to the proteins of surface of the virus by denaturing them [33].

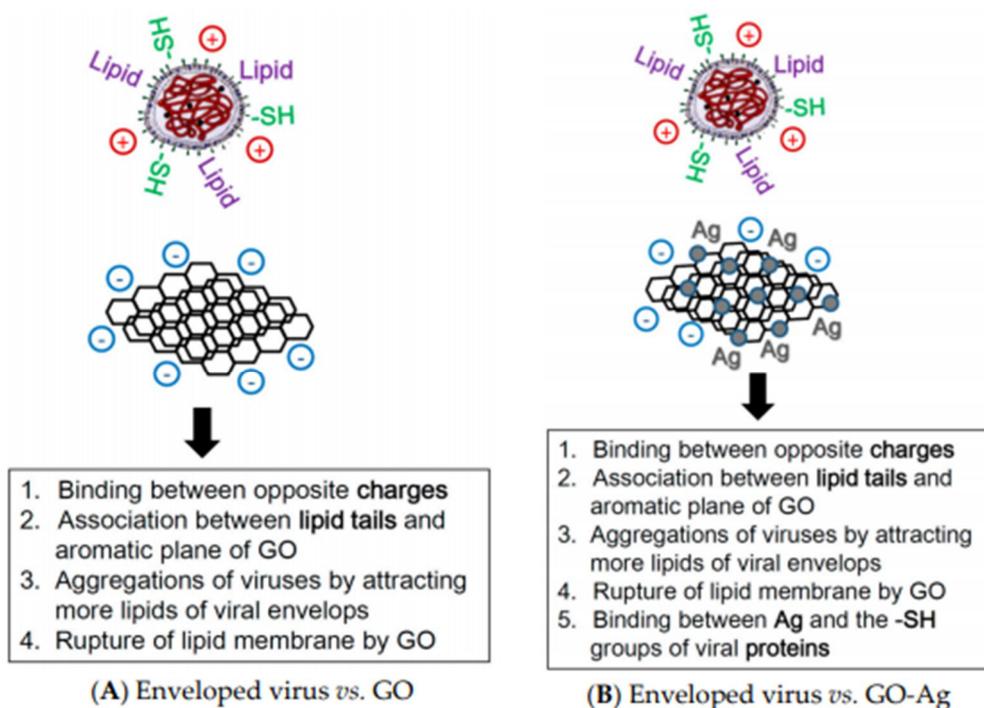


Figure 1.7: GO against the virus on the left and GO-Ag against the virus on the right [33]

In summary, including the shape considerations of the nanoparticles, Figure 1.8 shows the three different mechanisms of interaction between the nanomaterial and a virus correlated to the shape and the chemistry of the material [8].

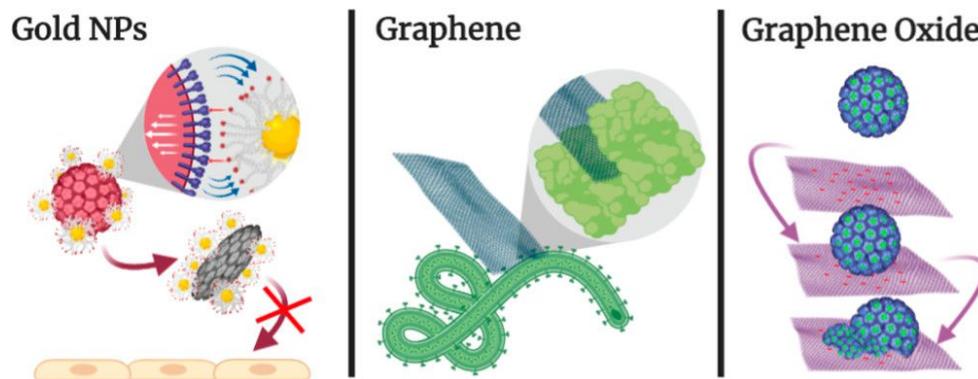


Figure 1.8: on the left an example of spherical shape: functionalized AuNPs binding with the viral envelope proteins; at the centre an example of mechanical rupture of the virus envelope due to the graphene sharp edges; on the right an example of the trapping and denaturation of the virus envelope due to the surface chemistry and the shape of graphene oxide [8]

As with nanoparticles, the surface area of these ultra-thin nanomaterials is widely modified to improve the antiviral efficacy [34]. A functionalization that increases the interaction between the surface of the nanomaterial and the virus, depends to the mechanism by which it occurs. For example, in the case of an electrostatic attraction due to the partial negative charge of a GO sheet, it is easy to implant on the surface of the sheet chemical groups that increase the partial negative charge [8]. Some chemical functionalization groups, on the other hand, could be exploited to introduce a further interaction mechanism in conjunction with the already existing one [34]. For example, Donsky et al. on the graphene sheets exploited the synergistic effect between the electrostatic and hydrophobic interactions, functionalizing the surface of the nanomaterial with long alkyl chains [34].

Chapter II

Metallic antiviral nanoparticles

The first nanomaterials considered in scientific research for their antiviral properties were the metals Ag, Au, and Cu. Generally, the previous literature constitutes an important factor in research. The named metals are the ones that scientists have been using in antibacterial research for years. The antibacterial potential of Ag on the wound surface has already been recognized since the 19th century [35]. Over the years, the study of these new antibacterial agents has been implemented by the fact that over time the bacteria can show resistance to their specific antibiotics. Unlike specific solutions, metallic nanomaterials are seen to exhibit interaction with the cell membrane of the bacteria, thanks to the bonds with the external proteins of the pathogenic envelope containing sulphur groups [36]. Since viruses also have similar proteins that they use to attach the host cell, these fewer specific solutions have also been introduced in the field of antiviral agent's research [35]. Also, for viruses they represented solutions applicable to a more diversified range of pathogens. In fact, the interaction and attack on the host cell occur in a similar way for many viruses, while the replication of the genome and the infection in the host system take place in different ways for each viral species [37]. Metallic nanomaterials have great antiviral potential due to their chemical, physical and biological properties and their versatile surface functionalization. In antibacterial research, Cu is the element that has shown a greater bactericidal action, but at the same time also greater cytotoxicity towards cells due to its instability. Ag has a slightly lower bactericidal potential than Cu and Au is the least toxic material among the three, but also the one with the weakest bactericidal action [8].

2.1 AgNPs

2.1.1 The interaction between AgNPs and biomolecules

2.1.1.1 AgNps and bacteria

Silver is the antiviral nanomaterial most studied by the scientists of the latter years. This agent has been introduced into medicine due to its extensive antibacterial characteristics [35].

Silver has been used extensively throughout human history, first in jewels and tools. Silver jewels was thought to bring health benefits to users and in ancient Indian medicine, it was used to treat some diseases. Later it was introduced in the field of medical devices and biomaterials,

for example about the creation of silver teeth and catheters. In the last century, many compounds with Ag have also been used in medicine [35].

It was said that the widespread use of antibiotics has contributed in part to the development of a microorganism's resistance to antibiotics and drugs, through some microbial modifications developed over time [36]. Over 70% of bacterial infections are resistant to one or more antibiotics generally used against the infection. This has led to the need for new drugs. Therefore, with the advent of nanotechnology, metals have been reused and renewed as bactericides. Each metal has a mechanism of action against the bacterium. There has been a meeting between historical cures and the new science [36].

Pure Ag nanoparticles were created. The toxicity of the metal is mainly due to its ability to interact with the chemical groups that make up the surface proteins of the bacterial membrane. Thanks to the nanometric size, the interaction between nanoparticles and pathogens is increased due to the consequent bigger specific area. The antimicrobial action of Ag was tested on various bacteria and fungi, subsequently on viruses [38].

According to the literature in general, Ag acts on bacteria [38]. Engineered Ag nanoparticles are toxic to the bacterium through multiple simultaneous mechanisms, unlike antibiotics. For this reason, Ag nanoparticles represent a good unspecific antibacterial solution [39]. The interaction of Ag nanoparticles with the biomolecules of various pathogens has not yet been widely understood. Various studies highlight the fulcrum of the interaction in the affinity between the Ag and disulphide or sulfhydryl groups of enzymes involved in metabolism or of membrane proteins [40]. Damage to the bacterial cell is given both by AgNps and by their ionic release: since Ag is considered a Lewis acid, it has a natural tendency to react with a Lewis base, such as P or S contained in the biomolecules of the pathogen. It is important to highlight the studies that have been carried out on the interaction of Ag nanoparticles and bacteria, because they could encourage studies on the AgNPs effects against viruses. The application of this nanotechnology to fight the spread and the consequent viral infections has only recently been carried out and has very often obtained good results [27] [40].

Scientists state that the most functional mechanism involved in the bactericidal activity of Ag nanoparticles is the one involving P or S atoms [40]. In bacterial cells, sulphur is found mainly in reduced form in the sulfhydryl groups (SH-, oxidation state -2) of the amino acids cysteine and methionine [15]. It must be said that Bacteria are divided into two fundamental groups, Gram-positive and Gram-negative. The different characteristics of the cell wall allow to distinguish the two groups. Gram-negative have an outer membrane with a double layer of lipids and glycolipids and associated proteins that is not present in Gram-positive [15]. Ag nanoparticles in general are more effective against Gram-negative bacteria, this could be given by the different outer membrane of the bacterial cell. The sequent considerations are on Gram-negative bacteria.

Escherichia coli is the most widely used model for Gram-negative bacteria. The envelope proteins, when they must fold correctly, involve the formation of one or more disulphide bonds

[41]. These bonds give greater stability to the outer membrane and are created by the oxidation of two neighbouring cysteine residues. Cysteine is one of the least residual amino acids found in proteins, and its unique chemical and physical properties often make it fundamental in biological activity. In addition to forming disulphide bridges in proteins, they have important functions in enzymatic activity thanks to their skills as nucleophiles. Cysteine residues in enzymes must be protected from oxidation to preserve enzymatic activity. In *E. coli* 40% of the envelope proteins have Cysteines. Most proteins have these cysteine residues involved in disulphide bonds and as for enzymes, only a small group use cysteine-based chemistry [41]. Despite their low presence, they are very critical elements in the bacterial cell, therefore the interactions of the nanometal with thiols and the disulphide bonds of cysteines in reduced and oxidized form respectively, could lead to important dysfunctions of the bacterial cell in the membrane and in some enzymes, up to its death [41]. Cysteine residues are also present in the envelope proteins of enveloped viruses. To understand these interactions, it is also necessary to study the differences between the interaction of AgNPs with cysteine residues in reduced and oxidized form [42].

In proteins, oxidized cysteine residues are the most common. So, the AgNps interact with the cysteine residues, the importance of this interaction effect is a function of the structure and conformation of the organothiols [42]. The initial binding between proteins and AgNps is independent of the oxidation state of the cysteine residues: the first forces involved are electrostatic and van der Waals forces, the nanoparticles have a slightly positive charge and the external lipopolysaccharide (LPS) bacterial membrane have partial negative charge [40]. The interaction with the reduced cysteine residues is favoured than that with the oxidized cysteine residues [42]. In the first case there is a direct reaction of the AgNP with the thiol group, in the second case, after an initial interaction, the disulphide bond is cleaved and it is subsequently absorbed on the Ag nanoparticle as thiolate. Furthermore, the disulphide bond is less exposed than the thiol group of reduced cysteine, because proteins use the disulphide bond to maintain a more globular shape, so it is located in the interior core of the protein structure. Thus, reduced cysteine can dissolve Ag nanoparticles more significantly than cysteine in oxidized form [42].

Several studies [36] [40] describe the action of nanoparticles on Gram-negative bacteria characterized by a first approach to the cell membrane, thanks to their large surface area they have a better contact with the microorganism, such as to disrupt the normal membrane permeability and such as to cause morphological changes on the membrane. They subsequently penetrate the membrane and can cause enzyme denaturation, compromising the cellular metabolism and leading to its death. Nanoparticles can also release Ag^+ ions in the presence of oxygen, which easily interact with electron-donor biomolecules, such as disulphide and sulfhydryl groups, altering the three-dimensional structure of proteins and blocking some active sites present in enzymes [39]. Ag^+ ions can compromise the sodium-potassium pumps transport of K^+ of microbial cells and can give insoluble compounds by reacting with DNA and RNA. Finally, the Ag nanoparticles can create oxidative stress in bacteria, generating ROS and other reactive species and causing damage to the DNA (DeoxyriboNucleic Acid) and cell membrane by hyperoxidation of proteins, lipids and other biomolecules [39]. Structural changes in the cell membrane with the formation of electron dense granules, formed by Ag and S, have been

demonstrated [43]. Feng et al. [43] show the presence of Ag^+ in the electron-dense granules that form on the bacterium *E. coli* and *Staphylococcus aureus*.

The optimal size for a greater effectiveness of the nanoparticles against bacteria is between 1 nm and 10 nm, while the shape that interacts the most is the one that exposes faces $\{111\}$, faces that interact directly with the bacterial surface. The octahedral, multiple twinned, icosahedral and decahedral form have mostly facets $\{111\}$ (figure 2.1), and have a good reactivity towards bacteria, visible in figure 2.2 [44].

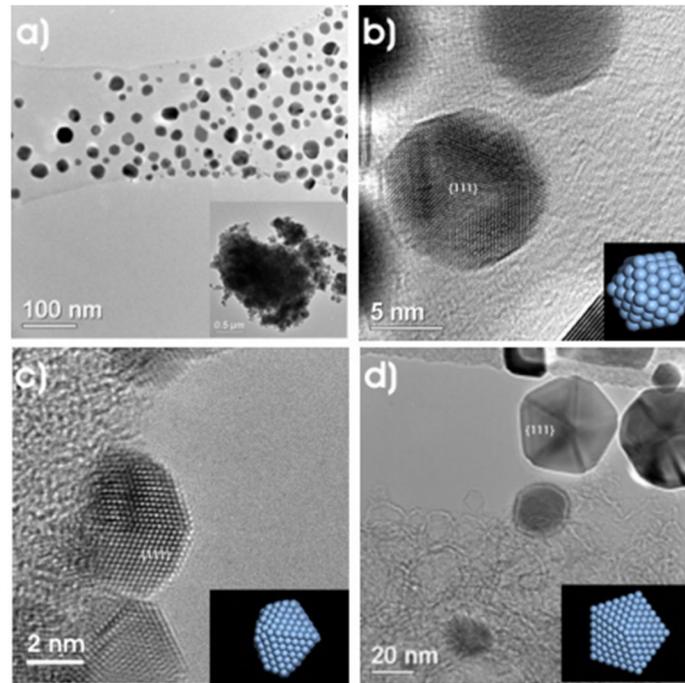


Figure 2.1: (a) TEM (Transmission electron microscopy) image of the silver nanoparticles released from a carbon matrix; (b) icosahedral particle, (c) twinned particle and (d) decahedral particle [44]

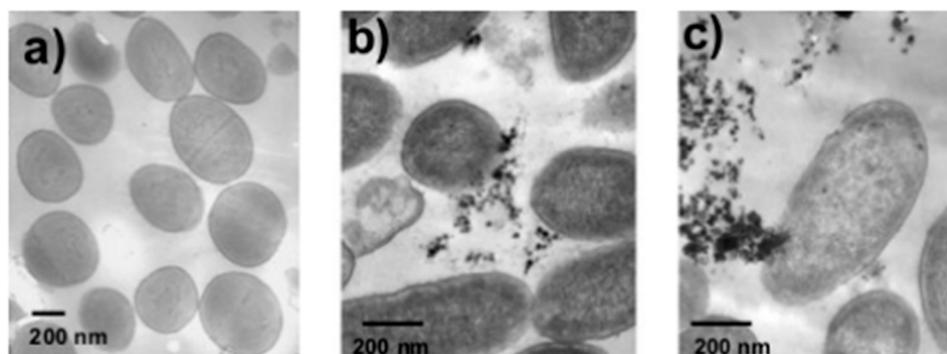


Figure 2.2: TEM images of a *P. aeruginosa*: (a) Control sample without Ag nanoparticles (b) and (c) samples previously treated with AgNPs. The Nanometal is inside the bacteria and it causes damages in the bacterial membrane [44]

Facets $\{111\}$ are more reactive than facets $\{100\}$ [27]. Pal et al. [27] studied different forms of Ag nanoparticles towards *E. coli*, the most effective ones were the nanoplates (visible in figure 2.3), consisting of a major content of facets $\{111\}$ compared to spherical nanoparticles and nanorods of Ag. These last two types of nanoparticles, in fact, show a ratio of $\{100\}$ surfaces to $\{111\}$ surfaces greater than nanoplates, spherical particles often have a cubic-like shape with facets $\{100\}$ or cuboctahedral shape, while the nanorods have facets $\{100\}$ on the sides and $\{111\}$ at their end [27].

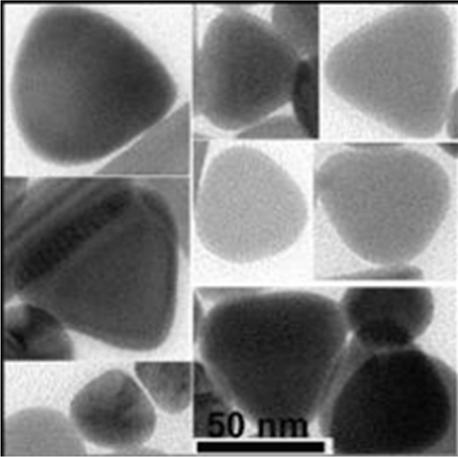


Figure 2.3: TEM image of the purified truncated triangular particles [27]

Pal et al. having experimentally observed the antibacterial efficacy of the nanoparticle shape with a major content of $\{111\}$ surfaces, cite a study performed by Hatchett et al. in which it is established that facets $\{111\}$ can absorb more thiulates than facets $\{100\}$ [27] [45]. Thus, it could justify the greater reactivity of the $\{111\}$ facets. Hatchett et al. observe the oxidative adsorption of HS^- from basic solutions on three low-index Ag electrode surfaces. Based on the atomic / ionic and van der Waals ray ratios between sulphur and Ag atoms, HS^- surface coverage is lower in $\{100\}$ surfaces [45].

Figure 2.4 shows the facets dependence on the Ag nanoparticles morphology.

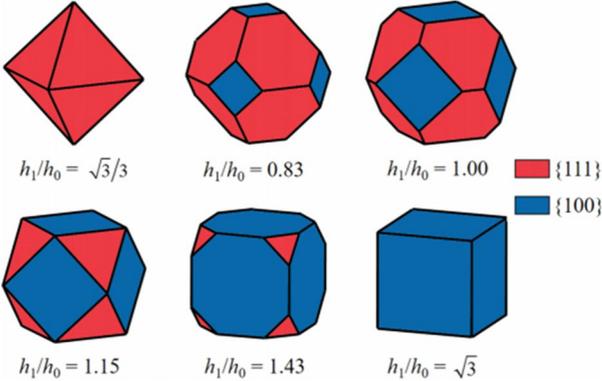


Figure 2.4: geometrical shapes of FCC nanoparticles from octahedral to cubic morphology [46]

In conclusion, the studies on Gram-negative bacteria show greater effectiveness for particles with a size between 1 and 10 nm and with a shape that favours facets {111}, such as Ag nanoplates. These nanoparticles initially interact through electrostatic and van der Waals forces, once absorbed on the surface of the pathogen they react with the biomolecules containing S or P [27].

2.1.1.2 AgNPs and virus glycoproteins

As in bacteria, also in enveloped viruses, sulphur is present in the form of sulphhydryl in the proteins' cysteine residues. The mechanism observed for the interaction between Ag nanoparticles and enveloped viruses involves the interaction of the nanometal with the virus's glycoproteins [35].

Glycoproteins are transmembrane proteins that often have a highly developed external domain. Often multiple monomers of these proteins combine to form characteristic structures, the spicules, visible in the electron microscope [15]. Many of these are involved in interacting with host cell receptors. The nanoparticles can interact with these glycoproteins in order to reduce, if not completely compromise, the possible attack of these pathogens towards host cells. Therefore, the interaction between Ag nanoparticles and viruses does not lead to the death of these microorganisms as in the case of bacteria, but to an inhibition of their infectivity: the virus loses or decreases its infectious power but remains alive. By neutralizing the infectivity of the virus, infection of other cells is prevented, and the spread of the virus is stopped [35].

Glycoproteins have cysteine residues, often involved in the disulphide bonds [47] [48] [49], which maintain the tertiary structure of these proteins for different steps of the replicative viral cycle. Some cysteine residues, localized towards the aminoterminal region of Human Immunodeficiency Virus-1 (HIV-1) gp120 glycoprotein, appear important because specialized envelope glycoproteins (fusion proteins) trigger fusion of the viral membrane with the cell membrane [49]. In the HCV (Hepatitis C virus) glycoproteins, the reduced cysteine residues are essential for the infectivity of the virus: the researchers propose a thiol-disulphide isomerization mechanism that occurs during virus infection, where the free thiol groups present form disulphide bonds, after the interaction with the surface of the target cell, to create a complex essential for the fusion of cell and virus membranes [50]. Also, in the case of the SARS (Severe Acute Respiratory Syndrome) coronavirus, a spike protein domain flanked by cysteine residues is important for activation of membrane fusion during virus infection [48]. These viruses are examples that show the presence of cysteine residues on the glycoproteins of the envelope. Therefore, based on the considerations made for bacteria on the interaction of Ag nanoparticles with reduced or oxidized cysteine residues, it would be appropriate to analyse the structure of the glycoproteins and of the envelope for each virus to be subjected to this type of analysis, to optimize the inhibition mechanism [35].

2.1.2 AgNPs against viruses

One of the first studies with Ag nanoparticles was focused on the interaction with the HIV-1 virus [51]. The HIV-1 virus has a lipid envelope like SARS-CoV-2, which has knobs of glycoproteins formed by trimers that have two subunits. One subunit is made up of the gp120 glycoprotein, which has disulphide bonds, striking sites for viral-nanoparticle interactions [23]. Elechiguerra et al. observed that the size of AgNPs attached on viral envelope was in the range of 1-10 nm [23]. Through the high angle annular dark field (HAADF) scanning transmission electron microscopy they analysed the interaction between the nanoparticles and viruses. Figure 2.5b shows the darker regions on the viral particle corresponding to the glycoprotein's knobs with a centre-to-centre distance of 22 nm on the viral surface. Figure 2.5a shows the virus treated with AgNPs (the lighter points). The Ag nanoparticles show a corresponding centre-to-centre spacing of 28 nm attached on the viral surface. The spacing between glycoproteins correspond to the centre-to-centre distance between AgNPs. Furthermore, nanoparticles larger than 20 nm showed no strong interactions with the viral particle. This important study could support the proposal of the mechanism of interaction between AgNPs and HIV-1 virus through the gp120 glycoprotein. To have the maximum number of nanoparticles attached to the gp120 glycoprotein knobs, the size of the diameter of AgNPs must be less than 20 nm [23]. Figure 2.5 shows the interaction between the nanoparticles and the virus and their spatial dispositions.

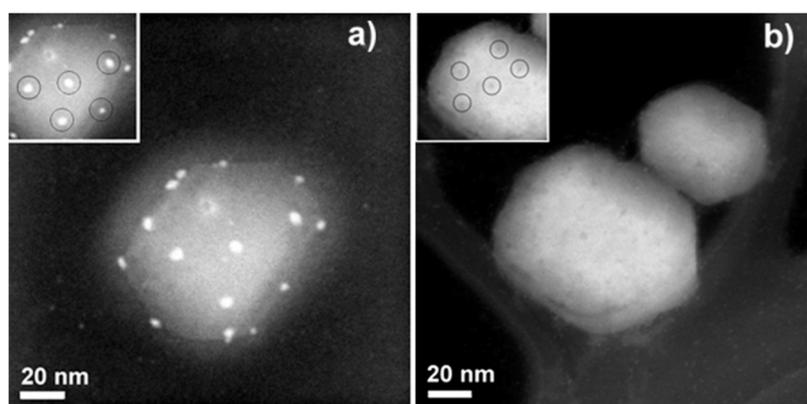


Figure 2.5: a) HAADF image of HIV-1 virus interaction with BSA-conjugated AgNPs; b) HAADF image of HIV-1 virus without AgNPs treatment [23]

The HAADF (High-angle annular dark-field) images in figure 2.5 show that the nanoparticles attached to the viral envelope are that those have dimensions of 1-10 nm: the smaller the diameter the stronger the bond [23].

Rogers et al. [52] investigated the effect of these nanoparticles on another enveloped virus, the Monkeypox Virus (MPV). Also, in this study they identified that the best inhibition on the infectivity of this virus was performed by 10 nm AgNPs, thus assuming an interaction mechanism similar to that observed by Elechiguerra on the HIV-1 virus [52]. The size of the nanoparticles must not be too small by a few nm as they could become too toxic for our cells, if these nanomaterials were, for example, inhaled in an excessive dose. In general, Ag nanoparticles, due to the low doses effective against microorganisms tested in different studies, have low toxicity. This characteristic is one of the factors that make these nanomaterials very

attractive for these researches, in addition to the fact that they would represent economic solutions [23].

Lara et al. [51] going on in the studies of Ag nanoparticles on viruses, they verified that these inhibited the infectivity of various strains of HIV-1, demonstrating that the antiviral action was general and non-specific. This was further confirmed by their action against viruses of different species [51]. Lv et al. [26] studied the effect of Ag nanomaterials in different forms. As was introduced in chapter 1, the nanoparticles are more effective than the nanowires against the transmissible gastroenteritis virus (TGEV), an enveloped virus belonged to the coronavirus family. The study reports the reduction of the TGEV viral titer at different concentrations of the Ag nanomaterials. The shapes studied are the spherical one, by nanoparticles with a mean size of 39 nm, and the rod-like-shape by two different diameters average nanowires, 60 nm and 400 nm, with the length dimension not in the nanoscale [26]. The mechanism of interaction suggested from the data could be again a direct interaction between the AgNMs (silver nanomaterials) and the surface proteins of the Transmissible gastroenteritis coronavirus (TGEV), such as TGEV S glycoprotein. The report, at the higher concentration of AgNMs, indicates the Ag nanoparticles as the most effective nanomaterials against the TGEV infection [26].

Most of the studies with Ag nanomaterials uses the nanoparticles.

The mechanism of interaction between Ag nanoparticles and virus surface proteins has been analysed in several studies with different viral species. As in the cases of HIV-1 virus and MPV virus, Tacaribe virus also has envelope's gp120 glycoproteins, which Speshock et al. identify as sites of attraction for AgNPs [53].

IFV influenza viruses have two main glycoproteins: hemagglutinin (HA) and neuraminidase (NA). In these viral species, HA binds the receptors of the host membrane [54]. The inhibition of the H3N2 virus by the spherical nanoparticles used by Xiang et al. could occur through the interaction of the nanometal with this glycoprotein HA [54]. In fact, the analyses carried out in the study show a reduction of the hemagglutinin virus titre of the viral particles treated with AgNPs. The antiviral action therefore seems to be linked to hemagglutination activity. Each hemagglutinin molecule has two disulphide bonds, which they bind to the host cell after their breaking. But such disulphide bonds could be the sites of interaction between AgNPs and the virus. This interaction could explain the destruction of the morphological structure of the virus, visible from the TEM images in figure 2.6 [54].

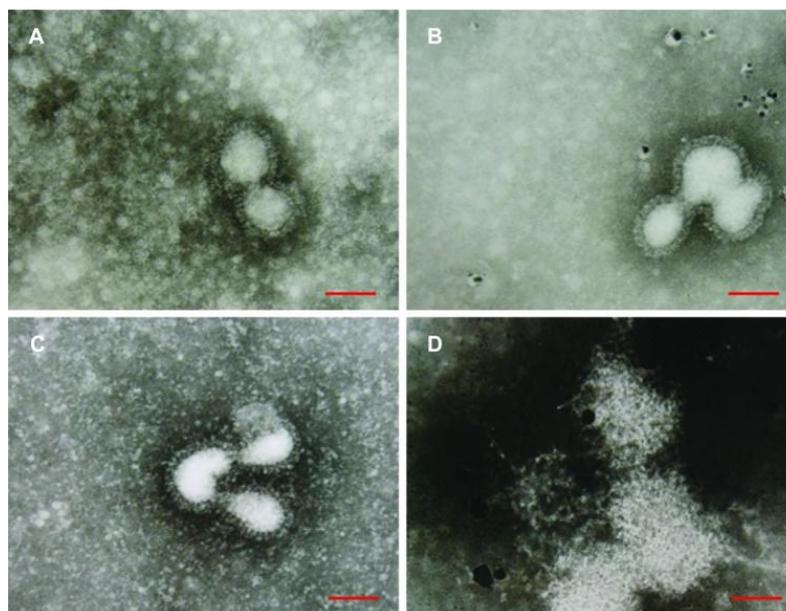


Figure 2.6: TEM images of AgNPs-treated H3N2 IFV. (A) Virus control. (B–D) Viral morphologic structures damaged with 50 µg/mL AgNPs for 30 minutes, 90 minutes, and 120 minutes, respectively [54]

The same type of interaction is also assumed with H1N1 virus, since it also has a reduction of the hemagglutinin titre virus [55].

2.1.3 Modified AgNPs against viruses

The potential antiviral action of naked Ag nanoparticles has been extensively verified on several viral species. These are nanoparticles that are very effective in inhibiting viruses, but they often tend to self-agglomerate and could be dangerous for environmental pollution, at high doses they could also be toxic to human health [8]. Careful attention should be paid to cytotoxicity analyses of these nanometals, as they would be applied in devices with oral or skin contact. For this, various coatings have been studied to be applied on the nanoparticles. The most used to reduce the cytotoxicity of AgNPs is poly (N-vinyl-2-pyrrolidone) PVP [8]. Lara et al. [56] use PVP-AgNPs against different strains of HIV-1. In the study, the inhibition action of the Ag nanoparticles increases with the incubation time of the virus with the nanometals. This could be given by the time required to first attach the gp120 glycoproteins and then break the disulphide bonds [56]. The antiviral action persists in the capped nanoparticles, with an efficacy that could be slightly less than that in naked AgNPs [56].

The surface treatments on the nanoparticles could also be carried out to increase the binding force between the antiviral agent and the virus, or to obtain a synergistic effect, given by the antiviral efficacy of the coating chemistry with the properties of the nanomaterials. The choice of the ligands depends on the antiviral mechanism exploited [57].

Orlowski et al. studied the effect of tannic acid-coated AgNPs against herpes simplex virus-2 (HSV-2) [57]. Tannic acid is the simplest and main hydrolysable tannin and tannins are

polyphenols capable of creating insoluble complexes with proteins and nucleic acids and of chelating metal ions. This strategy, therefore, creates a stronger inhibition with the virus envelope [57]. Baram-Pinto et al. studied the competition between host cell receptors and Ag nanoparticles with the glycoproteins of the HSV-1 virus [58]. In fact, the surface proteins of the virus form a bond with surface heparan sulfate (HS) during the attack on host cells. AgNPs capped with mercaptoethane sulfonate (MES), have sulfonate ends that mimic host cell surface HS [58].

Ag nanoparticles could also be coated with chemical species that would have their own antiviral activities, but which alone would be difficult to employ. Curcumin, for example, has instability due to its poor solubility in water, but good antiviral properties against respiratory syncytial virus (RSV). Therefore Gsuka et al. have created AgNPs capped with curcumin, able to interact with the RSV envelope glycoproteins, inhibiting the infectivity of the virus [59].

Among the metal nanoparticles, AgNPs have the greater antiviral efficacy, but their potential toxicity must be considered. This aspect will be analysed in a separate chapter.

2.2 *AuNps*

2.2.1 The interaction between AuNps and biomolecules

Metallic gold is a noble metal, like silver. The biocidal activity of Au is lower than the Ag activity and over the years it has been applied less than Ag in the field of antimicrobial agents. Au was exploited for the ease of synthesis and for its affinity to bind different ligands, in conjunction with its relatively low cytotoxicity [8]. Au is less reactive than Ag towards biomolecules, but like the nanoparticles analysed previously, it has a great affinity with S. The covalent interactions that lead to the formation of the Au-S bond, between the protein's cysteine residues and the nanoparticles, occur after protein absorption and first interaction on the surface of the AuNPs. As in the case of AgNPs, the initial interaction between AuNPs and proteins involves long-range or non-specific intermolecular forces such as electrostatic or van der Waals forces [60]. However, the surface interactions with proteins differ from those previously seen for AgNPs, also since the proteins absorbed on the AuNPs can form only a monolayer, while the AgNps can dissolve through the loss of Ag⁺ ions. The ions interact with the proteins, making available additional sites of interaction [42].

2.2.2 AuNPs against viruses

The synthesis of possible Au nanomaterials is preceded by the study of viral infection mechanism in host cells, to understand how to optimize the antiviral properties of the metal and how they could be applied against a specific virus.

The different strains of the influenza virus have been extensively studied. The mechanism of attachment and infection to the host cell is linked to the presence of the two main proteins HA and NA mentioned above [24]. The traditional drugs are NA inhibitor drugs, but over time, influenza viruses have developed resistance to these. Therefore, was studied the HA protein and its disulphide bonds as antiviral targets [24]. Ag nanoparticles are often modified because above to a certain dose they can present detectable toxicity [8]. Au nanoparticles do not need it. Kim et al. have studied the action of gold nanoparticles with a mesoporous structure (PoGNPs) [24]. In this way, the specific surface area is much greater than the surface area of non-porous nanoparticles of the same size. A higher specific surface area corresponds to a higher number of active sites which can react with the virus. The study compares the antiviral activities of porous gold nanoparticles with the efficiency of non-porous gold nanoparticles and of silver nanoparticles against H1N1 viruses and other strains of influenza viruses, H3N2 and H9N2 viruses. The silver nanoparticles considered have a 20 nm size average, while the two different types of gold nanoparticles have a 150 nm sized spherical structure. Generally, the antiviral activity of AgNPs is better than that of AuNPs, in this case also AgNPs are smaller, and this it should increase the antiviral effect. The antiviral activity of porous gold nanoparticles, on the other hand, is higher than that of non-porous gold nanoparticles and of AgNPs, despite the size higher than the latter. AgNPs have an antiviral effect higher than non-porous gold nanoparticles. This is due to the foam-shaped porous outer surface of PoGNP. The surface porosity of the gold nanoparticles leads to expose a higher number of active sites than those exposed by AgNps or at the same concentration. The PoGNPs, therefore, have a better availability of contact with the envelope of influenza viruses and, since Au has a strong affinity with the disulphide bonds present on the surface HA protein, they can inhibit viral activity. The interaction between the PoGNPs and the HA proteins of the virus inhibits the possible entry into the host cells [24]. Figure 2.7 shows the inhibition mechanism of the influenza virus with PoGNPs.

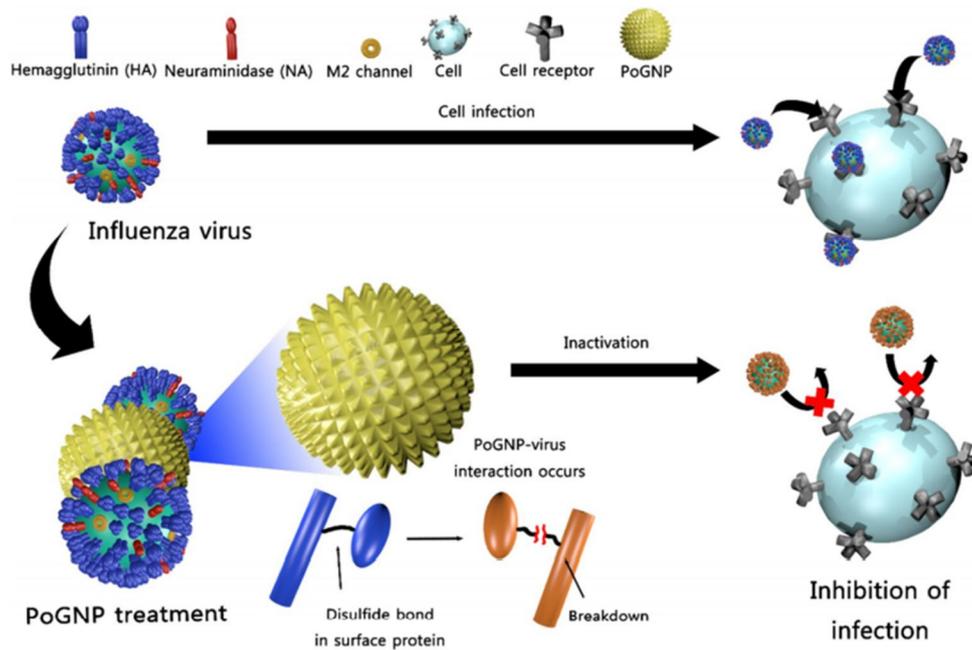


Figure 2.7: influenza A virus (IAV) inactivation with porous gold nanoparticle (PoGNP) [24]

Kim et al. suggest a possible antiviral application of these porous nanoparticles against viruses with spiked proteins, such as HIV and coronavirus, by cleaving their disulphide bonds [24].

2.2.3 Modified AuNPs against viruses

Gold nanoparticles can be superficially modified to enhance their antiviral activity, for example by reducing the risk of self-agglomeration as in the case of nanoparticles synthesized in the presence of gallic acid (GA) [61].

Most of the gold nanoparticles are used for the characteristics conferred by the chemical-physical properties assumed by the metal nanostructures. Therefore, they are often used to vehiculate different chemical groups which can inhibit or kill viruses. It is important that the basis of these ligands are gold nanoparticles, since often their biocidal activity depends on their orientation and arrangement given by surface facets of the nanometal. The Au among nanometals is used specifically for its minor cytotoxicity and for its inertness [8].

The chemical groups to apply on the nanoparticles depend on the method of inhibition chosen against a specific virus. Many scientists on gold nanoparticles have applied polyanions, consisting of polysulfates and polysulfonates, against influenza viruses [62] or BVDV (bovine viral diarrhea virus), surrogate for the study against the hepatitis C virus [63]. The polyanionic functionalized gold NPs seem to create an electrostatic interaction with the envelope of the influenza virus. The virus envelope shows a negatively charged lipid bilayer, so the surface of the virus should repel the anion groups, but the anion ligands interact with surface proteins, the viral glycoproteins, which have a residual positive charge [63].

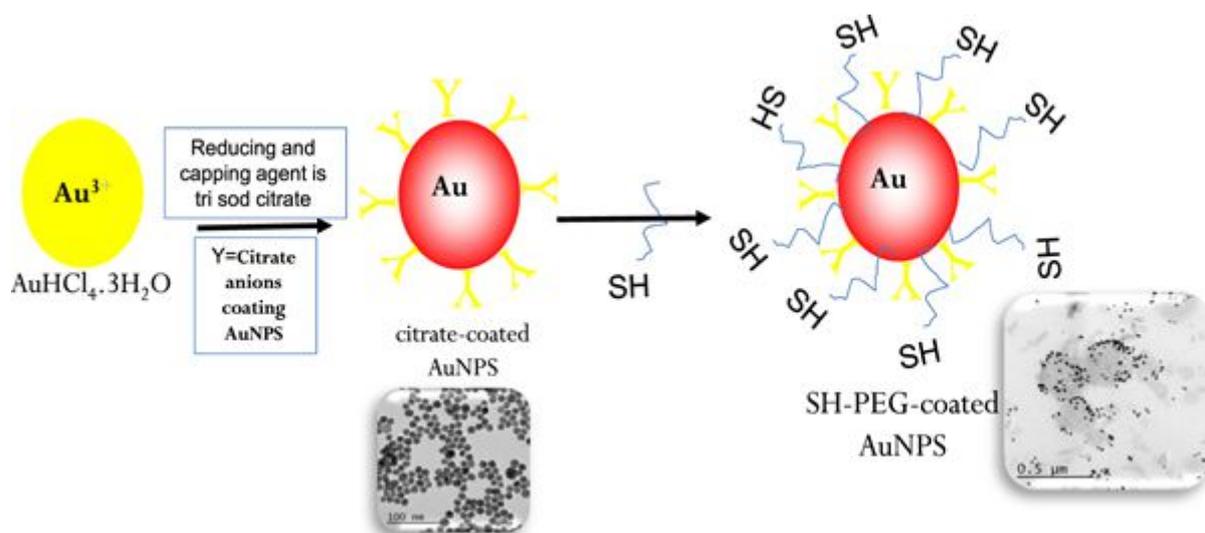


Figure 2.8: polyanionic Au nanoparticles [63]

The structures placed on the surface of the nanoparticles can have also polycationic dendritic forms, to interact with the virus. [64].

In this type of ligands not only the negative charge of the ends groups on the nanoparticles is important, but also their orientation. It is important to study the arrangement that these ligands assume on the surface of the nanostructured Au, based on the physical-chemical interaction. A more favourable orientation could lead to a greater antiviral action at the expense of a less negative charge: antiviral activity is not only governed by charge density, but also by the interaction between the functional group and the Au facets exposed on the surface of the nanoparticle [63] [62].

Papp et al. observed the presence of sialic acid residual on glycoproteins and lipids on the surface of the host cell, responsible for the interaction between influenza viruses and the cell [25]. In this study, therefore, they created sialic-acid-terminated glycerol dendron functionalized gold nanoparticles, to simulate the interaction that occurs during infection, competing with the host cell receptors. One of the most important evidences is the dependence of the type of attachment with the enveloped virus as a function of the size of the gold nanoparticles. AuNPs with an average size of 14 nm have a better antiviral activity than AuNPs with an average size of 2 nm. In fact, given the spherical shape of the viral particles, the 14 nm functionalized AuNPs allowed to have a better multivalent binding of several HA trimers to the nanoparticles [25].

Cagno et al. investigated a major upgrade for ligands emulating host cell reception sites with negatively charged heparan sulphate [65]. In fact, for example, AuNP functionalized with MES (linkers discussed previously for capped AgNPs), lose their inhibition efficacy against various enveloped viruses upon dilution. This is because it is a virustatic inhibition. Using as linkers on undecanesulfonic acid AuNPs (MUS-AuNPs), the inhibition of viral activity persists even after dilution (verified on HSV-1, HSV-2, RSV virus). MUS-AuNPs exhibit irreversible inhibition, due to the fact that in interaction with the virus they are able to change the structure of the viral

particle [65]. MUS are long and flexible linkers which create multivalent bindings with the enveloped virus, capable of inducing greater stress to the virion. Such stresses induced by MUS-AuNPs change the viral particle irreversibly. MES are linkers with sulfonic groups like MUS linkers, but with a shorter chain, which are unable to induce stress of this magnitude. MUS-AuNPs are virucidal nanoparticles, MES-AuNPs are virustatic nanoparticles [65].

Figure 2.9 shows the difference between virustatic and virucidal activity against an example of virus that normally interacts with heparan sulphate on the surface of the host cell during infection. Heparan sulphate (HS) is a linear polysaccharide which occurs as a proteoglycan (HSPG) [65].

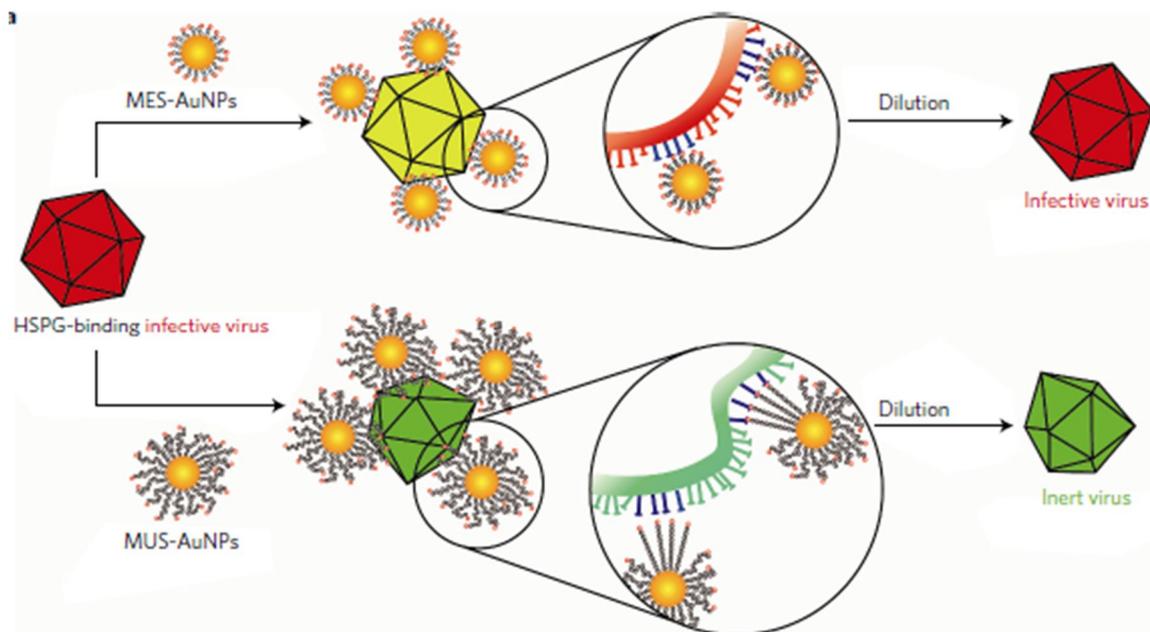


Figure 2.9: virustatic and virucidal activity against a HSPG-binding infective virus [65]

2.3 CuNPs

Cu is the third metal that has been most extensively studied for its bactericidal properties, but it is more unstable than the others two. Au and Ag are defined as noble metals, since they are resistant to oxidation in the air, while copper is easy to find in an oxidized state. Copper has a very strong microbicidal activity given by the release of Cu^+ and Cu^{2+} ions. The persistence of active pathogens on different surfaces has been studied, those in Cu and Cu alloys have the best action against bacteria and viruses, thanks to the oxide layer that forms above the surface. The corrosion and oxidation processes of metallic Cu and its alloys produce biocidal copper [66].

Based on the mechanism of the biocidal activity of copper, copper nanoparticles in the metallic state are not as interesting as those of ionic Cu. Therefore, the action of this metal against the virus is analysed in the next chapters, which include the study of copper oxide nanoparticles and other copper compounds.

Chapter III

Metal oxide antiviral nanoparticles

The interactions analysed between metallic Ag and Au nanoparticles and viruses could be useful to design and manufacture other models of antiviral nanomaterials. In the last years, in addition to nanometals, many compounds with metal ions, such as metal oxides, have been studied for their antiviral efficacy [67].

In general, metal oxide nanoparticles (MONPs) have several advantages:

- high stability;
- simple preparation processes;
- easy size and shape engineering;
- easy incorporation into hydrophobic and hydrophilic systems;
- easy functionalization by various molecules [68].

The metal oxides selected in the various studies have lower toxicological effects and better results in antiviral activities compared to other metal oxides [68].

According to the antiviral activity models analysed using Ag and Au nanoparticles, the surface of the new antiviral nanomaterials is expected to have strong physicochemical interactions with the proteins of the viral envelope. Once the nanoparticle has adhered to the viral envelope, it can prevent virus entry in the host cell and its replication, thanks to the interaction before the possible infection [69].

Metal oxide nanoparticles generally act through a second antiviral mechanism, such as the production of localized reactive species after the nanoparticle adhesion on the virus, with consequent degradation of the viral envelope receptors, the viral stability and the viral genetic material [68] [69].

Furthermore, in general, metal oxides are good candidates as coating materials, due to their chemical resistance and their efficacy against microbes. Thanks to the introduction of these nanomaterials, it is possible to overcome the limitations of traditional antiviral coatings [67].

3.1 Cu oxide antiviral nanoparticles

The Ag and Cu ions have the widest spectrum of antiviral actions. Cu oxides NPs are the simplest and cheapest nanoparticles formed by Cu compounds; therefore, they have different applications in industry [67].

Cu has powerful biocidal properties analysed in previous years especially against bacteria, in recent decades also the antiviral properties of this material have been studied [68].

Cu oxides nanoparticles and their biocidal activities include the two oxides corresponding to Cu +1 and +2 oxidation states: copper(I) oxide (Cu_2O) and copper(II) oxide (CuO), respectively. The two oxides in the expression of their antibacterial activity act through two different mechanisms [70]. Indeed, Meghana et al. [70] observed the different mechanisms of tryptophan capped copper(II) oxide (Trp- CuO) and tryptophan capped copper(I) oxide (Trp- Cu_2O) against the gram-negative bacterium *E. Coli*. The synthesized nanoparticles have sizes of about 30 nm for copper(II) oxide and about 40 nm for copper(I) oxide. The oxides nanoparticles, given by the two different oxidation states of Cu, have different crystalline systems: the CuO nanoparticles show a monoclinic crystallographic structure, while those of cubic Cu_2O have a face centered cubic lattice. In figure 3.1 it is possible to observe the TEM images of the two types of nanoparticles analysed [70].

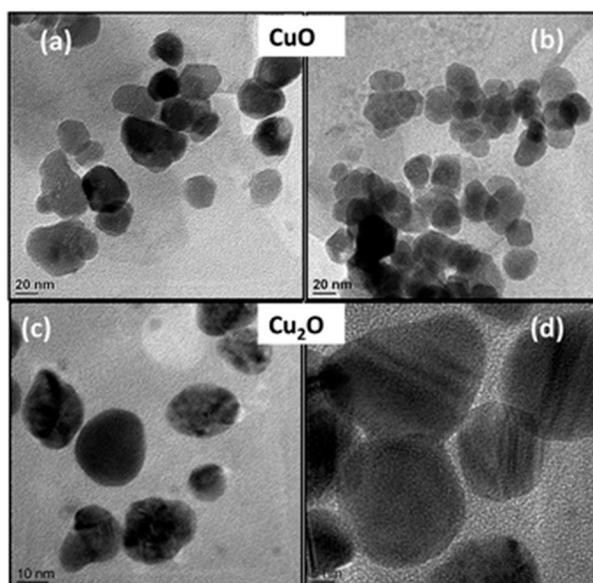


Figure 3.1: (a) and (b)-TEM images of Trp- CuO ; (c) and (d)-TEM images of Trp- Cu_2O [70]

Both nanoparticles cause cell membrane damages, and the subsequent analyses were carried out supposing that the nanomaterials could pass through nanopores formed by the bacterial membrane porines [70]. Based on their analyses, the authors of the article identify two independent and specific mechanisms for the two Cu oxides: Cu_2O would seem to have a great affinity with the intracellular proteins of the bacterium that are able to chelate Cu(I) ions, while

the involvement of reactive oxygen species (ROS) is more applicable for CuO. The bactericidal activity is more effective in the case of Cu₂O nanoparticles [70].

These observations on the greater effectiveness of Cu₂O nanoparticles compared to those of CuO were also observed on viruses [71]. A comparative study between the oxides was carried out to test the effectiveness of Cu oxides against influenza A viruses (H1N1) [71]. The particles considered do not have a nanometric dimension but fall within the range of micrometres: the copper(I) oxide particles have the diameter size in a range between 0.5 and 5 µm, while the copper(II) oxide in a range between 0.5 and 40 µm. In contact with the first particles, the influenza A virus shows a great reduction of the viral titer, while the contact of the virions with the solid state copper(II) oxide particles would seem to have little effect on the titer reduction, unlike the soluble compounds of Cu(II), which could denature the proteins present on the virus envelope by breaking the S-S bonds ($R-S-S-R + Cu^{2+} \rightarrow R-S-Cu-S-R, 2R-SH + Cu^{2+} \rightarrow R-S-Cu-S-R + 2H^+$). The action of the copper(I) oxide particles would seem to take place in the denaturation of the hemagglutinin HA protein present on the viral envelope, and generally the denaturation of a protein structure is caused by the partial cleavage of disulphide bridge [71]. To measure the S-S bond cleavage it is analysed the decrease in the concentration of thiols. The weak decrease observed would not seem to fully explain such a high antiviral activity. Therefore, it is hypothesized that these microparticles could attack virions through other mechanisms that could denature proteins, such as possible ionic interactions and disorders in the hydrogen bond network. [71].

The action of Cu oxides microparticles against influenza viruses was also analysed by Borkow et al. [72] in a study aimed at showing the anti-flu efficacy of N95 masks, which were obtained with particles of Cu oxides included in the polymer fibers. The authors assume that this effectiveness is due to the interaction of the copper ions with the virions that are trapped in the mask, but nevertheless they state that these mechanisms must be deepened [72]. The Cu oxides particles in the polymer fibers of the masks were obtained by the methods applied by Jeffrey Gabbay. In a patent application of which Gabbay is the author [73], is described the method of manufacturing antiviral and antibacterial polymeric materials with CuO and Cu₂O oxide powders. The size of the Cu oxides powder can vary according to the diameter of the fibers, so in the patent application between 1 and 10 µm, they never exceed 20 µm [73]. In these applicative studies the Cu oxides mechanisms are not distinguished [72].

Borkow et al. showed the efficacy of Cu oxides powder particles also against HIV virus [74] [75].

Borkow et al. used micrometric sized powders of Cu oxides in their studies and they assumed that the use of smaller sizes (in the nanometric range) of these particles could enhance the antiviral effects of the material. In fact, nanometric particles have many atoms at the corners and edges with high energy that facilitate the ionic release of the nanoparticles [74].

Particles of nanometric dimensions in the antiviral field have been applied and studied by Hang et al. [76] in a study involving the interaction of cuprous oxide nanoparticles against the

hepatitis C virus (HCV). These nanoparticles target the binding and entry step of infectious HCV virions into host cells. The size of the particles is 45.4 ± 6.8 nm and the scientists state that, based on the antiviral action shown, it would seem that their inhibition mechanism is not only due to the copper ions present in the solution, but also from a possible direct interaction between the solid state nanoparticles and the virion surface. This interaction could block the sites on the HCV envelope glycoproteins used for cell receptors binding [76].

Yughandar et al. [77], on the other hand, analysed the spherical copper(II) oxide nanoparticles, with dimensions ranging from 2 to 69 nm and produced by the extracted fruit of *Syzygium alternifolium* against the Newcastle Disease Virus (NDV). The growth inhibition of the NDV virus in the allantoic fluid increases in a dose dependent manner of CuO nanoparticles. The higher dose, however below the cytotoxic levels, shows no infection due to the NDV virus in the ovo test and the hemagglutination test is negative [77].

Cu oxide nanoparticles are very promising, especially for use in antiviral coatings and for their inclusions in PPE devices [72]. The antiviral mechanisms of action and the difference between the copper(II) and the copper(I) oxides should still be understood. What is certainly observed is the general positive result in the inhibition of enveloped viruses.

3.2 Fe oxide antiviral nanoparticles

Other promising and safe candidates among the antiviral metal oxides are iron(II,III) oxide (Fe_3O_4) and iron(III) oxide (Fe_2O_3) nanoparticles. The antibacterial activity of these metal oxides seems to take place through the production of reactive oxygen species (ROS) [68]. In this review it has been seen several times how the advent of nanotechnology has led to the development of antibacterial nanomaterials first and antiviral nanomaterials subsequently. Through nanotechnology, they have recently studied nanomaterials with enzyme-like properties.

The term "nanozymes" appeared for the first time in the paper of Manea et al. [78]. The authors observed that their triazacyclonane-functionalized AuNPs showed RNase-like behaviour, therefore they dubbed them "nanozymes" [78]. In the last decade, the study of *nanozymes* was more widespread, and to date these nanomaterials are formally defined as *inorganic nanomaterials with enzymatic-like intrinsic catalytic activities* [79]. However, these are new and recent materials, so a deepening in their research is still needed to increase their catalytic activity and specificity. Nanozymes are advantageous over natural enzymes for higher stability, low cost, recyclability, easier storage, and easy multi-functionalization [79]. In 2007 Gao et al. [80] published the landmark paper about iron(II, III) oxide nanoparticles and their intrinsic peroxidase- mimicking, from here many other nanomaterials with intrinsic catalytic activities, such as peroxidase, oxidase, catalase, hydrolase, and superoxide dismutase, have been observed [80] [79]. For example, also manganese(II, III) oxide Mn_3O_4 nanoparticles are capable of mimicking cellular antioxidant enzymes including superoxide dismutase, catalase, and

glutathione peroxidase. Nanozymes are part of an emerging research branch that connect nanotechnology with biology, useful to overcome the difficulties in the engineering of natural enzymes [79].

These nanozymes differ from natural enzymes also because it is possible to control their behaviour, modifying their various physical and chemical properties, and the typical nanozymes are iron(II, III) oxide nanoparticles (Fe_3O_4) [81].

Qin et al. [81] studied the behaviour of these iron(II, III) oxide Fe_3O_4 nanozymes in relation to the influenza virus. The synthesized nanoparticles showed a diameter of 200 nm and in a neutral environment, in contact with the H5N1 virus, they are able to activate lipid peroxidation on the envelope of the virus, consisting of a liposomal structure; it is therefore deduced that they have lipoxidase-like properties. These actions compromise the integrity of the lipid envelope, as shown in the TEM images in figure 3.2 [81].

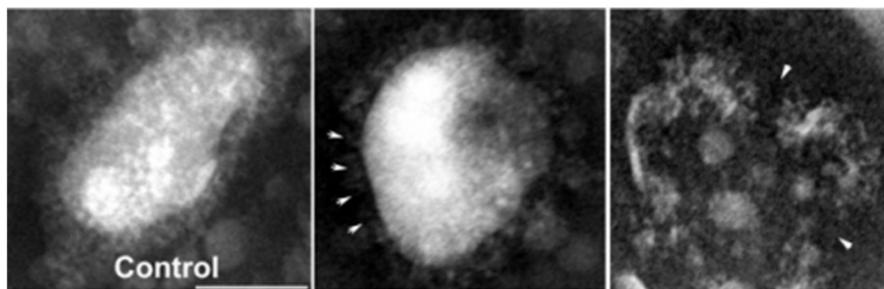


Figure 3.2: TEM images of IAVs treated with nanozymes of Fe oxide nanoparticles (the left image shows the virus control without nanoparticles treatment) [81]

Simultaneously with the lipid envelope degradation, the integrity of the proteins on the viral surface envelope is compromised, such as haemagglutinin HA, neuraminidase NA and the matrix protein M1. Nearby proteins could be compromised by a possible production of radicals from lipids. Viral particles treated with iron oxide nanozymes lose their ability to bind to host cell receptors and their infectivity is inhibited [81]. Figure 3.3 shows a summary diagram of the actions performed by the nanoparticles interacting with influenza viruses.

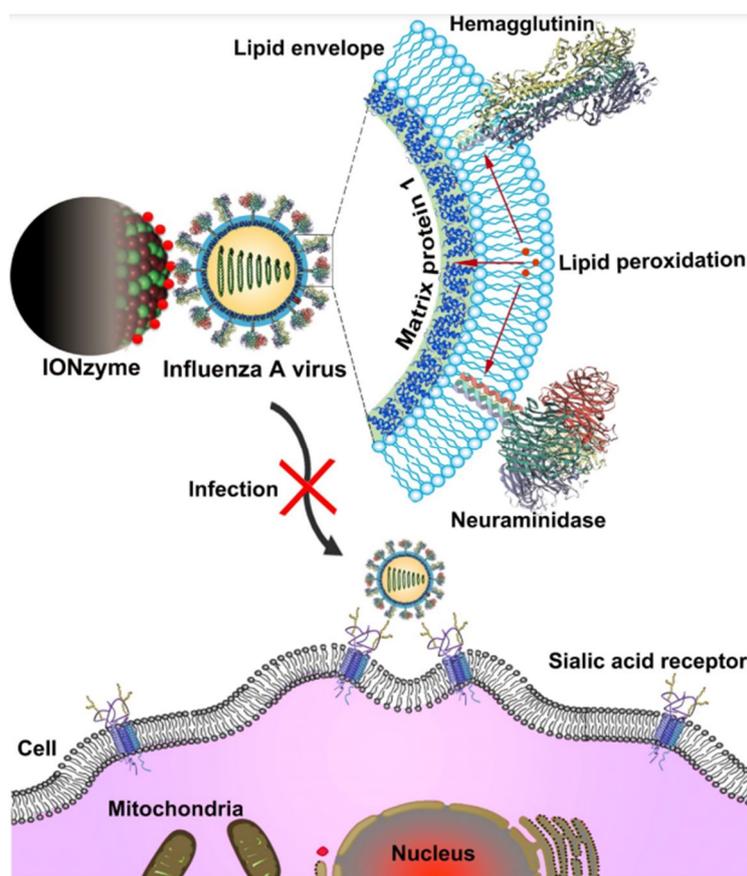


Figure 3.3: schematic description of the activities due to the interaction between the Fe_3O_4 nanoparticles and the influenza virus [81]

Iron oxide nanozymes are also effective against another coated virus, NDV, and other influenza virus subtypes [81]. Subsequently, these nanoparticles were applied to PPE masks, to test the inhibition of the influenza viruses absorbed on the device during its use. The results shown in the study are positive, so iron oxide nanozymes appear to be a good solution against enveloped viruses [81].

Since the iron oxide nanoparticles seem to act on the envelope that covers the virus, molecular docking studies have been carried out to analyse the behaviour of iron(II, III) oxide Fe_3O_4 and also of iron(III) oxide Fe_2O_3 towards the glycoproteins present on the envelope of the HCV virus and the spike protein receptor binding domain of SARS CoV-2 [82]. Docking study in molecular modeling is a method by which it could be predicted the preferential orientation of one molecule towards a second, during the formation of a stable complex. In this way, knowing the orientation of the molecule, it is possible to predict the strength of association or bond of the complex [82]. Abo-zeid et al. predicted the formation of stable compounds, especially between iron(II, III) oxide Fe_3O_4 and the spike proteins of SARS CoV-2 and iron(III) oxide Fe_2O_3 and the glycoproteins E1 and E2 of the hepatitis C virus HCV through the formation of hydrogen bonds. Subsequently, it is assumed that others antiviral mechanisms could be activated through the formation of reactive oxygen species ROS. This model would seem to confirm the inhibition of viral particles through interaction with iron oxide nanoparticles [82].

3.3 Ti, Zn and Sn oxide antiviral nanoparticles: the photocatalyst group

Some metal oxides possess an electronic structure which leads to semiconductor properties and, through energetic excitation given by electromagnetic radiation, they can generate radicals and reactive compounds, that interact with external species [68]. These photocatalysts in the form of nanoparticles have been widely used to obtain active surfaces against fungi and bacteria. This has led to deepen the research of these nanomaterials as antiviral agents for possible applications on surfaces of laboratories, hospitals, filters. Titanium(IV) oxide TiO_2 is the most studied photocatalytic oxide. The only disadvantage is that it is generally activated by UV light [68]. Zinc(II) oxide ZnO is the second photocatalyst oxide that has shown good bactericidal properties in previous studies, its activity depends on various factors including the presence of UV light, but unlike titanium(IV) oxide, it can also be activated by normal visible light [83]. The antiviral activity of these metal oxides is importantly increased under UV and visible light. The zinc(II) oxide activity against HSV-1 is drastically enhanced after creating additional oxygen vacancies under UV-light illumination. These nanomaterials mimic the viral binding ability of heparan sulfate (HS) on the host cell receptors [28]. Tin oxide also falls into this category of oxides with negatively charged surface [84]. Tin(IV) oxide SnO_2 nanowires studied by Trigilio et al. [84] are able to bind and trap HSV-1 before entry into host cells, UV light increased its negative charge, and the nanomaterial can attract the HSV-1 virus much higher.

This class of photocatalysts will be extensively discussed and detailed in chapter V.

Other metal nano-oxides have shown their antibacterial efficacy in previous studies, such as magnesium(II) oxide MgO , nickel(II) oxide NiO , aluminium(III) oxide Al_2O_3 or cerium(IV) oxide CeO_2 , but their antiviral efficacy should be still studied [68].

Chapter IV

Other antiviral compounds

4.1 Carbon-based nanomaterials towards viruses

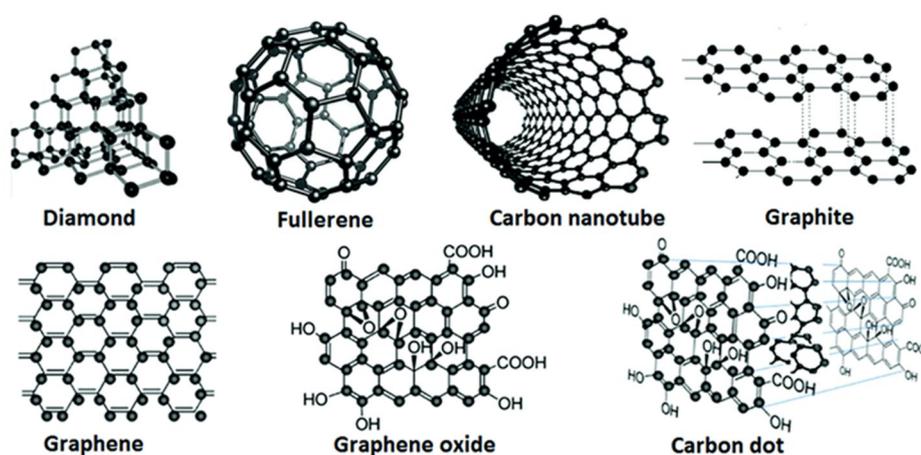


Figure 4.1: scheme of carbon-based nanostructures [85]

In the last past decades, the interest for carbon-based nanomaterials within the challenge for new antimicrobial solutions has grown. These nanomaterials have unique chemical-physical properties, such as stability, mechanical strength, corrosion resistance and electrical and thermal conductivity [86].

Carbon-based nanomaterials possess two fundamental properties for the nanobiotechnology field: good biocompatibility and easy functionalization. They have been studied in several application in nanomedicine, such as biosensing, optical imaging, diagnostics, and therapies [86].

These nanomaterials have different nanostructures dependent on carbon atom hybridization and their arrangement in the structure [86].

Fullerenes were the first C-based nanostructures discovered in 1985 and the first applied in antiviral research [87]. Subsequently, the nanostructure of graphene and carbon-based quantum dots (CQdots or CDs) was discovered and analysed in several studies against the spread of viruses. C-based nanomaterials also include carbon nanotubes and nanodiamonds. To date, in antiviral research, the former has not been taken into consideration for their potential toxicity.

Nanodiamonds can have antiviral activity, but their synthesis techniques include expensive purification works, due to the huge presence of impurities after the detonation synthesis method [87].

Fullerenes have been applied principally during the phase of interaction between the virus and the host cell: the mechanism that reduces viral infection is based on the direct interaction with the host cell and not with the virus particle [8]. Therefore, the two groups analysed in this thesis, able to block viral entry according to the conditions exposed in the first chapter, are graphene and its derivatives, and carbon dots. They both have limitations: generally, graphene and its derivatives are difficult to manage in size and shape, while carbon dots, although they are the easiest to achieve through few precursors and their carbonization, do not have a model to apply with viruses due to the potential infinite variety of these nanomaterials [8].

4.1.1 Graphene and its derivatives

Graphene consists of a single layer of sp^2 -hybridized carbon atoms in a honeycomb structure, with a C-C bond length of 0.142 nm [86]. GO is one of its most studied derivatives which contains C-O bonds in hydroxyl, carboxyl, carbonyl groups, it is different from graphene, it has high hydrophilicity and it is more useful in biomedicine [86]. GO is synthesized through the oxidation of graphite and its chemical exfoliation; therefore, the structure is constituted by graphene sheet with functional groups on its basal plane like phenol and hydroxyl groups, and at the edges like carboxyl groups. Studies with GO in the antiviral field are often accompanied using reduced graphene oxide (rGO), to observe the differences between the two nanomaterials under the same experimental conditions. To obtain rGO, the GO is treated in such a way as to eliminate its functional groups [88].

Graphene and graphene oxide are 2D nanomaterials able to adsorb on their surface biological molecules such as nucleic acids and proteins [86].

The biggest challenge to expanding the use and study of such nanomaterials in the antiviral field is the lack of synthetic protocols to precisely manipulate the structure of graphene sheets and accurately control their antiviral activity [34].

After the first studies on antibacterial graphene, several derivatives of graphene have been developed for the inhibition of microbes: in fact, they exploit the easy functionalization of the nanomaterial, the main subject of these studies is functionalized graphene oxide. The antiviral multivalence is increased by introducing more functional groups on the surface of the GO. To be able to modify the GO properly, it is necessary to know what affects the interaction between graphene derivatives and viruses, to intervene specifically [34].

The main mode and factors that make effective the interaction between these 2D nanomaterials and viral particles are:

- electrostatic interactions
- sharp edges
- capacity of virus wrapping
- hydrophobic interactions
- capacity of virus trapping [34].

The electrostatic interaction is the mode most studied in different works. The exploitation of this modality as an inhibition virus entry pathway has been considered over the years, following the observations of the viral infection mechanisms in host cells [34]. In this thesis the viruses taken into consideration have in common the presence of the lipid layer typical of enveloped viruses, therefore the first attachment of the virus on the cell is regulated by usual mechanisms, one of these is represented precisely by the electrostatic interaction with the surface receptors of the cell (for example this occurs with herpes simplex virus HSV, human immunodeficiency virus HIV, pseudorabies virus PRV, porcine epidemic diarrhea virus PEDV, influenza A viruses).

Sametband et al. [89] have studied the inhibition of the HSV-1 attachment to the host cell by functionalized GO. HSV-1 is a DNA-coated virus and the coating membrane is made up of about 12 different glycoproteins, including gB, gC, gD and the gH and gL complexes that mediate the entry of the virus into the host cell. The glycoproteins gB and gC are those responsible for the first attachment step, binding the heparan sulfate (HS) proteoglycans on host cell surface. HS is a negatively charged linear polymer that interacts with many proteins via ionic bonds. The affinity between HS and the glycoproteins of the coated virus is not extremely high, it serves to bring a greater number of virions closer to the target cell, which they will then be facilitated in subsequent bonds with more similar receptors [89]. In order to test the role of the negative charge in these interactions, Sametband et al. carried out the same experimental tests with GO and sulfonated rGO (rGO-SO₃), i.e. nanomaterials with different negatively charged groups, carboxyl for the former and sulfonate for the latter, but very similar ζ potential. Both nanomaterials have a thickness of about 1.1 nm and lateral dimensions ranging from nm to μ m. From the experiments they appear to have similar inhibitory activity towards the virus, therefore it would seem that their effectiveness does not depend so much on the chemistry of the functionalization groups, but on the charge density they present on the surface [89]. To investigate this hypothesis, Ye et al. [32] studied the interaction of GO and rGO against other enveloped viruses (PRV and PEDV). Using a cationic polymer, they verified that the antiviral efficacy of the nanomaterial could depend on its surface negative charge. In the experimental test, they carried out further checks, to exclude the possible involvement of the formation of a polymeric involucre around the GO sheet from the observed reduction in antiviral efficacy. The electrostatic interaction with the spike proteins of the envelope affects virus entry, but the particle remains intact. Figure 4.2 shows the two types of viruses analysed in the study after

one hour of incubation with GO: they show partial destruction of the envelope and spike glycoproteins [32].

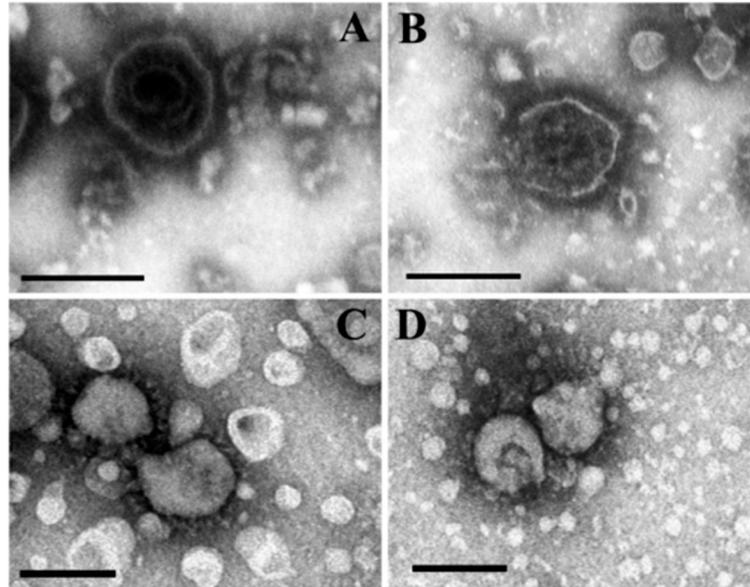


Figure 4.2: TEM images A) PRV control; B) PRV after the incubation with GO; C) PEDV control; D) PEDV after incubation with GO [32]

The destruction of the viral envelope is not due to electrostatic interaction, but probably from direct contact between GO and virus. GO can inactivate bacteria by direct contact with the sharp edges of the nanomaterial. The authors took into consideration this mode observed with bacteria, and to verify the influence of the structural characteristics of thin sheets, they carried out incubation tests of the viruses with graphite and oxidized graphite [32]. The ineffective results against virions would seem to confirm the theory that predicts the involvement of sharp edges in the mechanical destruction of the virus. The sharp edges are due to the structure of the ultra-thin and flat sheet with a thickness of 1 nm. The wrapping effect can also contribute to the inhibition efficacy of GO against these viruses [32]. This effect is considered in a study of Liu et al. [90] with GO against *E. coli*: the wrapping effect is exposed as a probable cause of the lateral dimension dependent antibacterial activity of GO. Liu et al. affirm that the GO with a larger lateral dimension can better compromise the cell viability of the bacterium since it covers it more easily [90]. GO in Ye et al. study has a size of 500 nm and the diameter of PRV and PEDV is approximately 200 and 100 nm, so there are conditions for an important contribution in the inhibition of the virus for the wrapping effect. Wrapping effect, like electrostatic interaction, is a virustatic mode of inhibition [32]. Donsky et al. observed the wrapping effect on HSV-1 virus with their functionalized nanographene sheets [34]. These nanographene sheets were functionalized with polyglycerol sulphate and alkyl chains of different lengths (nG-PGS-C_x, with x = 3,6,9,12,18), which to not be cytotoxic they must not exceed 12 atoms of C. Polyglycerol sulphate particles with a negative charge are effective against viruses interacting with HS on the host cell and nG-PGS-C₆ interacts electrostatically with the viral glycoproteins, wrapping HSV-1. In this study, there is another effect that increments the viral inhibition: a virucidal effect due to the alkyl chains. After the first electrostatic interaction, these groups can

create secondary hydrophobic interactions with the virions, leading to the envelope destruction. The high viral inhibition *in vitro* is due to the synergetic effect of electrostatic and hydrophobic interactions. The diameter size of nG-PGS-C6 is approximately 100 nm, so based on the previous statements, the wrapping effect exists, but it is probably not the one that most increases antiviral efficacy. With carbon-derived nanomaterials there is no universal correlation between size and antiviral efficacy, this trend depends on the functional groups present on the surface of the nanomaterial and on the prevailing inhibition mode [34]. Ziem et al. [91] studied the size-dependent inhibition of HSV by GO thermally reduced by graphite and functionalized with polyglycerol sulphate (TRGO-dPG-S). The different sizes of the nanosheets are between 100 nm and 1200 nm. The polysulfation was performed to compete with host cell HS. The results showed that the inhibition of these nanomaterials depends on both the degree of sulfation and the size of the nanosheet. *In vitro*, the positive charge of residual amino acids on gB and gC of the HSV-1 envelope interacts better with the nanostructures with a higher degree of sulfation, due to the higher density of negative charge. The contact area between the virus and the nanoarchitecture is wider if there is an energy gain given by the multivalent interactions able to compensate for the energy loss due to the bending of the nanostructure. The best condition would be that involving a higher ratio between interacting and non-interacting areas, so it has been proven that the smallest size of the nanosheets increases the effectiveness of viral entry inhibition [91].

The mechanical action of the sharp edges and hydrophobic interactions are the only two modes observed capable not only of inhibiting the entry of the virus, but also of rendering it harmless, destroying the envelope. Both modes can occur only after a first approach of the nanomaterial with the viral particle. In fact, for the action given by the sharp edges there must be physical contact between the two elements, while the hydrophobic interactions could involve very specific and not completely exposed hydrophobic parts of the virus, therefore also in this case a first approach between the nanomaterial and viruses is necessary [32] [34]. GC et al. [92] carried out a study using molecular dynamics simulation on the interaction between graphene sheets and VP40 protein of Ebola virus. This hexameric structure protein is involved in the formation of the viral matrix, the part that provides the shape and stability of the viral particle, in contact with the lipid envelope. The filaments that make up the Ebola virus matrix are given by the VP40 hexamers connected end to end through their C-terminal domains (CTDs) [92]. The CTDs interfaces contain hydrophobic residues highlighted in orange in Figure 21. These residues are not found exposed but are found more internally in the structure that covers the virus. Therefore, before graphene can attack the CTD-CTD interface outlined in figure 4.3, it must make sure to create a primary interaction with the viral particle. This could be achieved

through functionalization with groups capable of creating electrostatic interactions for example [92].

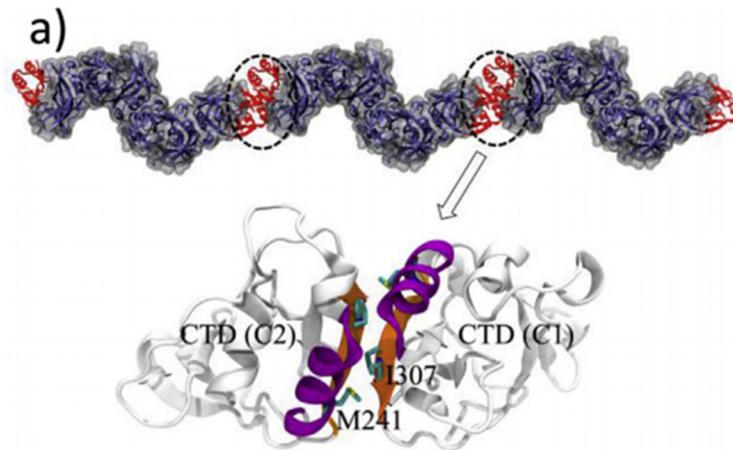


Figure 4.3: VP40 filament of Ebola virus with three hexamers connected end-to-end via tail CTDs. M241 and I307 are the hydrophobic residues [92]

GO with its aromatic polar oxygen-rich functional groups can support metal oxides, polymers, and inorganic nanoparticles. In this way, it is possible to create new nanocomposites with synergetic actions against viruses [93]. Du et al [93] and Chen et al. [33] analysed GO as support and stabilizing agent of Ag nanoparticles. These nanocomposites show good efficacy against porcine reproductive and respiratory syndrome virus PRRSV [93] and feline coronavirus FCoV [33]. GO sheets limit the agglomeration of nanoparticles and their dispersion in the environment [33]. Yang et al. studied GO functionalized with β cyclodextrin (CD) loaded with curcumin against the RSV virus [94]. By also introducing sulfonate groups the inhibition mechanism is further enhanced for the host cell mimicking properties. Curcumin is a natural polyphenol with low water solubility that has shown good antiviral, antibacterial, antifungal, and anti-inflammatory activities in general [94].

Graphene and its derivatives, despite their few applications in vivo and a limited search for their toxicity in vivo, could represent a potential solution for reducing the spread of viruses, for example through their application in mask coatings [95].

4.1.2 Carbon-based quantum dots (CQDs or CDs)

CDs are zero-dimensional carbon-based nanoparticles generally produced by the hydrothermal decomposition of “low cost” C-containing precursors. These nanoparticles have sizes smaller than 10 nm and are characterized by their ease of preparation, low toxicity, easy functionalization, and fluorescent properties. In various in vitro studies, the ability of CDs to form non-covalent interactions with the viral coating has been observed, thanks to the surface of these nanoparticles rich in carboxyl and hydroxyl groups. The limitations in the use of these

nanomaterials are the few in vivo experiments, so their application on masks or disinfectant surfaces is limited [8]. Like graphene derivatives nanomaterials, CDs are easy to functionalize and this property is exploited to increase their antiviral efficacy [8].

Often their functionalization can be carried out in a single step, in the CDs preparation phase precursors contain the molecule selected for the functionalization of the nanomaterial [8]. Ting et al. in this way they obtained cationic carbon dots with curcumin (CCM-CDs) [96]. Curcumin has also been applied to GO and, as previously mentioned, it is a polyphenolic compound that is used in the antibacterial and antiviral fields. The nanoparticles obtained from the pyrolysis of CCM have a diameter of about 1.5 nm, are rich in hydrophilic groups, and have a positive potential. Thanks to these properties they have observed that they are able to inhibit the viral activity of PEDV, taken as a model of coronavirus by the authors. This inhibition is supposed to be due to the formation of aggregates of CCM-CDs with PEDV due to electrostatic interactions: in fact, they have observed, through Raman displacement tests for PEDV, that these nanoparticles are able to modify the structure of the viral surface proteins [96].

Fahmi et al. synthesized other CDs to try to inhibit the entry and subsequent infection of the HIV virus [97]. The authors produced graphene-like structures rich in hydroxyl and carboxylate groups from citric acid pyrolysis, then they modified the C-dots with boronic acid molecules. From previous studies they have seen that materials containing boronic acid have a good response towards glycopeptides and glycoproteins, therefore they wanted to exploit this ability for the inertia between C-dots and HIV virus, knowing that the virion has on the envelope the glycoproteins essential for attachment with the host cell. C-dots, through the presence of hydroxyl and carboxylate functional groups on the edges, create non-covalent interactions with the molecules on the surface of the viral envelope, such as gp120 and gp41 glycoproteins. Boronic acid molecules, from carboxyl phenylboronic acid (CBBA), increase non-covalent interactions and lead to better inhibition activity. Figure 4.4 shows the inhibition scheme of CBBA-CDs against HIV entry [97].

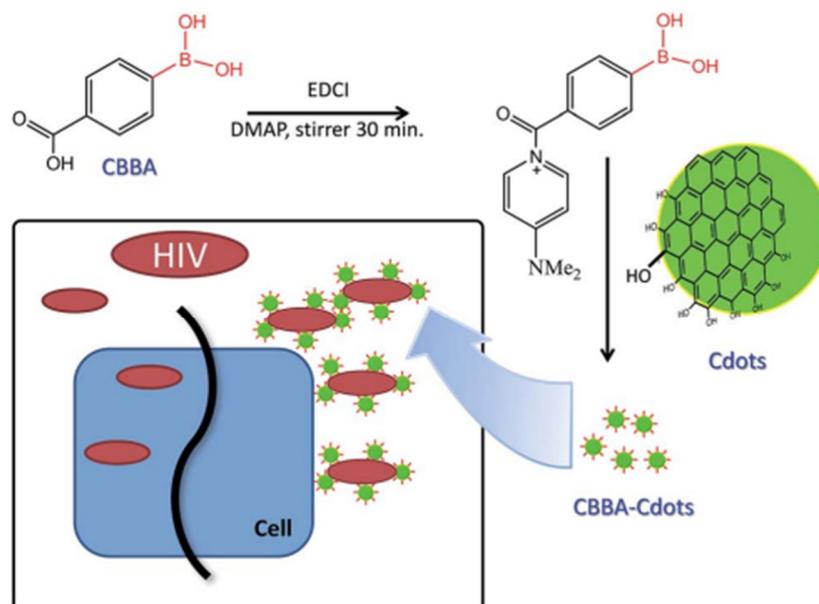


Figure 4.4: schematic representation of the inhibition actuated by CCBA conjugated carbon dots against the HIV entry in the host cell [97]

Some reports have suggested the use of benzoxazine derivatives for antimicrobial properties, so Huang et al. developed CDs from benzoxazine monomers (BZM-CDs) and evaluated their antiviral efficacy on several enveloped and non-enveloped viruses in vitro [98]. From the observed results, these nanoparticles can interact directly with virions, decreasing the infection of viruses. The mechanism of this interaction is still unclear, but probably involves the chemical species present on the envelope of the enveloped viruses, since nanoparticles are much more effective against them than non-enveloped ones. In fact, the authors verified the antiviral efficacy on additional enveloped viral species, increasing the hypothesis of the potential use of nanoparticles against enveloped viruses in general. Figure 4.5 shows the inhibition scheme for the enveloped viruses analysed in this study [98].

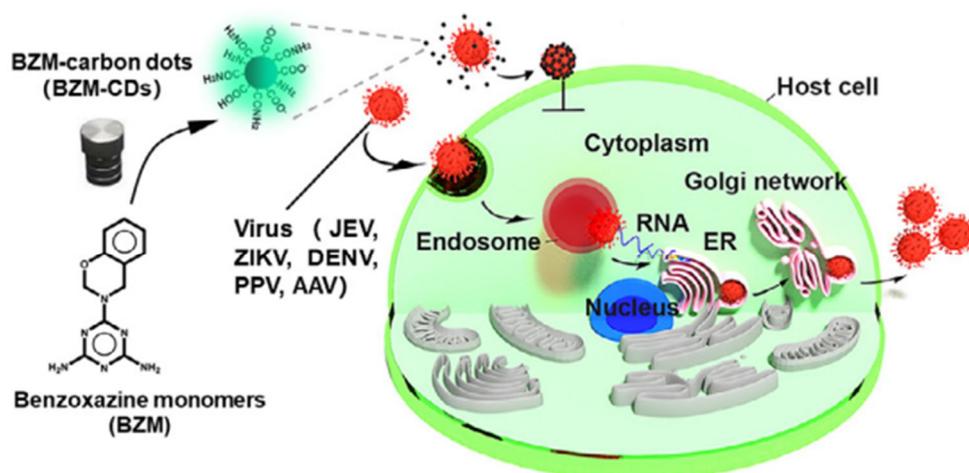


Figure 4.5: scheme of enveloped viruses (Japanese encephalitis virus JEV, Zika virus ZIKV, Dengue virus DENV, plum pox virus PPV, adeno-associated virus AAV) entry inhibition with BZM-CDs [98]

4.2 Porous silicon nanoparticles

Porous silicon (PS) is a form of silicon with a structure characterized by nano-sized pores. Porous silicon nanoparticles are porous silicon nanostructures. These nanomaterials are less toxic than metals and are easily biodegradable, due to their gradual dissolution in water through the formation of non-toxic silicic acid [99]. These SiNPs have been introduced in various biomedicine studies, such as therapies against cancer and diagnostic applications. Within the diagnostics, starting from the observation of silicon nanowires used in viral detection, Gonchar et al. have developed layers of porous in volume silicon nanowires with a diameter of 100 nm, the pore size of 5-10 nm, and a distance between the nanowires of 100-300 nm, situated on a silicon substrate [29]. The interaction between these layers and the H1N1 virus were tested by the authors. From the TEM and SEM (scanning electron microscope) images in figure 4.6, it is possible to see the virions interacting with the porous silicon nanowires layer. Virions are 100 nm in size can get stuck in the spaces between the nanowires. The interactions between the viral particles and the porous silicon are probably regulated by Van der Waals forces, so the glycoproteins of the viral envelope could adhere to the pores of the nanowires and get stuck there [29].

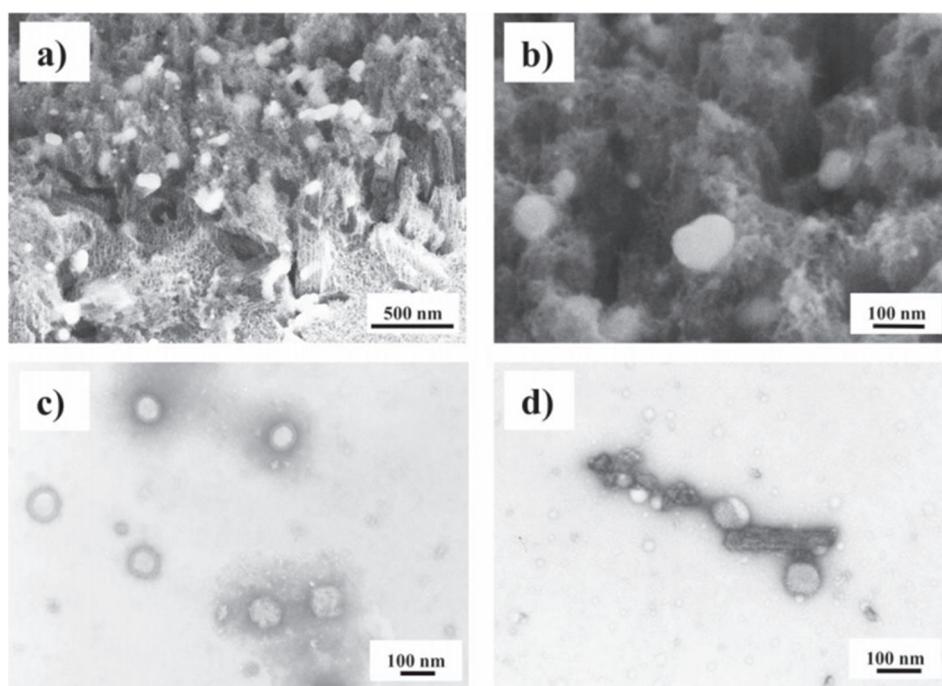


Figure 4.6: (a),(b) different magnifications of SEM images of porous silicon nanowires after the virus interactions (the white particles are the virions); (c) TEM image of H1N1 influenza virions; (d) TEM image of PSiNWs (porous silicon nanowires) and H1N1 virions interaction [29]

Osminkina et al. have deepened the interaction between porous silicon nanoparticles and enveloped viruses of RSV and HIV, expanding the possibility that these nanomaterials can be

studied for different applications in the biomedical field [99]. The authors link the antiviral activities of porous silicon nanoparticles (PSiNPs) and non-porous silicon (SiNPs). The size of PSiNPs are between 5 and 50 nm, the size of the pores of 2 nm, and most of the oxidized Si surface bonds ensure hydrophilic properties to the nanomaterial. Non-porous SiNPs bind weakly to the virus, since they are larger in size and have a smooth surface. The PSiNPs, on the other hand, as shown in figure 4.7, aggregate around the viral particles which are trapped in a PSiNPs network. The interactions between porous silicon nanoparticles and virions are strong and could be accompanied by charge exchanges between them [99].

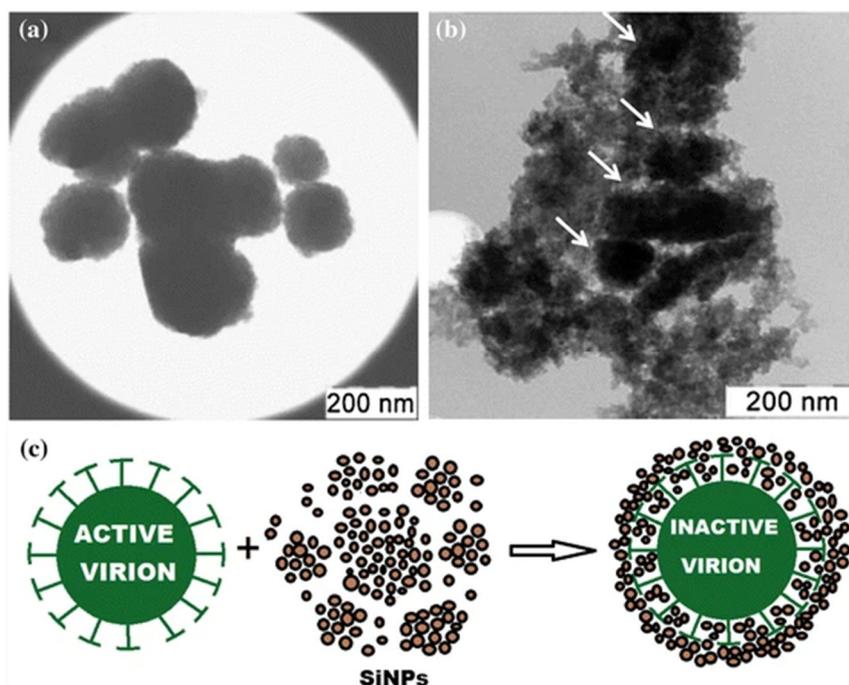


Figure 4.7: TEM image of RSV viral particles, **b** TEM image of RSV (indicated by the white arrows) after interaction with the porous silicon nanoparticles, **c** RSV trapped in a PSiNPs network [99]

The interactions between porous silicon nanoparticles and enveloped viruses are not specific and block the action of pathogens. Such non-specific interactions could make these nanoparticles potentially effective against other enveloped viruses as well. They are interactions to be explored [99].

4.3 Cu antiviral compounds

4.3.1 CuI nanoparticles

In the previous chapter, antiviral efficacy and the study carried out on copper oxide nanoparticles were explained. Copper oxides are stable compounds, but if added to a product

as antiviral agents, they can change its appearance due to their brown and black colours. Copper iodide compound could overcome this problem because it is white in colour and easily applicable on masks and other fabrics [100]. Therefore, Fujimori et al. analysed the interaction between these nanoparticles and the H1N1 influenza virus. CuINPs have a median diameter size of 160 nm and incubated in a suspension with H1N1, inactivate the virus. These nanoparticles can generate ROS, which can degrade glycoproteins such as HA and NA. Cu^+ released by the nanoparticles could also oxidize envelope virus lipids [100].

4.3.2 Potential antiviral efficacy of zeolite

The antiviral efficacy of Cu ions has been reported in several cases, and other nanomaterials that could exploit the activity of Cu ions are zeolites powders: mesoporous aluminosilicates of alkali earth cations [101]. Zeolite is a biocompatible and non-toxic substance widely applied in the biomedicine field, such as a catalyst or ion exchanger [102]. Bright et al. observed a positive response for Ag/ Cu zeolite powders with human coronavirus 229E and feline infectious peritonitis FIPV, but without observations on the interaction [101].

Chapter V

Photocatalysis and viruses

5.1 Antiviral photocatalysts

Photocatalysis has historically been studied for self-cleaning solutions and depolluting applications in the building sector, subsequently, in a lesser form, it has acquired a function in antimicrobial applications [103]. Photocatalytic materials in the presence of light are activated and can produce radicals and reactive compounds, that can react with chemicals and microorganisms in the surrounding environment. This effect is exploited to produce disinfectant surfaces and antimicrobial treatments in water [103].

In 1970 the photocatalytic properties of titanium(IV) oxide were reported for the first time. From here, these properties were analysed in various experiments and at the end of the twentieth century, they were also reported for other metal oxides, such as zinc(II) oxide. Once powdered, these metal oxides exhibit previously unexpressed photocatalytic properties. In fact, the depolluting and disinfecting effect of these materials is observed on their surface; therefore, it is optimized through the increase of the surface area to volume ratio [104]. Nanosized metal oxides such as titanium(IV) oxide TiO_2 and zinc(II) oxide ZnO belong to the category of semiconductors with photocatalytic properties for their specific electronic structure [104].

They have an electron-filled low energy valence band (VB) and an electron-free high energy conduction band (CB). The energy difference (ΔE) between the two bands defines the energy needed to excite an electron from VB to CB, and that is the energy of the band gap. Its own band gap characterizes each semiconductor. Therefore, the radiation with equal or greater energy than the ΔE between VB and CB, can have different wavelengths, specific to each nanomaterial. The photon energy of electromagnetic radiation $h\nu$ greater than or equal to ΔE , excites an electron from VB to CB, and creates the hole-electron pair ($h^+ + e^-$), called 'exciton', given by positively charged vacancy in the valence band and negatively charged electron in the conduction band. The electronic hole can induce oxidation reactions, it reacts with the water molecules H_2O and the hydroxide ion OH^- to form oxygen peroxide H_2O_2 or the hydroxyl radical $\bullet\text{OH}$, while the excited electron induces reduction reactions, it reacts with molecular oxygen to give superoxide anion $\bullet\text{O}^{2-}$. The chemical species created by the hole-electron pair are reactive oxygen species, called ROS (reactive oxygen species). ROS emerge on the surface

of the nanomaterial and can inactivate viruses, bacteria, yeasts, prions through advanced oxidation processes (AOPs) [104].

In the last decades, many AOPs have been considered as new strategies to solve various environmental problems, such as pollution, and for being used as antimicrobial species [105]. In figure 5.1 the production of ROS on the surface of TiO_2 and ZnO nano-oxides is schematized.

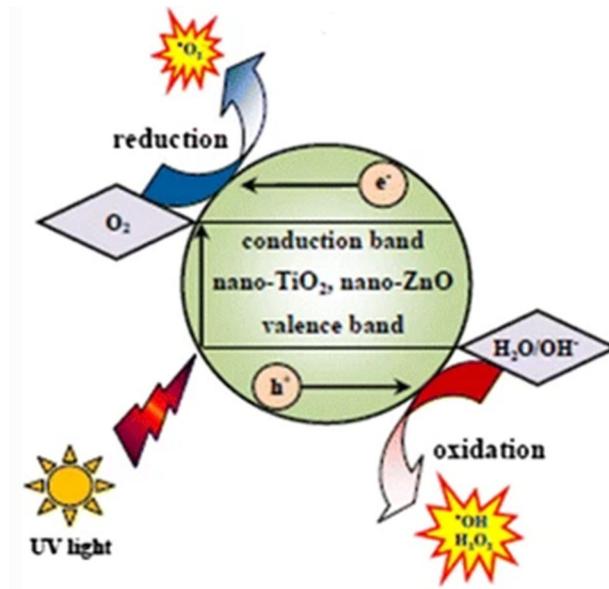


Figure 5.1: ROS generation on nano- TiO_2 and nano- ZnO [104]

Classic photocatalysts such as titanium(IV) oxide and zinc(II) oxide are activated by UV light. The UV component of the solar spectrum corresponds to 10-15% of the total solar spectrum and only 5-8% reaches the earth's surface in the form of UV-A (95%) and UV-B (5%). The UV-A component is the one exploited for photocatalysis processes; therefore this research field requires new solutions that can shift the absorption band of photocatalysts to longer wavelengths, above 400 nm, in the region of visible light of the sunlight spectrum, shown in figure 5.2 [103].

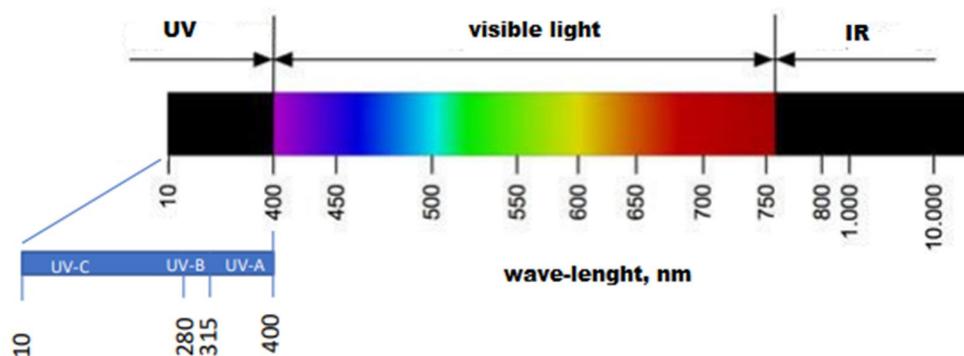


Figure 5.2: solar spectrum [103]

5.2 Antiviral titanium(IV) oxide TiO_2

5.2.1 TiO_2 polymorphism and photocatalytic mechanism

Titanium(IV) oxide exists in three crystalline phases: brookite, anatase, and rutile. The most common and most used are the anatase and rutile phases, as the brookite phase is less stable [106]. The crystalline structure of the oxide in rutile and anatase is given by chains of octahedra in which each Ti^{4+} ion is surrounded by six O^{2-} ions, while each oxygen atom is surrounded by three titanium atoms. The octahedron in the rutile phase is irregular with slight orthorhombic distortion, while in the anatase phase it is more significantly distorted, its symmetry is lower than the orthorhombic one. Anatase is the most photoactive and most applied phase [106]. Anatase exhibits its photocatalytic behaviour at an absorption peak at 385 nm. The activity against microorganisms is commonly explained through the action of ROS, such as hydroxyl radical or superoxide ions generated on the surface of the irradiated photocatalyst [105].

The band gap of anatase (3.2 eV) is greater than that of rutile (3 eV), therefore the former is excited by a more limited range of electromagnetic radiation than rutile [107]. Despite this, anatase has a better photocatalytic power and it is the most used in photocatalytic applications and also in anatase and rutile powders anatase represents the largest percentage, as in the case of Degussa P-25 powder, composed of 80 % anatase crystallites and 20% rutile crystallites [107]. Lutrell et al. analysed and compared the photocatalytic activity of anatase and rutile films and the photocatalytic activity of the different crystalline faces of the single rutile crystal [107]. The authors observed that the most active surface of the rutile (101) is however less active than the faces (001) of the anatase (the faces (001) of the anatase are not the most active). The better photocatalytic activity of anatase is given, at least in part, by its bulk properties: the charge carriers in anatase compared to rutile can originate deeper in the anatase bulk. The diffusion length of the charge carrier is one of the factors that most affect photocatalytic activity. The diffusion length of charge carriers depends on their diffusivity and on their lifetime (the time within which the hole-electron recombination takes place) [107].

The two biggest limitations of titanium(IV) oxide as a photocatalyst are its band gap and the consequent activation with electromagnetic radiation having wavelengths under 400 nm (UV range), and the rapid recombination of its charge carriers [108].

5.2.2 TiO_2 towards viruses

Titanium(IV) oxide irradiated by UV light can form ROS radicals to oxidize the C-H bonds and degrade the organic molecules with which interacts [109]. The envelope of enveloped viruses is very sensitive to the photocatalytic effect [110].

Zan et al. analysed the activity against hepatitis B virus HBV. The HBsAg antigen present on the virus envelope is responsible for the attachment of the viral particle to the hepatocyte receptors [109]. These antigens are constituted of amino acids, disulphide bonds and carbonyl groups: the photocatalytic reactions of the nano-anatase act on these groups and on the disulphide bonds in HBsAg proteins, compromising their structure and consequently their antigenicity. With UV light, surfaces coated with nano-TiO₂ inhibit the virus after a few hours and even with weak daylight irradiation, after more hours, it effectively inhibits the activity of virus antigens [109]. The peptide bonds present in HBsAg proteins are easily oxidized both by hydroxyl radicals and by electron holes created by the activation of photocatalysts [111].

In general, the photocatalytic inactivation of enveloped viruses is much more effective than that of non-enveloped viruses, this has been verified by several studies with influenza A viruses (IFV) compared with the inhibition of non-enveloped viruses, such as feline calicivirus FCV. These observations have led to the hypothesis of an inhibition mechanism involving the lipid membrane of enveloped viruses. It could occur a peroxidation of the phospholipid components, responsible for the significant damage to viruses [110].

Cui et al. verified the inhibition of this nano-photocatalyst in the form of spindle-shaped anatase nanoparticles of 50 nm [112]. The efficiency of the nanomaterial also depends on the intensity of UV light: the higher the intensity of the UV light, the greater the inhibition of the HA activity recorded after the virus interacted with the surface of the nano-photocatalyst [112]. Guillard et al experimented the antiviral photocatalytic activity of the nano-oxide using a photoreactor that can inhibit H5N2 in aerosol [113].

It is important to verify that even at low intensity of UV irradiation the antiviral photocatalytic activity is effective since it is necessary to model the intensity of indoor light in the daytime. Nakano et al have seen that by reducing the intensity of the UV-A light used in the experiment to the lowest limit, the inactivation of the H1N1 virus is still observed after 16 hours [1].

Antiviral photocatalytic activity is not specific. Therefore, the nano-oxide can attack the envelope proteins and the binding sites used by the virus during the attachment with cell receptors. Nakano et al. observed an initial dramatic degradation of viral proteins, followed by their complete digestion. By destroying surface viral proteins, infectability decreases and subsequently photocatalytic reactions could attack the nucleic acid of the virus [1].

Mazurkova et al. observed an antiviral activity of titanium(IV) oxide nanoparticles independent of the photocatalytic activity against the H3N2 virus [114]. The authors tested the action of TiO₂ nanoparticles of different sizes in suspension with the virus. The suspension with the smaller nanoparticles, between 4 and 10 nm, destroys the envelope after 15 min of incubation. As the images in figure 5.3 show, the nano-oxide particles adhere to the viral envelope, then they glue together the surface spinules and the envelope is broken. After 5 hours it is shown a complete fragmentation of the viral particle. The antiviral activity of nanoparticles would seem to come from direct contact between the virus and nano titanium(IV) oxide and this mechanism should be investigated [114].

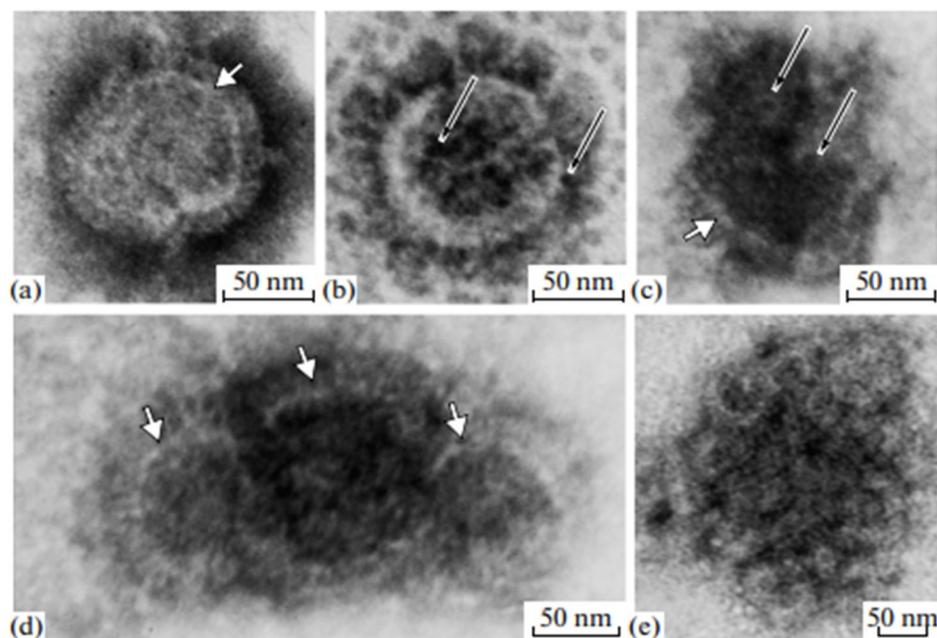


Figure 5.3: TEM images of the interaction between titanium (IV) oxide nanoparticles of 4-10 nm and particles of H3N2. The viral envelope is indicated with white arrow, while black arrows indicates nano-TiO₂ : (a) H3N2 virus, (b)TiO₂ NPs on the virus after 15 min of incubation glued surface spinules of the virus (c) destruction of the viral envelope, and (d, e) destruction of the viral particle after 3 and 5 h [114]

5.2.3 Modified TiO₂

5.2.3.1 TiO₂ composites with better antiviral efficacy

The activity of the titanium(IV) oxide nanophotocatalyst can be increased by the presence of a mechanism that may involve a primary interaction with the viral particles on the nanomaterial surface, subsequently subjected to the mechanisms of photocatalytic oxidation of the semiconductor [115]. Monmatrapoj et al. analysed the antiviral action against H1N1 virus of a nanocomposite consisting of anatase nanocrystals embedded in hydroxyapatite crystals [115]. Hydroxyapatite HA (Ca₁₀(PO₄)₆(OH)₂) is a type of calcium phosphate ceramic widely used in the biomedical field due to its chemical composition like that of bones and teeth. It is a biocompatible and non-toxic material; it can adsorb bacteria and viruses. Once the viral particles are adsorbed on the surface of the hydroxyapatite crystals of the nanocomposite, the complex is irradiated by UV light, in order to activate the production of ROS on the surface of the anatase crystals. Above all, hydroxyl free radicals and peroxide can react with the surface proteins of the virus, the phospholipid membrane, and the nucleic acid, in order to destroy or inhibit the virus [115].

Another way to increase the antiviral efficacy of titanium(IV) oxide nanoparticles is to create nanocomposites with metal nanoparticles that have shown good antiviral activity for various virus strains, as in the case of Ag nanoparticles [116].

5.2.3.2 Visible light induced photocatalyst TiO₂

The greatest limitation of the practical application of the titanium(IV) oxide nanophotocatalyst is its activation with UV light, which constitutes only a small percentage in sunlight [103]. For this reason, there is a need to develop new active photocatalysts in the visible range [106]. These photocatalysts can be created from titanium(IV) oxide through some modifications. Titanium oxide doped with metal cations or non-metal anions introduces intra-band gap states between VB and CB, that decrease the energy difference between the bands to create hole-electron pairs, to excite the nanomaterial with radiation of wavelength greater than 400 nm [106].

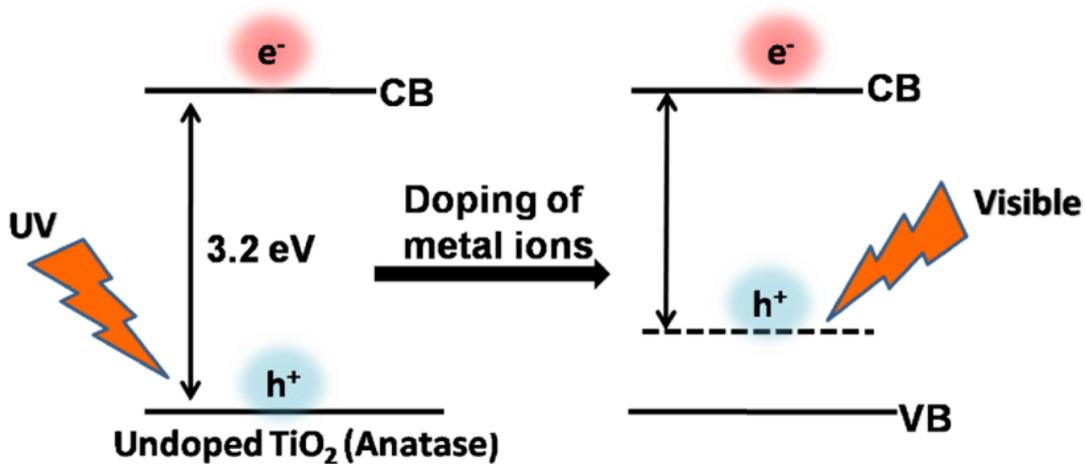


Figure 5.4: the band gap modification of metal cation doped TiO₂ [117]

Choi et al analysed (Fe, Mn, Mg) -doped titanium(IV) oxide against the H1N1 influenza virus [118]. The absorption peak of the doped semiconductor has a redshift at 420 nm, so it can inhibit the activity of the virus with only the visible portion of the solar spectrum [118].

5.3 Antiviral zinc(II) oxide ZnO

The nanosized zinc(II) oxide exhibits good bactericidal properties both against Gram-negative and Gram-positive bacteria when exposed to ultraviolet radiation [104]. As in the case of titanium (IV) oxide, once activated it can form reactive oxygen species (ROS). The radiation capable of exciting the electrons from the valence band is always found in the ultraviolet portion since the ΔE is 3.37 eV. It has been applied lesser in applications against the viral spread [104].

Mishra et al. and Antoine et al. studied the virustatic effect of zinc(II) oxide micro-nano-materials against HSV-1 and HSV-2 viruses respectively [119] [28]. Both viruses base their attachment mechanism to the host cell on the electrostatic interaction between positively

charged glycoproteins present on the lipid envelope and the negatively charged heparan sulphate (HS) of the cell receptors. The structure of the zinc(II) oxide micro-nano-materials have nano-spikes that mimic induced filopodia (the cytoplasmic projections on the surface of the host cell induced by the virus). They are ZnO tetrapods, interconnected hexagonal rods that mimic the structure of a sea urchin capped with nanoscopic filopodia. The thickness of these hexagonal rods is between 100 and 200 nm, and lengths between 1 and 5 μm [119]. The sea urchin structure and the negative charge given by the oxygen vacancies provide the micro-nanomaterial with the ability to mimic cellular receptors HS and create a virustatic trap. If these structures are subjected to UV irradiation for 30 min or 1 h, the distribution of the negative charge present on the surface increases and consequently their inhibitory properties towards viruses [119] [28]. Figure 5.5 shows the mechanism of these micro-nanostructures.

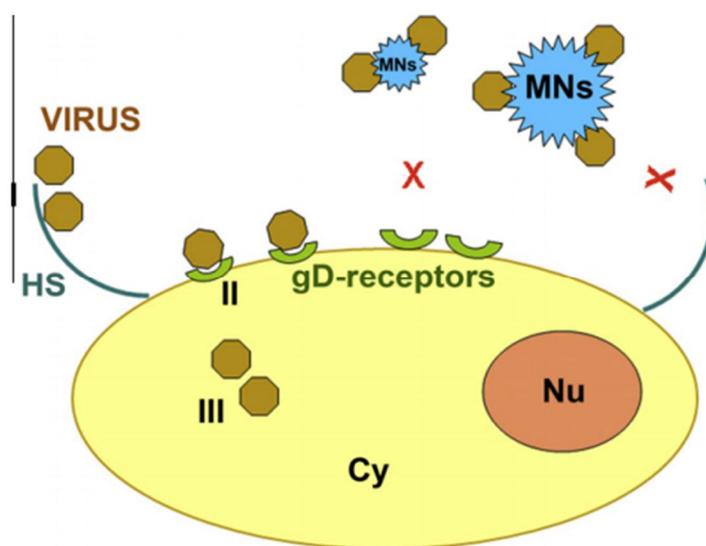


Figure 5.5: mechanism of HSV-1 entry inhibition by ZnO tetrapods [119]

Oxygen vacancies and defects in semiconductors become centres where electrons are collected and they help the distribution of the photogenerated negative charge [120].

5.4 Antiviral tin(IV) oxide SnO_2

Trigilio et al. investigated structures similar to those analysed in the previous paragraph for zinc(II) oxide, but with tin(IV) oxide [84]. The antiviral efficacy has been tested on the HSV-1 virus and the nanoparticles were snowflake type structures having nanowire lengths from mm to cm, while their diameter had a range between hundreds of nm and μm . Small tin(IV) oxide crystals with a size of 50-100 nm were distributed over the entire surface of the nanowires. Their inhibitory activity consisted in the virustatic trap of viral particles that derives from the

negative charge present on the surface of the nanowires, increased by the 1 h pre-treatment with UV light [84].

5.5 Antiviral platinum-loaded tungsten(VI) oxide Pt-WO₃

In the research for new active photocatalysts in the visible field, Sumitomo Chemical Co. has developed the ILUMIO photocatalyst, platinum-loaded tungsten(VI) oxide [121]. Takehara et al. analysed the photocatalytic activity of this photocatalyst against the H1N1 influenza virus [121]. ILUMIO absorbs visible light up to 470 nm and has been studied as an antibacterial. After 2 h under irradiation, even with a UV filter, the viral titer of the virus in contact with the photocatalyst goes below the detection limit. Many photocatalysts require UV lights or several hours of irradiation, but not in this case. ILUMIO could represent a good solution for indoor antiviral surfaces. [121] [122].

Chapter VI

Potential interaction between particulate matter (PM) and viruses

In the previous chapters, the possible interactions of enveloped viruses with the cited antiviral nanomaterials have been discussed. With the pandemic caused by the spread of SARS-CoV-2, the correlation between environmental pollution and the high diffusion of Covid-19 cases has been deepened [5]. Among the pollutants in the atmosphere there is the particulate matter (PM), which has a wide range of sizes, from micrometres to nanometres. One of the biggest issues of the viral spread to be resolved is the potential interaction of particulate matter with viral particles and its acting as a carrier [5]. If so, as discussed in the previous chapters, the interaction between viruses and nanoparticles should be investigated.

To date, the question is still open since the analyses on the particulate matter are scarce, but there are many studies that report various evidence supporting this hypothesis [5].

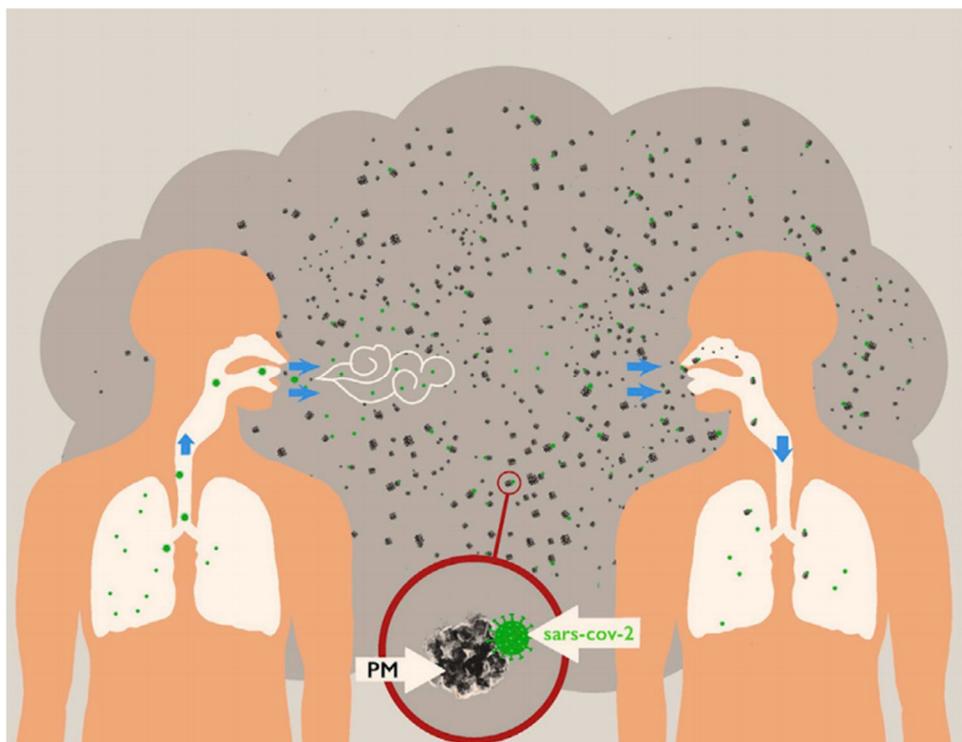


Figure 6.1: Potential role of particulate matter PM as a SARS-CoV-2 carrier [5]

6.1 Modes of viral transmission

Before going into the discussion of a possible interaction between PM and virus, it is necessary to know how the virus can be transmitted.

The main modes of transmission valid for many viruses are the direct or indirect ones given by droplets larger than 5 μm [123]. Direct contact can occur by physical contact between two subjects, while indirect contact can occur with a surface on which infected droplets are deposited. These droplets may contain SARS-CoV-2 if produced during the breathing, speaking, sneezing, and coughing of an infected person. Among these exhaled droplets there are also those smaller than 5 μm that remain longer in the aerosol in the air. There is also a definition of "droplet nuclei" which represent the remnants of smaller droplets [123]. In the literature, airborne transmission and aerosol transmission are often interchangeable, but they do not identify the same thing [123]. Aerosol transmission involves the particles that carry viruses in the aerosol, while airborne transmission identifies the transport of the aerosol operated by the air movements. The droplet transmission is the widely recognized one, while airborne/aerosol transmission is still under discussion because, in addition to the smaller droplets with SARS-CoV-2, the transmission of the microbes on PM could be added [123].

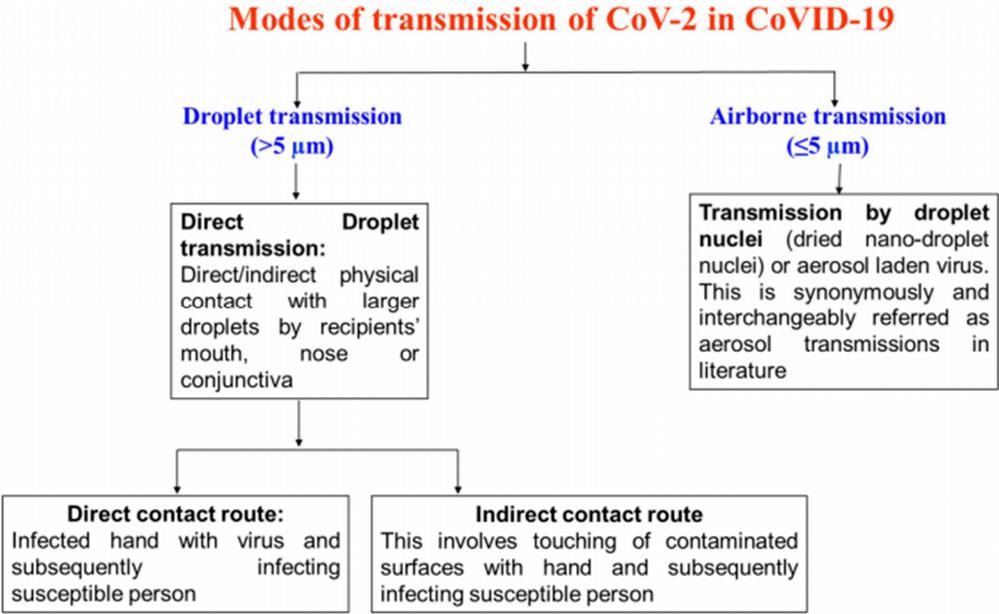


Figure 6.2: scheme of SARS-CoV2 modes of transmission [123]

Airborne transmission may have lower infectious power than that given by droplet, but is able to stay longer in the atmosphere, therefore it could cause secondary infection, especially in indoor environments [123].

Viruses such as SARS-CoV-2 can survive outside the human system even for a few hours, depending on the surface they are on, they could remain active in the aerosol for about 3 hours, with a half-life-time of 1.1 hours [124]. Smaller aerosol droplets through air movement could mediate long-range human-to-human transmission [125]. These, remaining longer suspended in the air, in fact, can be transported for distances greater than 6 meters [126]. However, while

the study of droplet transmission is well documented, that of airborne/aerosol transmission is limited [126].

Qu et al. in addition to the transmission modes described above, suggest the aerosol transmission from contaminated sewage, basing their hypotheses on studies carried out in Hong Kong in 2003, which correlated a failure of the sewage system to the large number of SARS infected cases coming from the buildings surrounding the leak [125]. They suggest other hypothesis of transmission via airborne dust or PM, a further mode that would mediate long-range transmission of the virus [125]. In fact, the smallest PM particles have a significantly longer life in the air than the particles given by respiratory droplets [126]. The airborne transmission involving the PM as a carrier for the virus is still much discussed, since it would be necessary to know several parameters that are still uncertain today, including the virus charge on the PM, the viability and lifetime of the virus on the PM, minimum dose of the virus to infect and transmit the disease [127].

6.2 Particulate matter PM: definition and indexes

Particulate matter (PM) is the term used to refer to the microscopic solid and liquid particles present in the air, their characteristics and their composition vary depending on the geographical area and the climatic conditions in which they are found [128]. The differences of PM due to the geographical area depends on the variability of the sources of particulate matter [128]. The sources of PM can be both natural (such as volcanic emissions, sand, rock erosion, pollen) and anthropogenic (such as incinerators, asphalt, motorized traffic, aircraft engines, exhaust gases, industrial fumes, domestic heating) [129]. The particulate matter could also form when condensation of low vapor pressure substances occurs, which could subsequently react with the gases of primary pollutants [129]. The PM generated directly from the sources described above is of primary origin, it contains carbon and organic compounds, metals, metal oxides, and ions. The one generated by chemical and physical reactions with the gases present in the air is of secondary origin [130]. The primary pollutants gases could be SO₂, NH₃, NO_x which convert into sulphates, nitrates, and ammonium ions; PM can also contain microorganisms, especially the one generated by livestock is the most biologically active [131].

So the PM is not given by a single pollutant but is a complex mixture of particles suspended in the air with different physical, chemical, and biological characteristics [131] [128]. These particles are floating in the airflow and their sublayer could facilitate the survival of the virus for hours and days [132]. Reassuming, PM could consist of a carbon core covered by sulphate, nitrate, organic chemicals, metals and crystal elements on which bacteria and viruses and toxic heavy metals would be adsorbed. These complex mixtures are made up of soluble and insoluble fractions, the soluble ones contain water-soluble ions and organic acids, while the insoluble ones are those responsible for higher cell mortality and contain kaolinite, calcium carbonate

and some organic carbon [133]. However, there is no precise composition, but it depends on the geographical location, weather, temperature, wind, relative humidity, and UV rays [133].

The PM particles according to the aerodynamic diameter are classified into:

- “coarse” if they have a diameter greater than 2.5 μm ;
- “fine” or “thin” if their diameter is between 100 nm e 2.5 μm ;
- “ultrafine” if they have a diameter up to 10 nm;
- “nanoparticles” if they have diameter up to 2 nm or few nm [129].

This classification is used in PM indexes that measure the amount of particulate matter in a certain volume of air: there are multiple PM indexes that indicate the amount of particulate in different size ranges. The most common indices are $\text{PM}_{2.5}$ and PM_{10} , which measure particulates smaller than 2.5 μm and smaller than 10 μm , respectively. To date there are no indices for ultrafine particulate matter or nanoparticles, as it is not yet possible to distinguish the number of particulates with smaller dimensions. PM_{10} , for example, is unable to provide information on the particle size distribution included in the sampled $\mu\text{m}/\text{m}^3$ [129].

WHO has established maximum tolerable limits of the indices, both for the annual mean and for the quantity of particulate produced in a day. In Europe these limits are different for PM_{10} and $\text{PM}_{2.5}$:

- PM_{10} must not exceed 40 $\mu\text{m}/\text{m}^3$ as an annual mean and 50 $\mu\text{m}/\text{m}^3$ or more than 35 times a year as a daily mean.

-il $\text{PM}_{2.5}$ must not exceed 20 $\mu\text{m}/\text{m}^3$ as an annual mean.

The limits are used to regulate the amount of toxic pollutants in the air. In fact, especially the smallest PM particles, enter very easily into tissues and cells, carrying toxic substances and probably viruses and bacteria [129].

6.3 A possible correlation between pollutants and viral diseases

For several years, pollution has been related to the incidence of human diseases, including those of viral origin [129]. In 2013 the International Agency for Research on Cancer (IARC) identified the pollutants of combustions in the “group 1-definetly carcinogenic”. The particulate matter would not be the cause of the virus spread, but it could make an important contribution [129]. Carugno et al. correlated the elevated levels of PM_{10} and hospitalization for the

respiratory syncytial virus (RSV) in Lombardy [134]. Also Ye et al. support this correlation between PM₁₀ and the RSV case incidence, adding that children, subject to RSV infection, have a high respiration rate so they could increase PM inhalation [135]. Cui et al. analysed the air pollution index (API) of 5 different regions in China, between April and May 2003: the index of the major pollutant was PM₁₀ and showed a positive correlation to the case fatality of SARS recorded in that period and in those locality [136]. Martelletti et al. correlate the exceeding of the legislative limits of the indices PM_{2.5} and PM₁₀ in Northern Italy during February 2020 with the case fatality of Covid-19 and the spread rate of the SARS-CoV-2 virus. Setti et al. observed a linear relationship between daily PM₁₀ data and Covid-19 cases in Italy [137]. The contagion curves in less polluted regions have trends compatible with human-human transmission calculation, while the contagion curve relating to the Po valley shows an anomalous increase. The PM₁₀ levels recorded in the Po Valley region seem to confirm the possible boost effect of atmospheric particulate matter [137]. Chen et al. have correlated the spread of influenza A virus with Asian dust storms (ADS) [138].

The relationship between particulate matter and viral disease could be explained by two factors:

- Oxidative stresses caused by the inhalation of PM which would lead to negative effects on the immune system and which would facilitate viral infection.

- PM as a potential viral carrier [129].

Laxmipriya et al. on the air pollution with SARS-CoV2 speak about “unseen evil twin of the virus” [139].

6.3.1 Toxic effect of PM on human health

Air pollution and PM could cause oxidative stress on human cells and interfere with the antiviral response of the immune system [129]. Pollutants could reduce the activity of macrophages capable of phagocytizing pathogens and they could compromise the pulmonary cleaning activity. Therefore, often exceed PM levels in more polluted cities may have increased the susceptibility of the population to more severe symptoms and respiratory complications of the disease [129] [125]. PM seems to act against the angiotensin-converting enzyme 2 (ACE2) receptor which operates as a viral entry point into the cell [140].

6.3.2 PM as a viral carrier

The elevated PM levels in cities and periods related to the spread of a viral disease have often been used to explain the greater virulence of the spread in those locations [129]. Setti et al. claim that atmospheric particulate matter is known to act as a transport vector for many

chemical and biological contaminants, including viruses [141]. Viruses could attach to particulate matter by coagulation, in order to remain in the air for hours, days or weeks and spread for long distances. The PM could also present a substrate that allows the virus to remain in the air in vital conditions for more time. The viability of the virus depends on external factors, such as temperature, UV rays and relative humidity [141]. Evidence of increased concentration of bacteria, fungi or fungal spores during desert dust storm suggests involvement of air pollutants in local bioaerosol levels [138]. Therefore, the aerosolization of the dust from the ground and the organic aggregates in sea spray could facilitate the long-range transport of bacteria and viruses into the atmosphere [142]. Evidence of airborne bacteria attached to dust or embedded in organic particles has been shown, while there is less evidence of dust carrying viruses [142]. Sedlmaier et al., to investigate the properties of PM as a carrier virus, produced and analysed an aerosol with influenza A virus (subtype H10N7) contaminated fecal fine PM_{2.5} [143]. The authors observed that on PM_{2.5} the infectivity of the influenza A virus at 20 ° C was less disturbed, while normally above 4 ° C it should be more unstable [143].

Qin et al., thanks to recent advances in DNA extraction from airborne samples and thanks to metagenomic library preparation, observed the presence of DNA on airborne PM samples collected over a 6-month period in 2013, a period in which there was an increased incidence of diseases in the city of Beijing [144]. The DNA extracted from the particulate matter belonged to a great diversity of microbial species, including bacteria, fungi, and viruses. This research certainly deserves to be investigated and the authors underline that such evidence found on particulate matter "should be treated as only exploratory evidence of potential risks" [144].

Setti et al. found the first evidence of SARS-CoV-2 on PM [137]. They analysed 34 PM₁₀ samples from an industrial site in the province of Bergamo, the first Italian city most affected by the Covid-19 pandemic in 2020, collected between 02/21/2020 and 03/11/2021. The analysis confirmed the presence of SARS-CoV-2 RNA on part of the collected PM₁₀ samples. Despite having found evidence of RNA on the particulate matter, they should investigate the virus viability. In fact, the virus should be infectious and in a sufficient concentration on PM₁₀ to consider the particulate as the carrier responsible for the boost effect of the contagion [137]. Nor et al. have demonstrated the presence of SARS-CoV-2 RNA on indoor PM_{2.5} collected in different wards in a hospital in Malaysia for Covid-19 patients, but remains to be verified if the traces of RNA come from intact viruses or from non-infectious virus particles [126].

Borizova et al. propose the exclusivity of virus-PM interaction for enveloped viruses. In fact, thanks to their lipid envelope, they are able to create unspecific interactions with different surfaces, including PM [145]. SARS-CoV-2 can invade the central nervous system inducing neurological diseases. PM can also interact with the lipid components of the brain nerve terminal plasma membrane. Since the origin of the lipid envelope of enveloped viruses comes from the host cell, the envelope of the SARS-CoV-2 that infected the central nervous system could have the same characteristics of the lipid components capable of interacting with PM. Therefore, in a water surrounding the types of PM that interact with the brain nerve terminals could interact with the SARS-CoV-2. In addition to carrying the virus and facilitating its entry into the respiratory system, the PM could compromise the integrity of the cell plasma

membrane, allowing the virus to enter the cell without the participation of ACE2 receptors [145].

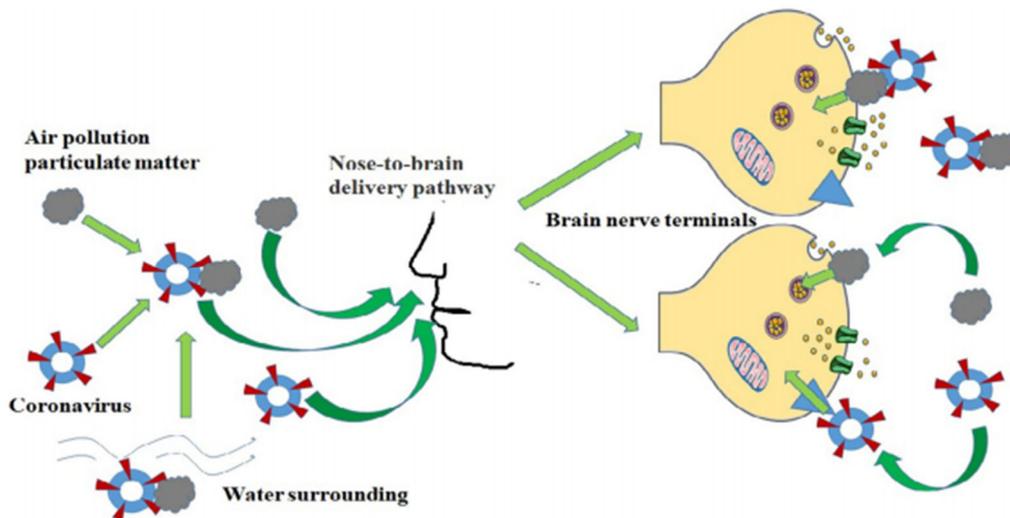


Figure 6.3: PM and SARS-CoV-2 effect on brain nerve terminals [145]

Di Girolamo with an analytical model investigates the potential role of the airborne transmission with PM as a carrier for SARS-CoV-2 [128]. The author makes various assumptions and based on these he estimates the number of coalescing events of breathing/coughing droplets on PM₁₀ and PM_{2.5}. The evaluation given by this model would seem to confirm the incidence of PM as a SARS-CoV-2 carrier on the spread of infections, but the results are based on the assumptions made for the calculations of the analytical model, therefore the degree of uncertainty about the results is large [128].

Assuming the presence of intact and infectious SARS-CoV-2 on PM, the transported viral titer will not be very high, but the cluster formed by virus and PM could increase infectiousness power of the pathogen [146]. In fact, PM particles could increase the persistence of the virus in the upper respiratory tract, thanks to the larger cluster size compared to single droplets. In addition, clusters larger than 5 μm could deagglomerate once inhaled and form smaller virus-landed PM able to deposit in the pulmonary alveoli. Figure 6.4 shows the size of the inhaled PM clusters and their respective deposition mechanisms in the respiratory system [146].

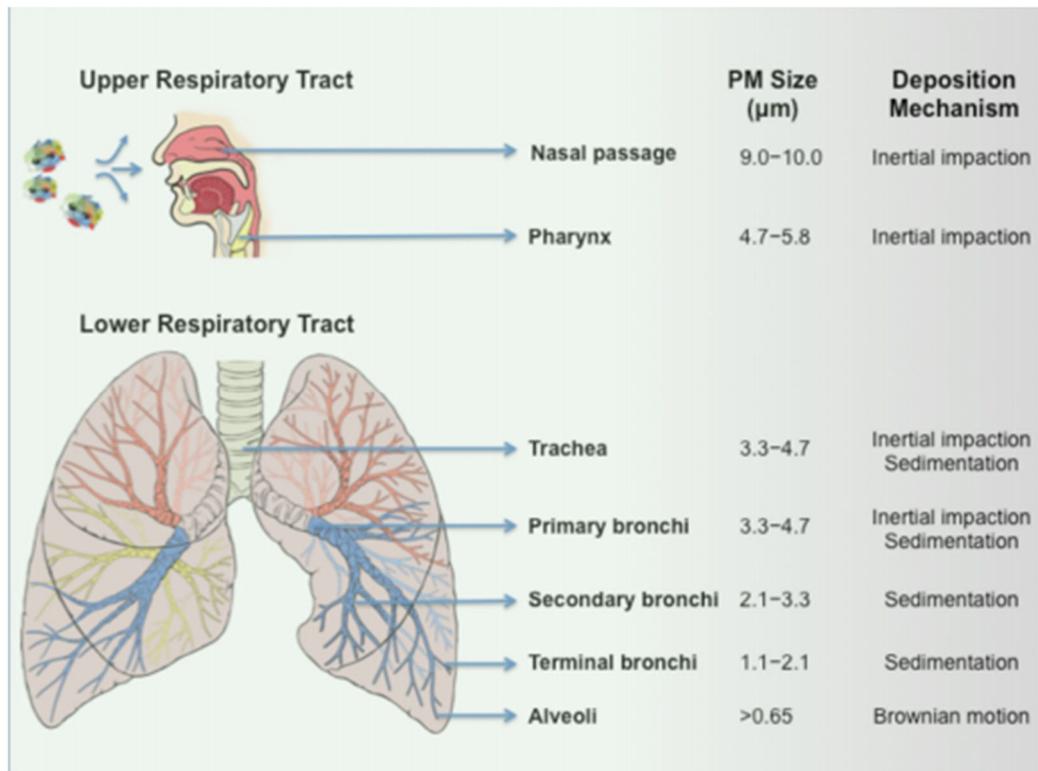


Figure 6.4: SarS-CoV-2 and PM cluster inhalation and deposition in the respiratory tract [146]

Other scientists deny the hypothesis of PM as a potential viral carrier since there is not enough evidence for this correlation [5]. The scientific community requires more research on the transportability of the virus on PM, on its infectiousness, viability and lifetime on the particulate matter [5]. In fact, the various correlations between the levels of the measured PM indices and the spread of viral diseases could be exclusively due to the already confirmed weakening of the immune system and of the biological barriers exposed to atmospheric particulate matter [5]. Bontempi states that it is not possible to conclude that a spreading mechanism for Covid-19 occurs through the air with PM as a viral carrier [147]. In fact, higher levels of PM₁₀ were recorded in Piedmont than in Lombardy and the number of Covid-19 cases was lower. They report that PM certainly has a negative impact on human health, thus making the population more vulnerable to the virus outbreak, but its contribution as a viral carrier is yet to be proved [147]. Belosi et al. confirm the ineligibility of the results obtained on viral carrier aerosol, since the concentration of RNA found on PM is too low for it to be considered a valid contribution to the spread of the disease [127].

In conclusion, it can be said that airborne transmission that sees the PM as a viral carrier is still a very controversial issue within the scientific community and that needs further study [127].

Chapter VII

Considerations and conclusions on antiviral nanomaterial applications

From the previous chapters, it is assumed that research on the potential application of nanomaterials as new antiviral solutions is being increasingly considered [19]. The potential application of new materials must always be accompanied by studies on their possible toxicity and safety towards human and animal health and on their environmental impact, considering their future production at the industrial level [148]. In this last chapter will be presented general considerations on the toxicity of antiviral nanomaterials and, subsequently, their possible applications.

7.1 Nanotoxicity

The increasing development of nanotechnology is still accompanied by a great lack of investigation into the potential toxicity of nanomaterials in the short and long-term [19]. In fact, nanotechnology is studied and applied in various fields, such as electronics, biosensing, sunscreens, paints, and food industry and cosmetics, therefore living beings and the environment are subject to increased exposure to nanomaterials [149]. Considering their possible applications as antiviral solutions in personal protective equipment, both the effect on human health and on the environment given by the consequences of the nanowaste must be taken into account [148].

Nanowaste is a new type of waste and like all other types, it will have to undergo several studies on its recyclability [150]. Figure 7.1 shows the steps from nanomaterials used in nanotechnologies, to their waste and possible recycling modes [150].

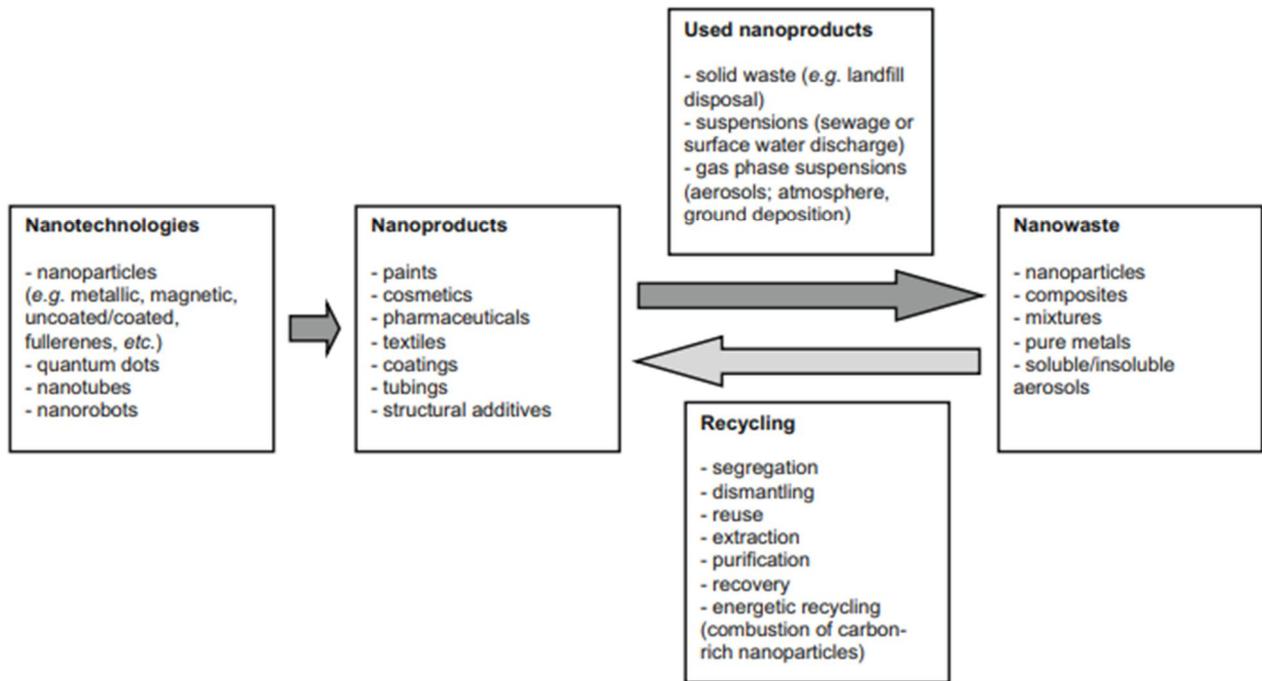


Figure 7.1: green nanoscience scheme on nanowaste [150]

To date, the risks to human health and the environment are still unclear [148].

The nanowaste given by washing fabrics modified with antiviral nanoparticles has a specific concentration of NPs that can be dispersed in the environment. This nanoparticles concentration depends on the characteristics of the modified fabrics, the type of washing, and the washing liquid used [148]. The fabrics are in direct contact with the skin. Before going into production, they must undergo several tests that analyse the potential irritation of the skin, in general it is said that they must be dermatologically tested. These tests must also consider the delicacy of the skin and its superficial changes [148].

Personal protective equipment also includes masks, therefore the possible inhalation or ingestion of nanomaterials must also be considered, if they are not well fixed in the material that constitutes the device. For this reason, it is necessary to deepen these studies with simulation tests, such as bioprinting techniques or reconstructed human epidermis (RhE) [148]. The developments must be accompanied by the creation of guidelines for the correct use of these new protective devices, for the safety of consumers, and to avoid further problems related to pollution. Palmieri et al. have schematized in figure 7.2 the possible behaviours to be adopted for the correct use of nanomaterials-based personal protection equipment [148].



Figure 7.2: scheme of behaviours for the NMs-based PPE correct use [148]

To establish the risk associated with a nanomaterial, several in vitro tests must be carried out on different organs cells. The damages that could be observed in this case are mainly those to the DNA and apoptosis [149]. Subsequently, in vivo tests are performed, for example on mice or rats, administering the nanoparticles through inhalation, ingestion, or intra-peritoneal injections. Here the effects on mitochondria, cell membranes, and genetic material are studied [149].

Nanotoxicology and nanosafety are two branches of scientific research that must accompany the development of nanotechnology [6]. Only in recent years some changes and more considerations towards nanosafety occurred. Among all there is that of the European Commission Regulation 2018/1881 on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) on nanoforms of substances, applied since 01/01/2020 [6].

Therefore, guidelines for nanosafety towards humans and the environment are still being defined today. This aspect of research must never be underestimated or not taken into consideration. In fact, often in health emergencies, scientists are looking for solutions to be applied as soon as possible to contain the spread of the problem, but scientific solutions for such emergencies must undergo often complicated and lengthy evaluation procedures. To date, the products that could represent the new antiviral solutions are subjected to the various control steps much faster than usual. Haste in research must not compromise the benefit-risk ratio of these new products [6]. This relationship has already been underestimated in the past, as in the case of the drug thalidomide in the 1960s, and this has led to serious consequences on human health [6].

The test that has always been carried out in the studies reported in the previous chapters is the cytotoxicity test. After this test it is usually established the lethal dose 50 (LD₅₀), i.e. the dose of the nanomaterial administered capable of killing 50% of a sample population [150]. The cytotoxicity test, which is done *in vitro*, analyses the effect of the nanomaterial in the short term [150].

In general, the main issues to be considered in studies on the toxicity of a nanomaterial are:

- the chemical-physical characteristics of the nanoparticles, such as the shape, size, chemical composition and any surface treatments;
- dose metrics (usually $\mu\text{g} / \text{mL}$);
- comparison with the toxicity of different nanomaterials;
- standardization of the experimental setup [151].

AgNPs seem to be the most potentially toxic nanoparticles compared to other nanomaterials, such as iron(II, III) oxide or titanium(IV) oxide. In fact, in *in vitro* tests, AgNPs caused dose-dependent mitochondrial dysfunctions, DNA damage, apoptosis and necrosis [19]. The cytotoxicity and genotoxicity of these nanoparticles depend on the nanosize-concentration and the exposure time [19]. If they are inhaled and ingested beyond a concentration limit, they could cause lung failure, increased heart rate and a decrease in blood pressure [148]. Ahmed et al. hypothesized that AgNPs, if inhaled, could cause damage to lung epithelial cells or alveolar macrophages even at non-cytotoxic doses [149]. The cytotoxicity of these nanoparticles is greatly reduced if they are coated with other molecules. The difficult step is to report and research the *in vivo* effects of chronic low dose exposure to these nanomaterials [149].

Hence metal oxides appear to be less toxic than metal nanoparticles such as AgNPs [19]. Titanium(IV) oxide has been declared non-toxic several times, also because it does not appear to be able to cross the deepest levels of the skin, but there is still evidence of its potential toxicity [19]. Titanium(IV) oxide, like zinc(II) oxide, for its antimicrobial properties, is often used in the form of a nanoparticle in cosmetics, so it must be subjected to skin irritation tests [152].

With these oxides capable of generating ROS when stimulated by UV radiation, phototoxicity should also be tested [152]. Park et al. and Nica et al., based on their results, considered the inflammation observed on dermal and pulmonary cells to be negligible [152] [153]. The effects of oxidative stress are present, but they are dose-dependent, so at low doses it is possible not to consider them important [152] [153].

Between the carbon-based nanomaterials, the only ones to have been subjected to exhaustive toxicological assessments are the carbon nanotubes, which, as it was already mentioned in chapter 5, have been declared potential carcinogenic [6]. Figure 7.3 shows the nanotoxicity effects due to the metal nanoparticles.

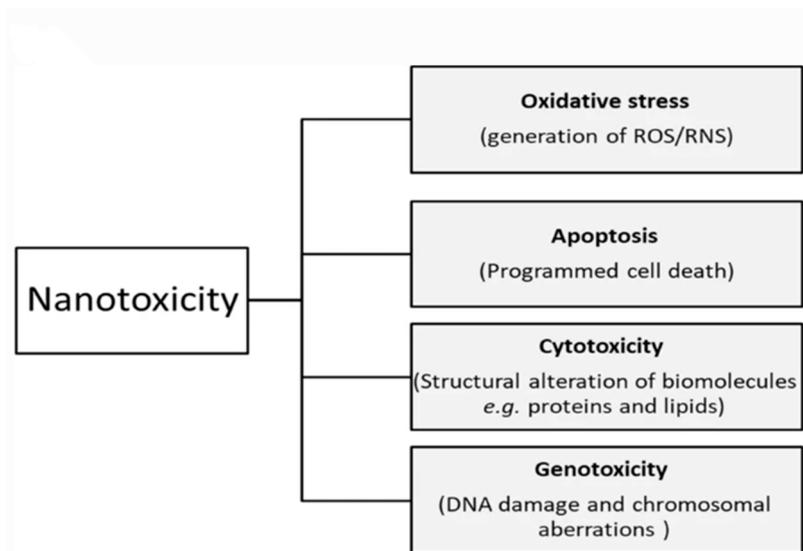


Figure 7.3: nanotoxicity effects of metal nanoparticles [154]

Future studies must consider improved ADME (adsorption, distribution, metabolism, and elimination) effects and more experiments on the exposure of nanomaterials in the long term [151]. It is difficult to draw up precise rules or doses not to be exceeded for each nanomaterial since the nanotoxicity of these elements depends on several variables, that could change for each application. [153]. What could be done is to establish guidelines to test the potential toxicity of a specific nanomaterial in a specific application [153].

7.2 Nanomaterial-based applications against viral spread

The current pandemic is not the first and it will not be the last. During a health emergency of this kind, if vaccination is not possible or does not yet exist, the best way to prevent the viral spread is to protect yourself from infection [3]. Therefore, personal protective equipment such as masks, gloves, protective suits, and scrubs, i.e. all the elements that make up the personal

protective equipment (PPE), become very useful [155]. In the previous chapter, the possible methods of transmission were described, which imply the need for respiratory protection and protection against direct contact. The devices that have the task of providing respiratory protection intervene in a complex system that must consider various aspects:

- they must prevent the release of any infected droplets;
- they must avoid the potential infection given by the virus in circulation;
- they must have a good air filtration capacity [156].

In general, respiratory devices such as masks or respirators must be able to protect against infection both from the outside and from the inside, so as not to increase the spread.

Personal protective equipment represents good antiviral solutions since they do not involve the use of drugs [156]. To ensure breathability through the masks, pores must be large enough to ensure the passage of air, for this reason, viral nanoparticles smaller than 100-300 nm are able to pass through [157]. Viral nanoparticles are often found in aggregate states or in larger droplets, therefore the performance given by filtration would not be so bad if it were always accompanied by the correct use of these devices [156]. Often, however, there is improper usage of PPE, during their use and especially in undressing step or taking off the mask. If you touch its outer surface when you remove the mask, you can come into direct contact with the deposited infected droplets, triggering the potential secondary transmission mode. Nanotechnology and nanomaterials in this field would be very useful, as they should be able to inhibit the viruses with which they have interactions, or even destroy them. Therefore, they could avoid the potential secondary mode of transmission [156].

Nanomaterials can be embedded in the fibers that make up PPE. Figure 7.4 shows the characteristics required for the design of new nanomaterials-based respiratory protection devices against the viral spread [156].

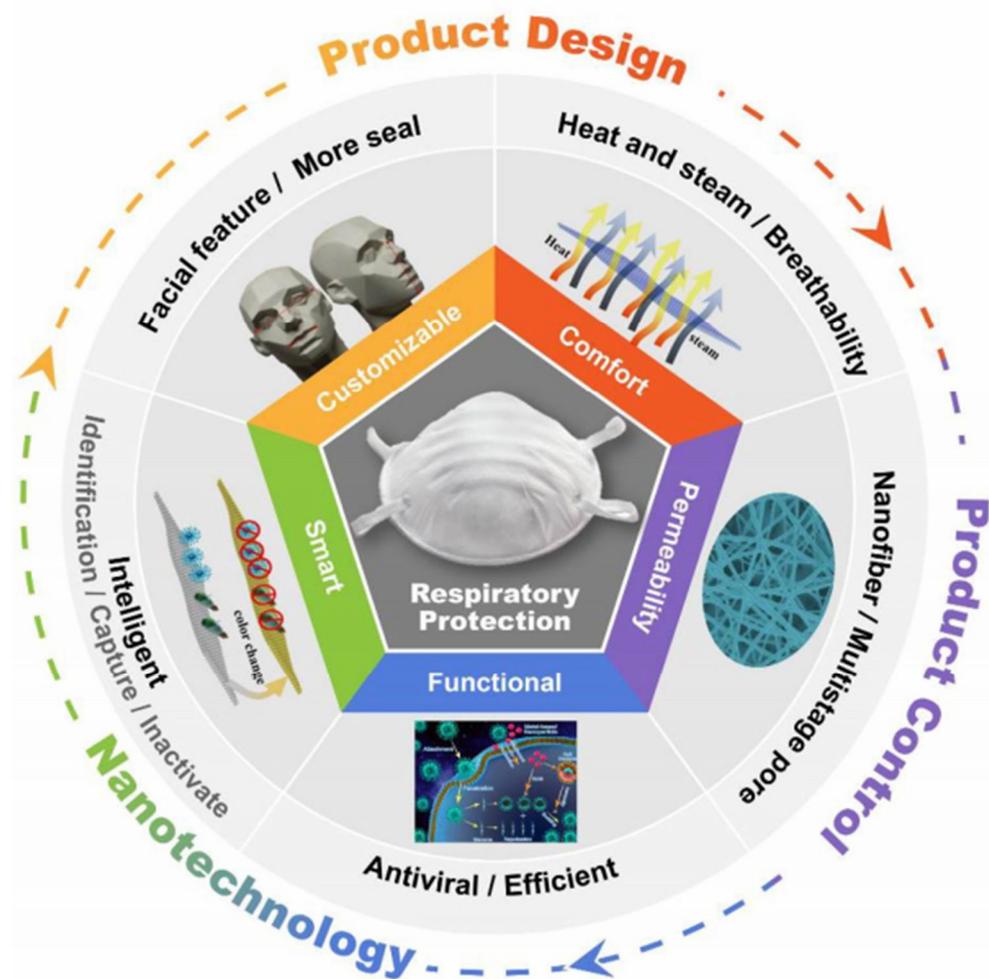


Figure 7.4: characteristics of the nanomaterials-based respiratory protection design [156]

Antiviral nanomaterials can be applied not only in PPE but also in nanocoatings, to avoid the spread by secondary transmission, given by contact with surfaces contaminated by infected droplets [156].

This thesis deals with nanomaterials capable of acting on the virus before it can infect host cells, so their potential applications are those that do not foresee an infection already in progress, but protection from the potential infection. Devices that could protect against potential viral infection, as well as antiviral PPE and nanocoatings, could be antiviral air filters, wound dressing, or water filters. WHO has established that 50% of biological contamination in indoor environments is given by air handling systems and the accumulation of microorganisms in them. The nanomaterials previously treated could be included in the air filter and inhibit the growth of these microorganisms. AgNPs have already been applied in air filters, in antimicrobial catheters and gel for topical use, such as that for burn wounds [19].

Another strategy that involves the use of antiviral nanomaterials could be to synthesize new disinfectants and sanitizers that can remain on the surface for a long time, resisting continuous

washing and friction [157]. The Nanotech Surface Company, for example, has formulated a disinfectant based on titanium(IV) oxide and Ag nanoparticles which has recently been used to clean buildings in Milan. Other disinfectants could exploit the production of light-enhanced stress oxidation [157].

Nanotechnology is an excellent solution to increase the antiviral function of PPE since it is able to intervene without changing other characteristics of these devices: in the case of masks, it increases the antiviral efficacy without decreasing breathability [157].

The respiratory devices can be divided into 3 categories based on the filtration capacity:

-single-use face masks, consisting of only one layer, do not filter very well.

- respirator masks N95 and FFP2, consisting of 4 layers (non-woven layer, activated carbon layer, cotton layer, and non-woven layer plus the optional valve to regulate the breathability) and adapt very well to the face.

-surgical masks, consisting of 3 layers (filter layer between the two non-woven layers), are the most common and are able to block the largest droplets [148].

The nanomaterials that have already been used in face masks are copper oxides, carbon-based nanomaterials, AgNPs, and titanium(IV) oxide. These are embedded in the polymers usually used in mask layers, such as polypropylene PP, polyethylene PE, polyester, fiberglass paper, and polycarbonates. The incorporation of these antiviral nanomaterials could bring other qualities, such as in the case of graphene, mechanical strength, and conductivity [148].

Sportelli et al. have been working on antimicrobial nanomaterials since 2004, and in this period they have focused on antiviral ones [3]. They reviewed antiviral solutions capable of preventing contamination and contagion. They studied the embedding of metal nanoparticles in polymeric matrices and treatments capable of creating nanocomposites for overshoes, surgical gowns, hair cups, and respirators [3]. Borkow et al. have already experimented masks with copper oxide nanoparticles against the influenza A virus [72]. In conclusion to their review on the possible applications of antiviral nanomaterials, they write: “Can inorganic nanoparticles be useful in affecting the early viral lifecycle stages? The specific question is: is it possible to realize active nanomaterials able to inhibit the binding and fusion of viruses on the host cell? We believe that it is certainly possible to find a nanotechnological solution for these quests. Our idea is to realize hybrid antiviral nanomaterials by functionalizing metal nanoparticles with typical antiviral drugs, with enhanced synergistic efficacy. These synergistic nanoantivirals might offer a great help in increasing the efficacy of PPE and improving the safety of common touch surfaces” [3]. Therefore, for them the best solution would be a solution that provides antiviral effects of some drugs capable of forming metal complexes [3].

AgNPs have also been applied to cotton or silk textile fibers against bacteria and have shown very high antibacterial efficacy. AuNPs are limited in their large-scale use in PPE by their higher cost [156].

Once the potential antiviral nanomaterials have been studied and selected, their most useful applications must be identified and subsequently the treatments to applied them [158]. A treatment with AgNPs that was developed by HeiQ, a Swiss-based company, the HeiQ Viroblock NPJ03, has been tested on facemasks and has proved to have the ability to reduce the viral charge on masks. This AgNPs treatment prevents them from being a potential infected droplets carrier. This textile antiviral treatment provides at least 99.99% protection, both against coronavirus (229E) and influenza viruses [158]. A treatment for gowns with a dispersion of 9 nm titanium(IV) oxide nanoparticles was also studied and applied. The gloves treated in this way showed antiviral efficacy against the hepatitis C virus, hepatitis B, and HIV [158].

These systems that involve the application of antiviral nanomaterials still have numerous challenges to face, such as production costs, potential toxicity and environmental effects issues [157].

7.3 Conclusions and future perspectives

The development of antiviral nanotechnology will have to go hand in hand with nanosafety and nanotoxicity studies [6]. As it has been pointed out several times previously, the study on antiviral nanomaterials and their potential application on fabrics or other materials used in public spaces, such as hospitals, must be deepened [19]. Nanotechnology-based sanitizers would be optimal for avoiding the spread of viral infections [19].

This development should lead to the production of cheap, easy and quick to obtain and easy to apply antiviral nanomaterials in different PPE devices through safe and non-polluting treatments [19]. Developments in the study of the mechanism of action against viral particles could help the effectiveness of the design of nanomaterials to obtain the highest antiviral efficacy and the lowest possible cytotoxicity and nanotoxicity. In nanotechnology research, the benefit-risk ratio must always be considered [6].

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