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Nano-based antiviral coatings to combat viral infections

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ABSTRACT

In the recent past, epidemics and pandemics caused by viral infections have had extraordinary effects on human life, leading to severe social and financial challenges. One such event related to the outbreak of the SARS-CoV-2 virus has already taken more than 917,417 lives globally (as of September 13, 2020). The nosocomial route of viral transmission has also been playing a significant role in the community spreading of viruses. Unfortunately, none of the existing strategies are apt for preventing the spread of viral infections. In order to contain the viral transmission, the principal target would be to stop the virus from reaching the otherwise healthy individuals. Nanomaterials, due to its unique physical and chemical properties, have been used to develop novel antiviral agents. In this review, we have discussed several nanotechnological strategies that can be used as an antiviral coating to inhibit viral transmission by preventing viral entry into the host cells.

Contents

1. Introduction

The World today faces some unprecedented challenges with regard to combating viral emergencies with the outbreak of COVID-19. With an ever-rising death toll, already surpassing 917,417 people globally (as of September 13, 2020), COVID-19, the latest of the three pandemics caused by the coronavirus family in the last two decades (including the SARS outbreak in 2002 and MERS outbreak in 2012), has affected about twenty-nine million people around the World, as per the latest report by WHO [1,2].

Historically, viral outbreaks have been one of the primary causes of morbidity and economic losses, highlighting our ever-remaining lack of preparedness to combat these sudden emergence or re-emergence of viral diseases [3–5]. Enteric viruses like Caliciviruses(Noroviruses), Rotaviruses(RVs), Astroviruses(AVs), Enteroviruses(Polioviruses, Kobuviruses), Hepatitis A Viruses, Hepatitis E Virus, Enteric Adenoviruses, Coronaviruses, Toroviruses, Picobirnaviruses, etc. transmit by the orofecal route, that means, through either direct contact with an infected person or through consumption of/contact with contaminated food or water [6,7]. Ebola virus (EBOV), HIV, etc. also transmit by unprotected physical contact and bodily fluids of an infected person [8–11]. Sexually transmitted viruses(STVs) like Human Papilloma Virus (HPV), Human Immunodeficiency Virus-1(HIV-1), Hepatitis B Virus(HBV), Hepatitis C Virus(HCV), Herpes Simplex Virus-2(HSV-2), etc. infect the human hosts through mucosal route [12]. Several reports suggest the transmission of HBV

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and HCV, especially among the healthcare workers by mucosal-cutaneous (accidental encounter of infected blood or other biological material of an infected person with the mucus membrane of eye, mouth or with the intact/nonintact skin of a healthy individual) or percutaneous (a healthy individual receiving an injury with a sharp contaminated object like needles, piece of glass, etc.) exposures [13]. Arthropod-borne viruses (arboviruses) such as West Nile virus(WNV), Japanese encephalitis virus(JEV), Dengue virus(DENV), Chikungunya virus(CHIKV), Yellow fever virus(YFV), Zika virus(ZIKAV), Mayaro virus(MAYV), Bluetongue virus(BTV), Venezuelan Equine Encephalitis virus(VEEV), etc. can also transmit to humans or agriculturally important domestic animals via insects [14]. Moreover, viruses belonging to the families of Paramyoviridae(Measles, Parainfluenza), Pneumoviridae(HMPV, RSV), Coronaviridae(HcoV, MERS-CoV, SARS-CoV), Picomaviridae(Rhinovirus), Adenoviridae(Adenovirus), Orthomyxoviridae(Influenza virus), etc. infect the respiratory tract of the host. These viruses may infect different species of animals and may vary in their host range and virulence factors. However, one major characteristic common to all of them is that they infect and replicate inside the host cells and subsequently shed, which ultimately leads to transmission to other individuals. Cross-species barriers have been of somewhat help in protecting humans from acquiring highly pathogenic viral strains from other infected animals, like the avian influenza-A virus. However, some mutations in the viral genome that may cause a spillover event from an animal to a human might bring about pandemic scenarios with immeasurable health and economic impacts [15–17].

Viral transmissions may occur by three different routes: Contact (indirectly by fomites through hygiene malpractices, directly through body fluids such as blood or semen, or through insect bites), Droplets, and Aerosol [18]. According to WHO, the particle size of 5 μm is considered as a cutoff size for particle diameter to distinguish between droplet mode and aerosol mode of transmission. Another factor that differentiates between these two modes is the terminal settling velocity of air. Particles that are less than 5 μm tend to remain in the air for an extended period. Contrarily, the particles having a size of more than 5 μm cannot stay airborne for long. They tend to settle upon a surface within $\sim$1m of their site of generation, provided the velocity of air in that particular area does not exceed the terminal settling velocity of air. Thus, particles with a diameter of 100 μm are reported to remain airborne only in cases where air velocity was more than the terminal settling velocity of the particle. The rate of desiccation of the particles is also an essential factor that needs to be considered. There are cases reported where larger droplets that initially had fallen out of air get desiccated and decrease in size to become airborne again. This phenomenon significantly increases the virus transmissibility because each of these droplets or aerosols are packed with millions of microorganisms to which humans are exposed, who are otherwise very susceptible to catch the infectious disease even when they are exposed to a tiny number of virus particles [18–21].

Nosocomial transmission of viral infections has also been reported in several incidences [22]. Nosocomial infections are acquired through the hospital settings or other healthcare facilities, either during the patient's stay at the hospital, during hospitalization, or hospital visits. Though diseases that are already in the incubation period before the person visits hospitals are not considered nosocomial, community-acquired viral infections are a significant source of nosocomial infections [22,23]. Hospitalized patients are generally much more susceptible to infections as a consequence of either their decreased immunity or invasive medical procedures that are followed in a hospital, creating new routes of infections [22]. Few standard nosocomial modes of viral transmission are through either direct contact with the infected healthcare workers, visitors, family members, or through contaminated fomites. Poor hand hygiene practices (like not disinfecting hands properly after attending to a patient) among healthcare workers are also a paramount cause of nosocomial respiratory virus transmission. Moreover, reports suggest that low temperature and low humidity condition, most frequently observed in healthcare facilities equipped with central air conditioning units, sustain the MERS-CoV in such a way that it can still be recovered from surfaces of steel or plastic, even after 48h [24]. Both SARS-CoV and SARS-CoV-2 were also reported to be recovered from the surfaces of inanimate objects like plastic and stainless steel even after 72 h [25]. Aerosol generating procedures or AGPs like tracheostomy, bronchoscopy, cardiopulmonary resuscitation, etc. can worsen the nosocomial spread leading to super spreading events [24,26]. Thus, there are several reports where hospital-related amplification of human transmission potential was observed for viruses, like SARS(Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome) coronaviruses [24,27–30]. In fact, nosocomial transmission accounts for approximately a third of total MERS-CoV cases globally [24]. The percentage was reaching as high as 100% of the total encountered cases of infection in Al-Hasa, Saudi Arabia, during the period of April 1 to May 23, 2013 [28]. In South Korea, too, during the MERS outbreak of 2015(May 20 to July 5), 100% of all cases (a total of 186 cases) were related to hospital settings [29]. In cities like Singapore, Toronto, Beijing, and Vietnam, the hospital-related transmission of the SARS-CoV contributed to the lion's share of cases from February to May 2003 [27,31–34]. 74% of all the cases of SARS-CoV infections in Singapore, as well as 100% of all the cases of SARS outbreak in Toronto, were related to hospital settings from February 25 to May 11, 2003, and February 23 to April 15, 2003, respectively. [27,31,32]. In the most recent coronavirus outbreak by SARS-CoV-2, nosocomial transmission has also been reported [35]. Technically, nosocomial respiratory viral infections are those where the number of days of a patient's hospital admission to the onset of symptoms surpasses or stays within the range of the incubation period of the identified virus. However, in cases of sudden viral outbreaks like SARS-CoV-2, where there is a limited characterizing data of a zoonotic virus of such a high virulence, it is tough to track the viral infection map. Moreover, a large number of asymptomatic carriers worsen the situation by helping the viral transmission to attain a pandemic nature [36,37].

In order to contain nosocomial infections or viral transmission in general, there are a number of suggestions from the World Health Organization and the Centers for Disease Control and Prevention (CDC) [22,38,39]. Person to person transmission can be checked by performing proper hand decontamination, maintaining good personal hygiene, using appropriate protective clothing, masks, and gloves. Routine cleaning of the hospital environment by hot or superheated water treatment of suitable surfaces or chemical/heat sterilization of the potentially infected surfaces are required to be performed along with other prescribed guidelines to prevent transmission through the environment. Proper use and handling of personal protective equipment (PPE)s by the health care personnel, adequate decontamination for medical instruments needs to be followed [26]. Furthermore, to avoid viral infections that can cause an outbreak, early identification is the key [22]. However, for viruses like SARS-CoV-2, where the incubation period for the onset of symptoms can be as high as two weeks, it might become challenging to contain the spread [40].

In such a scenario, nanotechnology comes to our rescue with several functional nanoparticle-based antiviral agents as well as with nanomaterial coating strategies to prevent the virus from infecting susceptible individuals [3,41]. It can also provide us with affordable and easy to maintain solutions to reduce viral

transmission drastically. A fascinating nature of antiviral nanomaterials is its nonspecific mode of action, which is its ability to inactivate various kinds of microbes through a single platform. Moreover, the multivalent nature of nanomaterials makes them a suitable candidate that can interfere with viral attachment and, thus, subsequently interfere with viral entry into the cell [42]. However, for vector-borne viral transmissions, like arthropod *Aedes aegypti* borne dengue virus (DEN-2), control measures of which only include effective vector control strategies, nanomaterial intervention as an antimicrobial strategy, such as a surface coating, would be of limited use [43]. In this review, we are going to discuss the potential nanotechnological solutions that can be used as antiviral coatings to prevent the advancement of several viral infections.

2. Mechanism of virus-receptor interaction

The primary target of any potential antiviral coatings would be to neutralize the virus before it can invade the host cells to hijack the host cell machinery. Understanding the mechanism of virus-receptor interactions would thus play a significant role in developing an antiviral nanomaterial. In general, the role of viral receptors is diverse. They function as coordinators of virus entry as well as a regulator of multiple downstream signaling events that would eventually help the virus enter the cell. There are specific attachment proteins on the surface of the virion that usually mediate the viral receptor interactions as being represented through Fig. 1. The nature of these attachment proteins can differ depending upon the virus shape (spherical or icosahedral) and the composition of the viral coat (enveloped or nonenveloped) [44]. Viruses can directly bind and fuse with the host cell's plasma membrane, usually after interaction with a specific membrane receptor, for penetration into the target cells [44–46]. It may also get internalized through endocytic pathways after binding to the surface receptors and then fuse with or disrupt the endosomal membrane [45,47,48].

Whatever the mode of entry may be, the initial encounter with the host cell receptors is a critical regulatory step of viral infection and can vary from nonspecific to highly specific. The viruses might first interact with a low affinity-high avidity interaction followed by one or more high-affinity ones with secondary or tertiary receptors. These initial low-affinity interactions are often mediated by carbohydrates such as sialylated glycans or sialic acids(SAs). A variety of modified SAs is present throughout the animal kingdom. In humans, the most commonly found one is 5-N-acetyl-neuraminic acid(Neu5Ac), which is further modified by α2,3- or α2,6 linkages with the Gal or GalNAc residues of the main sugar chain and are known as α2,3- or α2,6 linked SAs, respectively. The α2,6 linked SAs are expressed primarily in the upper respiratory tract, while the lower respiratory tract predominantly harbors the α2,3-linked SAs. These patterns of distribution of different varieties of SAs play a significant role in host selection, disease progression, as well as the severity of the infection. For example, in the event of the influenza-A virus (ensheathed by Hemaglutinin(HA) and Neuraminidase(NA)) infection, HA mediates binding with terminal α2,3- or α2,6 linked SA, which are connected to galactose residues of glycoproteins and glycolipids on the host cell surface. This interaction is followed by a fusion event of the viral membrane with the endosomal membrane. The human influenza virus primarily interacts with the α2,6 linked SA of the upper respiratory tract while the avian influenza virus uses α2,3 linked SA of the lower respiratory tract. Thus a host jump is possible for the avian influenza viruses through a deep inhalation where avian influenza may find its way to the lower respiratory tract of a human, subsequently interacting with an appropriate choice of SA for its invasion to the host cell. This kind of host jumps is also possible through reassortment of viral gene segments in the reservoir hosts (like swine, avian species like chickens or ducks) containing both α2,3- or α2,6 linked SA, leading to the evolution of the influenza virus by bringing changes in receptor interaction properties [45].

Moreover, studies suggest that for many kinds of viruses, binding with SAs is not sufficient for viral entry. For example, influenza-A virus entry also requires subsequent interactions with C-type lectin receptors (CLRs) like DC-SIGN(CD209) and L-SIGN(CD209L) for the virus entry [50].

Coronaviruses, on the other hand, mediates the virus-receptor interactions through extended Spike(S) proteins, a ~180-200kD, hollow, trimeric, type-1 transmembrane protein, that gives these viruses their names. The N terminal extracellular region of S protein is divided into two subunits, S1 (for receptor binding) and S2 (for membrane fusion) [51]. N-terminal domain (NTD) of the S1 subunit mediates the virus attachment to the sugar-based receptors, whereas the protein-based receptors interact through the C-terminal domain (CTD). However, this does not hold for all the members of the Coronaviridae family. MERS-CoV, as other beta-CoVs, mediates virus binding to the host cell through SAs, with an increased preference to α2,3 linked SAs. However, its CTD later interacts with dipeptidyl peptidase 4(DPP4) to complete the viral invasion to the host cell. CoVs like SARS-CoV and more recently SARS-CoV-2 directly mediate virus entry through directly interacting CTDs with the host cell angiotensin-converting enzyme 2 (ACE2), majorly detected on lungs as well as cells of the small intestine [45,51,52].

Thus, knowledge of the virus receptor interaction plays a critical role in developing novel antiviral strategies. We will be discussing some research works that have primarily aimed at preventing the virus from entering into the host cells through various nanotechnological approaches. These approaches have the potential or have already been used as a nanomaterial coating, that can be utilized to control transmission virus infection.

3. Nanomaterials as viral entry inhibitors

Functional carbon dots as antiviral agents: Several antiviral therapeutics are recently being developed around the World, based on carbon dots or carbon quantum dots, initially discovered for its colorful and bright fluorescence resembling the conventional semiconductor quantum dots [49,53–55].

In one such approach, curcumin derived cationic carbon dots (CCM-CDs) were used as multisite inhibitors for enteric coronaviruses [54]. CCM-CDs were hydrothermally synthesized using curcumin and citric acid as precursors, and its effect was investigated on viral entry. The study reported that CCM-CDs were inhibiting the entry of PEDV (Porcine Epidemic Diahorrea virus) into the Vero cells in a robust concentration-dependent manner when PEDVs were preincubated with CCM-CDs, i.e., before they are allowed to infect the cells. Subsequent studies have further implied that the positive charge of the CCM-CDs was resulting in aggregation of the virus, reducing the viral infectivity (inhibition efficiency over 50% at the concentration of 125μ g/mL). On the other hand, when different concentrations of CCM-CDs were injected after the PEDVs could attach to the cell surface, no effect of CCM-CDs was observed in viral penetration. With the help of Raman spectral analysis, the study also reported that the interaction with positively charged carbon dots could induce certain changes in viral protein structure, leading to the aggregation and reduced viral infectivity. These results further substantiated the role of CCM-CDs on viral entry and their effectiveness as a potential antiviral agent.

Benzoxazine monomer derived carbon dots (BZM-CDs) were also investigated for determining its effectiveness in reducing

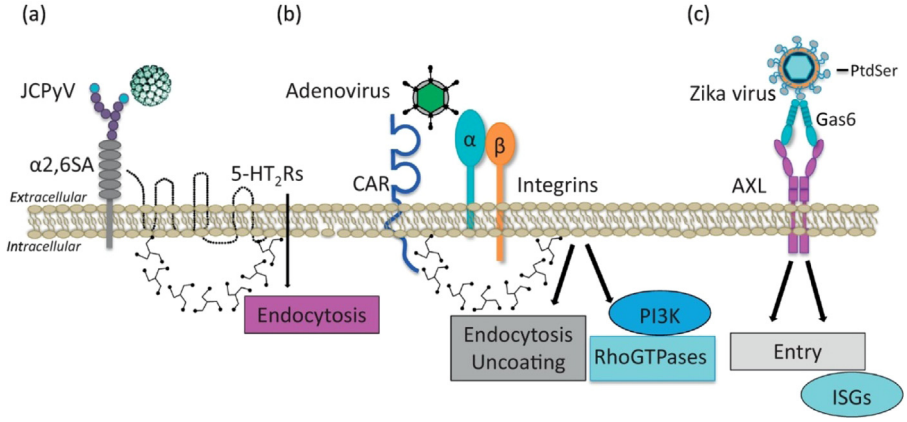

Fig. 1. Virus-Receptor interaction, a crucial step in the viral infection cycle. Three classes of receptors are shown: (a) Sialic acid, (b) Cell adhesion molecules, (c) PtdSer receptors, along with downstream signaling events associated with each class of receptors upon viral interaction. Copyright 2018, Elsevier Ltd.
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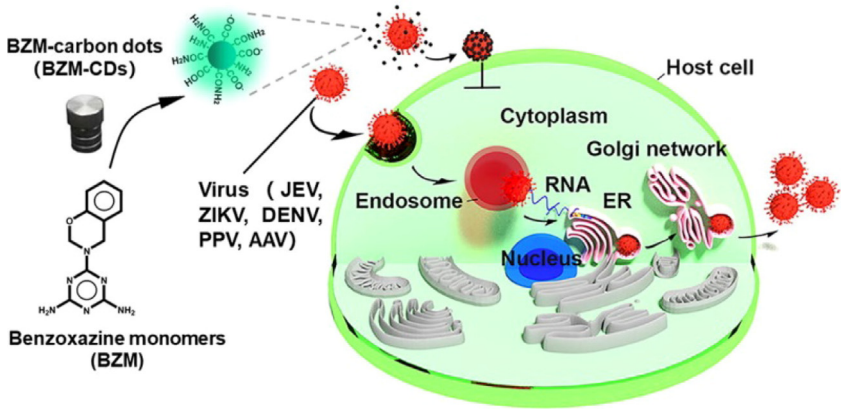

Fig. 2. Benzoxazine monomer derived carbon dots (BZM-CDs) and its mode of action in inhibiting viral entry inside cells. Copyright 2019, Elsevier Inc.
Source: Reproduced with permission [49].

viral infectivity, as shown in Fig. 2. [49]. It was reported that BZM-CDs were successful in inhibiting the entry of the flaviviruses (ZIKV, JEV, and DENV) and the nonenveloped viruses (PPV, AAV) by directly interacting with the virions. BZM-CDs of 4.4 ± 0.6 nm were hydrothermally synthesized using benzoxazine monomers in combination with NaOH. It was further investigated for its antiviral properties using BHK-21, Vero, HEK-293T as well as IBRS2 cells. It was observed that BZM-CDs inhibited viral entry in a concentration-dependent manner, which was confirmed by both plaque reduction assay and TEM. The heterocyclic compound, benzoxazine, helped BZM-CDs to directly interact with the virus leading to the disruption of the cellular receptor interaction [49]. Another aspect of the study was to investigate the broad-spectrum effect of BZM-CDs, thus ensuring whether it could be used as a suitable agent for antiviral therapeutics in a broad range of commercial as well as hospital settings.

In a more recent study, Tong et al. investigated the effect of Glycyrrhizic acid-based carbon dots(Gly-CDs) upon the invasion potential of PSRRV(Porcine reproductive and respiratory syndrome virus) using African green monkey kidney(Vero) and Porcine Kidney(PK-15) cells [55]. Through TEM, they concluded that Gly-CDs could directly interact with the PSRRVs, adding to the virus's reduced infectivity due to Gly-CD treatment. However, the exact mechanism through which glycyrrhizic acid imparted its antimicrobial activity remained unclear, several studies report the interaction of glycyrrhizic acid with the cellular membrane and its ability to alter the membrane properties which could lead to the antimicrobial phenomena [56].

3.1. Metal nanoparticle-based antiviral strategies

There are several reported ways by which metal nanoparticles can exert its antimicrobial activity, as being illustrated in Fig. 3a [57]. By reactive oxygen species (ROS) generation, by physical abrasion of the membrane incurred due to interaction with nanoparticles, by loss of membrane integrity due to nanoparticle binding, by the release of metal ions from the nanoparticles, etc. are to name a few [58].

However, silver nanoparticles (AgNPs) have attracted ever-increasing attention due to its multifaceted mode of antimicrobial activity leading to unprecedented biomedical significance [60]. Fig. 3b illustrates the diverse mechanism of antimicrobial action of silver nanoparticles along with other metal nanoparticles, including copper, cobalt, and zinc. Along with the ability to bind to the viral membrane and destabilize the membrane, silver nanoparticles exhibit their antiviral activity by interacting with the viral envelop protein like gp120 of the HIV-1 virus, thus inhibiting viral entry [61]. In an investigation on the antiviral activity of AgNPs against nonenveloped viruses like poliovirus, it was observed that silver nanoparticles inhibited the infection by directly interacting with it, thus not allowing the virus to bind with the receptor moiety on the cell surface [62]. The monovalent silver ions released from a nanoparticle that acts as a reservoir was also reported to be an active antimicrobial agent [63]. The released silver ions (Ag+) was found to have a very high affinity towards the thiol- or amine-bearing biomolecules, causing an irreversible aggregation. This phenomenon, also known as the

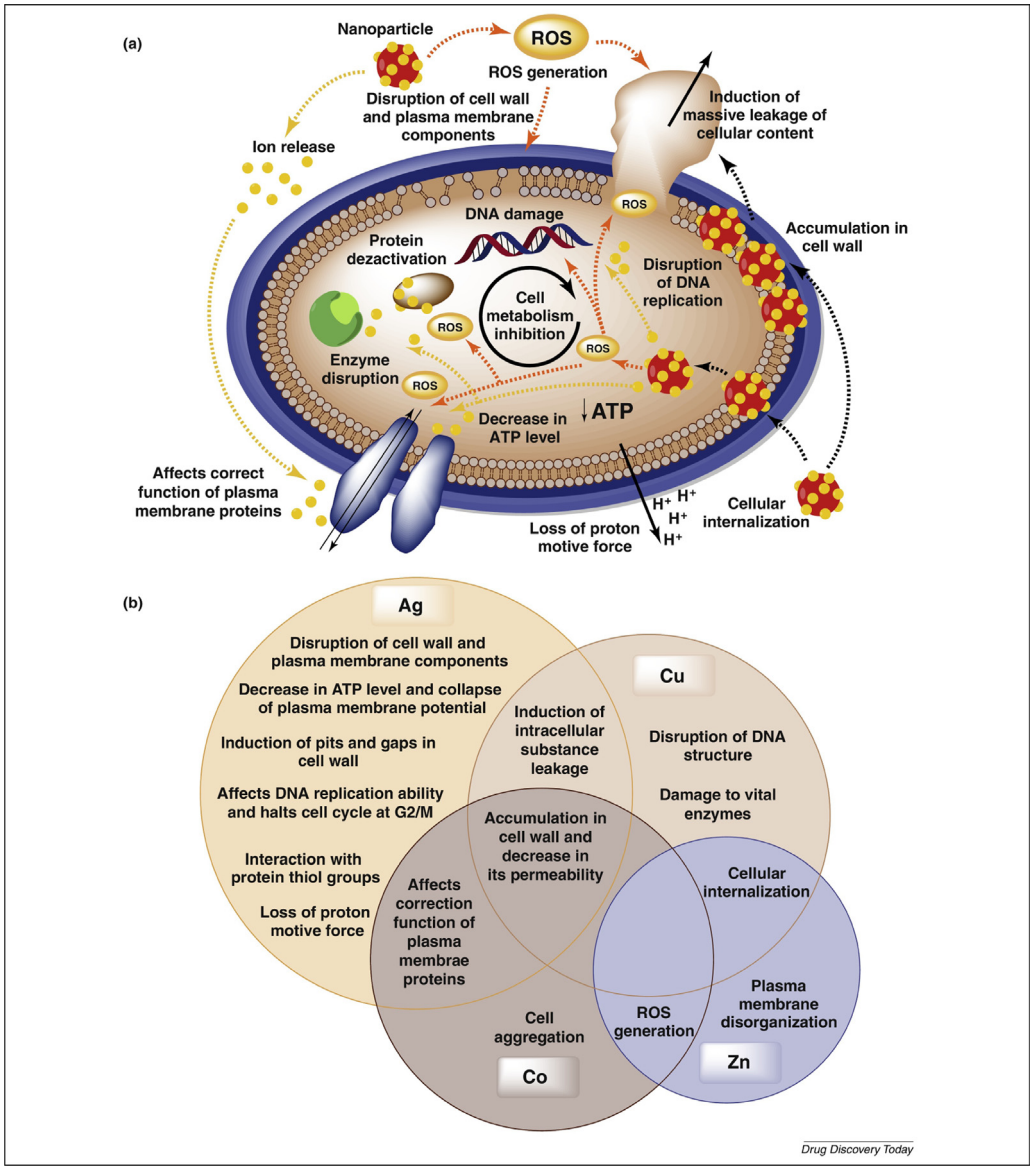

Fig. 3. (a) Different antimicrobial mechanisms of metal containing nanomaterials; (b) Detailed mechanism of antimicrobial activity of specific metal nanoparticles including silver (Ag), Copper (Cu), Cobalt (Co) and Zinc (Zn). Copyright 2016, Elsevier Ltd.
Source: Reproduced with permission [57].

oligodynamic effect, prevalently results from heavy metals, more prominently by silver [64]. Apart from ROS generation related to Ag+ ion release from AgNPs, there is ample evidence of Ag-NPs entering a cell and subsequently turning the DNA into a condensed form, thus halting its replication [57,65]. These multivalent antimicrobial strategies of silver nanoparticles make it an excellent choice for the fabrication of nanocoating for various biomedical applications. One such example is to coat cotton fabric with AgNPs to incorporate antimicrobial properties in the material [66]. Seino et al. reported another compelling study on the antibacterial activity of silver nanoparticles that were radiochemically synthesized onto textile fabrics. These AgNP-coated fabrics would thus be an excellent choice for use in hospital clothing fabrication [61,67]. Very recently, some convincing results from Guilherme et al. suggest the process of making AgNP functionalized polycotton fabric by the pad-dry-cure method. The composite has shown a substantial degree of inactivation of the SARS-CoV-2 virus as well, along with other pathogens, in a very short period [68].

With approval from the American Environmental Protection Agency (EPA) for being one of the "antimicrobial materials with public health benefits", copper has also been known to possess antimicrobial properties from times immemorial [69]. Copper (Cu) nanomaterials thus have been extensively tested and used for its antimicrobial properties. In an investigation by Fujimori et al. it was observed that monovalent copper ions (Cu^{+}) exerted antimicrobial activity based on the production of hydroxyl radical (·OH) or O^{2-} in aqueous media, resulting in degradation of protein [70]. Another mechanism reported by Rafi et al. was based on the opposite electronic charge of the Cu^{2+} ions and the microbial membrane, which led to altered enzyme functions or solidification of protein structures. These altered membrane functionalities subsequently resulted in the inactivation of microbes. The authors also suggested the production of hydrogen peroxide at the surface of microbes due to the recycling redox reaction between the leached out Cu^{2+} and Cu^{+} ions [71]. Several reports also suggested that Human Coronavirus 229E (HuCoV-229E) and alike retroviruses were also inactivated due to the action of copper ion release and ROS generation [69].

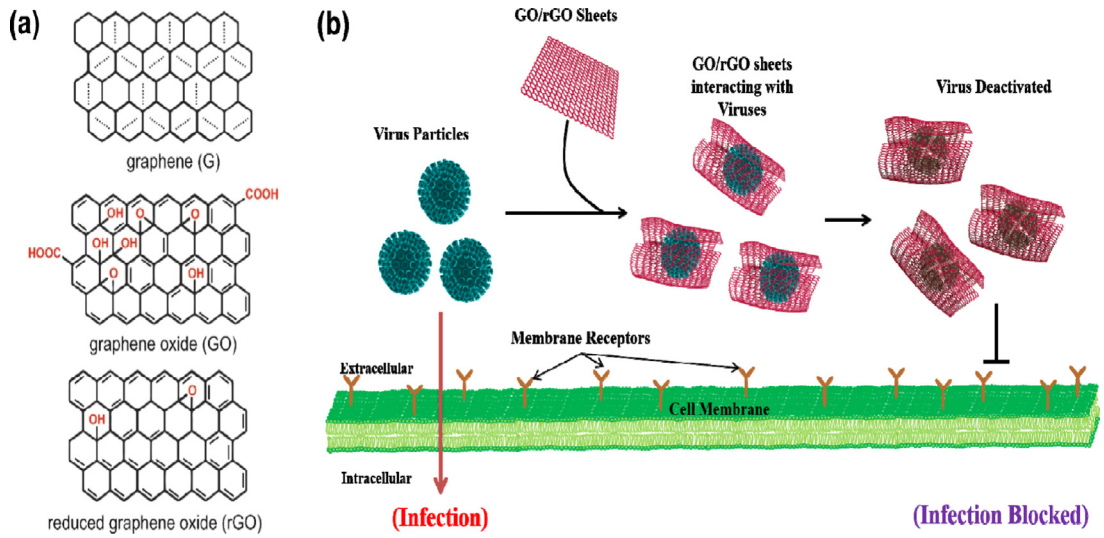

Fig. 4. (a) Structure of graphene (G), graphene oxide (GO) and reduce graphene oxide (rGO) [59]. (b) Antiviral activity of Graphene oxide and reduced graphene oxide nanosheets by interacting with the virus particles in a charge-based mechanism and subsequently inactivating the viruses through PDI.

Other metal nanoparticles like Zinc oxide (ZnO), cobalt, gold nanoparticles, were also reported to exert antimicrobial properties. A recent compelling study by Ghaffari et al. reported the inactivation of the H1N1 influenza virus by ZnO nanoparticles [72]. Gold(Au)/Copper Sulfate(CuS) core–shell nanoparticles were also reported to be an active antimicrobial material by Broglie et al. [73]. Another study by Gianvincenzo et al. demonstrated the antimicrobial properties of amphiphilic sulfate ended ligands coated gold nanoparticles [74]. Moreover, gold nanoparticles bound on the surface of mesoporous silica nanoparticles were reported to be showing peroxidase or oxidase-like activities, thus producing ROS to inactivate microbes through oxidative stress [75].

These and several other investigations substantiate the fact that these antimicrobial nanomaterials are potent agents that can be effectively used for reducing the risk of spread of microbial infection by inactivating the microbial load. Thus, the use of such functional nanomaterials as a coating at public places, offices, touch surfaces, doorknobs, mattresses, hospital equipment could result in a substantial decrease in the spread of the microbe. Delgado et al. reported the development of a plastic material with antimicrobial properties. The researchers embedded copper oxide nanoparticles in polypropylene in order to develop antimicrobial polypropylene(PP)/Copper oxide nanoparticles(CuOPs) composite [76]. Suggestions have also been made for the use of copper-based nanocoating in dentistry and other medical practices (for coating the surfaces of clinics, coating of the rotary equipment used in dentistry, handpieces, air–water syringes, and other medical equipment) to check the spread of the SARS-CoV-2 virus [69].

3.2. Graphene oxide-a multi-mechanism antiviral nanomaterial

Graphene(G), which was first procured from mechanical exfoliation of graphite, is a single atomic plane of graphite (Gt) and is constituted of sp^2 bonded carbon atoms [77]. Graphene oxide (GO), which can be chemically exfoliated from graphite oxide (GtO), is a graphene sheet with additional functional groups associated with its structure, like phenol hydroxyl groups, epoxide groups on its basal plane and carboxyl groups at its edges. On the other hand, elimination of the functional groups on the GO, by thermal annealing or chemical treatment results in the formation of reduced graphene oxide (rGO) [78]. Structurally, GO possesses carbon atoms in a hexagonal lattice arrangement to form a 2D, single atomic thick structure with multiple oxygen-carrying functional groups, as being represented in Fig. 4a. In addition to its vast tenability, resulting from the presence of various functional groups on its surface, graphene oxide is also reported to be less cytotoxic, making it a promising antimicrobial agent [79–81]. Furthermore, the unique physical and chemical properties have made graphene and its derivatives (graphene oxide (GO) and reduced graphene oxide (rGO)) excellent nanomaterials for multidimensional applications.

Antiviral activity of graphene oxide nanosheet structure was investigated by Ye et al. using porcine epidemic diarrhea virus (PEDV, a DNA virus) and pseudorabies virus (RNA virus). They reported the broad-spectrum antiviral activity of GO was due to its negative charge and layer structure. In order to determine that the charge present on the go nanosheets was contributing to its antiviral activity, they wrapped the graphene oxide sheets with PDDA (cationic polymer) and PVP (neutral polymer). They observed that GO-PDDA composite completely lost its antiviral activity while GO-PVP retained it. Thus the negative charge on GO made the interaction with the viruses more probable before viral entry inside cells, leading to viral inactivation [79,80]. Comparable antiviral activity of GO and (rGO) nanosheets further substantiated the fact that the functional groups upon GO are not essential for its operation. In another experiment, when they used graphite(Gt) and graphite oxide(GtO) flakes along with graphene oxide sheets, they found that the antiviral activity was very less for the Gt/GtO flakes as compared to GO/rGO sheets. From AFM studies, it was observed that GtO had much larger height as compared to GO/rGO, which may have resulted in the reduced activity of the flakes. They also found that the GO/rGO nanosheets tend to wrap around the virus particles because of the opposite charges, and the sharp edges on the nanosheets rupture the viral envelope as well as the capsid protein layer leading to its inactivation as illustrated in Fig. 4b [79].

In another study by Du et al. graphene oxide–silver nanoparticle composites were investigated for its antiviral activity by inhibiting viral invasion, using PRRSV and PEDV. They observed a better antiviral effect upon AgNP incorporation with GO sheets. AgNP-GO composite was reported to be inhibiting AgNP agglomeration, thus increasing its bioavailability for better antiviral effect, adding to the sharp edge induced antiviral activity of GO [81].

Graphene oxide is reported to be exhibiting another mechanism for its antiviral activity. Owing to its superior thermal conductivity, GO nanosheets were investigated under near-infrared radiation (NIR of 808 nm) for its high light to heat conversion potential [82]. Sulphonated magnetic nanoparticles functionalized with reduced graphene oxide(SMRGO) were synthesized and preincubated only for 10 mins with herpes simplex virus type-1 (HSV-1) particles. The results showed that electrostatic interactions with the sulfonated magnetic nanoparticles led to viral capture with subsequent photothermal inactivation by GO sheets [82].

Thus, graphene oxide-based antiviral strategies can be an excellent choice for viral inactivation in different scenarios.

3.3. Photodynamic inactivation of microbes and recent advancements

Reported almost 130 years back by Niels Ryberg Finsen through his landmark treatment of *Lupus vulgaris* [42], the mechanism of using light to generate reactive oxygen species (ROS) has been reported to treat cancer (Photodynamic therapy, PDT) or to kill microbes (Photodynamic Inactivation, PDI). Though most of the applications of PDI as an antimicrobial therapy remained a controversy ever since its creation, the flare of research around the World from the last decade has substantiated the enormous potential this technology holds.

There are three pillars of the PDI mechanism: a photosensitizer (PS), light, and oxygen. One major characteristic that needs to be taken into account is that the effect of PDI is local, meaning that all three components need to be present at the same place. Fig. 5a illustrates that when the light of appropriate wavelength strikes a PS, it forms an excited singlet state or S1 state. From this S1 state, the molecule can return to the ground state in two ways: fluorescent emission of radiation or relaxation via internal conversion (radiationless); or it might form an excited triplet state(T1) by spin inversion (Intersystem crossing, ISC). As opposed to the other route to the ground state, which is almost instantaneous, the T1 state gives ample time to react with the neighboring molecules to form the entities required for PDI. Two types of events (photoreaction) might occur after attaining T1 state: type I and type II. In Type I photoreaction, the PS transfers an electron to other substrates (biomolecules) generating radicals, most prominent of which is superoxide anion radicals. These radicals then create hydroxyl radicals in a multistep process called Haber-Weiss/Fenton reaction. In type II photoreaction, singlet oxygen ($^1O_2$) is produced while the PS molecule returns to S1. Both of these photoreactions generate reactive oxygen species(ROS) and are involved in viral PDI [42,83].

There are three targets in the viral PDI mechanism: virus protein, nucleic acids (DNA/RNA), and viral lipids, as being represented in Fig. 5b. Viral lipids create an additional target moiety for enveloped viruses making it more vulnerable to PDI mode of viral inactivation. Some PS might get intercalated to the viral DNA/RNA (though not necessarily always) and cause oxidative transformations, which ultimately lead to DNA fragmentation, SSB (single-stranded breaks), or cross-linking with proteins. Oxidative damage to oxidation sensitive amino acids in the protein backbone, such as methionine, tryptophan, histidine, etc. might lead to structural modifications to the viral envelope proteins. Moreover, direct interactions between viral protein and PS may lead to protein misfolding, subsequently affecting viral function. It should also be noted that oxygen is not always mandatory for generating $^1O_2$. Some PSs like Psoralens can make $^1O_2$ in an oxygen-independent phototoxic mechanism [42,83].

Curcumin [84], polyglycerol functionalized graphene sheets [85], C_{70} and silver nanoparticle loaded block copolymer (BCP) thin films [86], etc. are few currently developed PDI technologies. A detailed review of the photodynamic inactivation strategies has been extensively discussed by Wiehe et al. [42]. Most recently, the viral capture and inactivation mechanism was beautifully combined in a study by conjugating both virus attachment and ROS generating PS modules in a single platform [87]. Jeong et al. synthesized 6'-Sialyllactose-Chitosan-Chlorin e6 (SCC) multivalent structure by linking chitosan to the reducing terminal of sialoyllactose, followed by conjugating it to Ce6 (Chlorin-e6). They targeted it against the influenza A (H1N1) virus. As previous knowledge entails that viral HA binds sialoyllactose glycoproteins in the host cell membrane in a multivalent fashion, SL moiety in SCC helped in multivalent viral recognition with the help of chitosan. Chitosan provided the backbone to SCC by allowing multiple SL to get conjugated in a single platform, creating a multivalent viral recognition system. In addition to that, Ce6 generated ROS to inactivate viral infectivity by destroying the viral architecture [87]. Moreover, to cater to the tailored requirement of different photosensitizer molecules for the different wavelengths of light, Lim et al. used near-infrared (NIR)-to-visible upconversion nanoparticles (UCNs). These UCNs not only acted as a carrier for the attached photosensitizers but also participated in PDT by transducing the NIR to visible light, appropriate to photoactivate the attached PS molecule [88]. Another recent investigation by Kim et al. reported the use of C_{60} fullerenes coated aminated SiO_2/stainless steel mesh for remote bacterial and viral inactivation based on the PDI effect of fullerenes. Along with many technical advantages associated with the environmental application of fullerenes such as mechanical as well as thermal robustness and high quantum yield for $^1O_2$ generation, surface modification with amine-terminated groups enable C_{60} Fullerenes to attain visible light activity [89]. Immobilized C_{60} fullerene functionalized aminated silica-coated stainless-steel mesh (C_{60}-SiO_2-NH_2 SS) thus enabled the researches to deactivate MS_2 bacteriophages remotely, through $^1O_2$ generated by the fullerene moieties, making it a promising strategy to be used as an antimicrobial surface coating.

Another mechanism by which antimicrobial reactive oxygen species (ROS) generation takes place is through photocatalysis. When the light of the appropriate wavelength bombards the photocatalyst molecules, a valance band electron of the molecule gets excited to the conduction band (e^-_{CB}) by creating a positive hole in the valance band (h^+_{VB}).This occurs if the absorbed photon energy is greater than the bandgap of the molecule. The excited conduction band e^-_{CB} reacts with molecular oxygen to generate superoxide radical ($\cdot O_2^-$), or hydroperoxide radicals ($\cdot HO_2$). Simultaneous oxidation of water (H_2O) takes place at the valance band positive hole (h^+_{VB}) to generate hydroxyl radicals and hydrogen ion (H^+) [90]. The ROS molecules generated through the photocatalytic mechanism has already been reported to be an efficient antimicrobial agent. Several investigations have reported the photocatalytic antimicrobial activity of Titanium dioxide (TiO_2) nanorods/nanoparticles and Zinc oxide (ZnO) nanorods/nanoparticles [90,91]. However, these TiO_2 or ZnO nanorods/nanoparticles were reported to be UV driven photocatalysts. Visible light driven (VLD) photocatalysts are one of the most promising nanomaterials for its ease of use. For example, α-Fe_2O_3 (iron oxide) thin film and nanorods/nanocolumn array is one such VLD photocatalyst, reported to have antimicrobial characteristics. Sharma et al. also reported cadmium sulfate (CdS) nanomaterials as an effective antimicrobial VLD photocatalyst [92]. Recently, tungsten trioxide based photocatalysis was integrated with Copper nanocluster treated fabric by Ghezzi et al. to make a hybrid filter to inactive SARS-CoV-2 virus in indoor environments [93]. Thus visible-light-driven (VLD) photocatalysts make an excellent nanomaterial to be used as an antimicrobial coating to surfaces in the outdoor as well as indoor conditions.

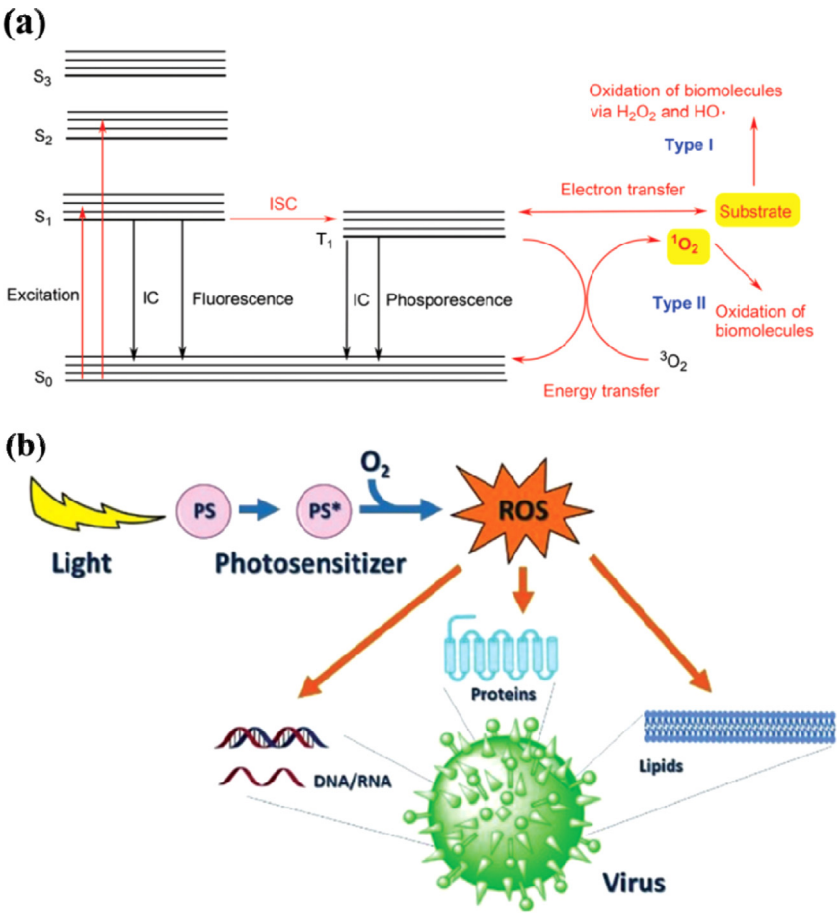

Fig. 5. (a) Modified Jablonski diagram representing ROS formation mechanism upon photoexcitation. (b) Three viral targets (DNA/RNA, Protein, and Lipids) of ROS generated by photosensitized molecules upon illumination to deactivate viruses.
Source: Reproduced with permission [42].

4. Antimicrobial nanomaterial coating strategies

Antimicrobial nanomaterials can be used as a coating through various approaches. In an investigation by Foster et al. chemical vapor deposition (CVD) was used to put CuO/TiO_2 dual-layer nanocoating on a glass surface to check the antimicrobial activity of the coated surface [94]. Several nanomaterial coating strategies have been developed so as to incorporate antimicrobial properties into stainless steel since it is frequently used in making medical tools, hospital surfaces, household as well as commercial establishments [95–97]. One such approach was the electrophoretic deposition of Lysozyme–Silver nanoparticles upon medical instruments by Eby et al. as being shown in Fig. 6a. The study reported the use of antimicrobial properties of both lysozyme and silver nanoparticles by synthesizing lysozyme-AgNP colloidal solutions and electrophoretically depositing it upon scalpel blades and needles. Lysozyme worked as a reductant and colloidal stabilizing agent in AgNP synthesis. Using ATR FT-IR, the authors confirmed that there was no change in the lysozyme protein backbone due to electrodeposition. [95]. In another study, stainless steel was covalently modified with antibacterial peptides (Nisin, Trp11, and 4K-C16) onto plasma polymerized surface in a two-step process, as was reported by Vreuls et al. Continuous plasma polymerization of stainless steel was performed with allyl glycidyl ether monomer and antimicrobial peptides were covalently attached by the facile reaction between the amine groups of plasma polymerized steel surface and the peptide amine groups. Because of the covalent attachment of the antimicrobial peptides, the surface functionality was long-lasting and was unaffected by cleaning treatments using non-ionic detergents. Moreover, it was also confirmed that the antimicrobial properties of the antimicrobial peptides remained unaffected upon covalent attachment. [96]. Antimicrobial peptide functionalization was also performed in a multistep process using chitosan, terephthaldehyde, and nisin/magainin on AISI 316 L stainless steel surface. Sulphochromic acid-treated stainless steel (SS-SC) surface was consecutively drop deposited by 0.05% chitosan, terephthaldehyde, and antimicrobial peptide solution (0.01 mg/mL) respectively resulting in SS-SC-Chi-Tere-Nis/Mag surfaces which is illustrated in Fig. 6b. Chitosan, being a rich source of hydroxyl and amine groups, made the grafting of the bioactive compounds possible. These functionalized surfaces showed remarkable antimicrobial activities in addition to being environmentally friendly and safe for human health [97].

Another approach by Castro-Myoraga et al. reported the use of electrospun poly (3-hydroxybutyrate-co-18 mol%-3-hydroxy valerate) (PHBV18)/AgNP nanofiber mats for coating PHVB3 films. After fabricating PHVB3 films by compression molding using hot plates hydraulic press (180 °C, 1.8 Mpa for 5 mins), AgNP incorporated PHBV18 nanofibers were thermally treated using a hot press(150 °C for 2mins) that resulted in a uniform and smooth film due to fiber coalescence. After treatment of the PHBV3/PHBV18/AGNP films at 100% RH and 37 °C for 24 h, no active FCV (feline calicivirus) was observed along with 0.86 log reduction in MNV (murine norovirus) infectivity. Entrapping AgNPs in PHBV18 also stabilized the AgNPs into its polymer matrix and significantly reduced the aggregation of AgNPs [98].

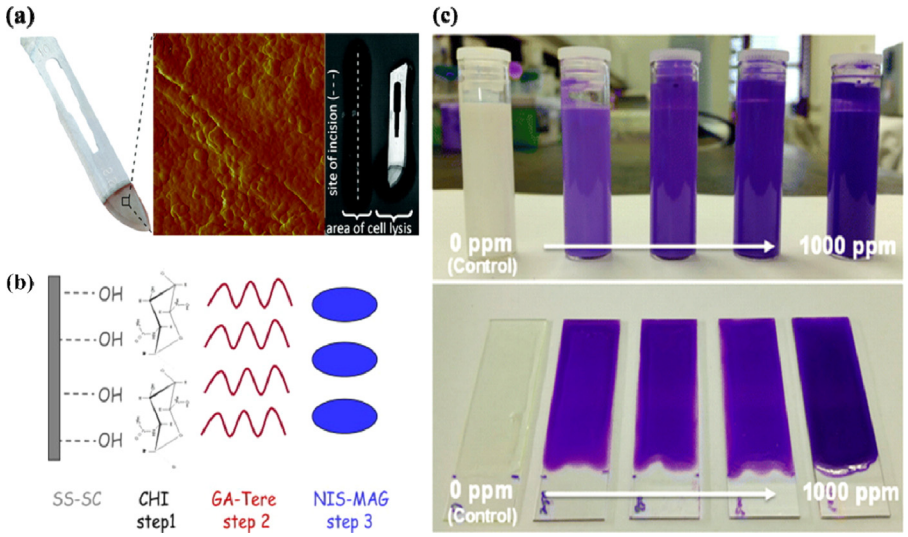

Fig. 6. Antimicrobial Coating strategies, (a) electrodeposition of lysozyme–silver nanoparticles upon surgical blades. Reproduced with permission [95], Copyright 2009, American Chemical Society; (b) Three-step adsorption procedure of antimicrobial peptides upon sulfochromic acid-treated stainless steel surface, covalently conjugated with glutaraldehyde/terephthaldehyde treated chitin. Reproduced with permission [97], Copyright 2011, Elsevier B. V.; (c) Antimicrobial paint prepared using crystal violet in commercial acrylic latex, Reproduced with permission [99], Copyright 2015, American Chemical Society.

In one recent report by Hwang et al. white light-activated antimicrobial paint (WAALP) was developed using crystal violet (CV) as a PDI based antimicrobial agent, shown here in Fig. 6c. CV, generating $^1O_2$ and ROS upon light exposure, was incorporated into commercial acrylic latex and applied upon polyether-based thermoplastic polyurethane (PTPU) to test its antimicrobial activity. There was little leaching of CV molecules up to 100 h when CV-paint coated samples were tested in PBS immersed state, while after 100h, no additional leaching was detected. Moreover, it was also reported that the PDI activity of CV was well retained even after it was mixed with acrylic latex [99]. Another recent invention by Watanabe et al. reports the development of an antibacterial, antiviral coating film using acrylic melamine paint, polyvalent carboxylic acid, phosphoric acid, and a quaternary ammonium salt. Carboxylic groups of polyvalent carboxylic acid interact with the quaternary ammonium group of QA salts, thereby linking two or more QA salt molecules to a single polyvalent carboxylic acid molecule. The large molecular weight of the ionic-bonded multiple QA salt molecules does not allow the leaching of QA salts from the coating and prevents the deterioration of antimicrobial properties of the film in water. Phosphoric acid was used as a curing accelerator because curing of acrylic melamine films are inhibited by the presence of QA salts in it and results in decreased solvent resistance and surface hardness. The resulting antimicrobial coating demonstrated excellent antimicrobial properties along with remarkable water resistance [100].

However, investigations on nanomaterial coatings need to consider the fact that the coated surfaces like doorknobs, buttons, medical equipment, etc. would be in direct contact with human skin. Thus, the toxicological effect of the nanomaterials being studied needs to be taken into account. Several nanomaterials like CuO nanoparticles are considered safe because of their very low cytotoxicity [69,101]. Another factor that contributes to the nanomaterial toxicity evaluation is the lethal dose (LD_{50}) value. For silver nanoparticles, the LD50 value can be as high as 1600 mg kg^{-1} for rats (oral dose) [102]. Therefore, thorough investigations on toxicological effects need to be performed while developing the antimicrobial nanomaterial-based coatings. However, quantitative evaluation of TiO_2 and ZnO nanomaterial penetration/permeation by Kimura et al. reported that there was no observable permeation of the nanomaterials for intact skin. Very little or no permeation was reported through the stratum corneum [103]. Thus, nanomaterial-based broad-spectrum antimicrobial coatings can be a potential and safe alternative to combat the viral or microbial transmission.

5. Conclusion and future perspective

With the spread of viral diseases gaining significance worldwide, nanotechnology has been of immense help in developing many broad-spectrum antiviral therapeutics. Combinatorial approaches in devising antimicrobial solutions are recently gaining popularity. We have observed advancements in the in-silico experimentations, like molecular docking, providing useful insights and directions towards the development of newer and efficient antimicrobial materials. For example, Abo-zeid et al. performed molecular docking model studies to track and understand the interactions between IONPs (iron oxide nanoparticles: Fe_2O_3 and Fe_3O_4) and spike protein of SARS-CoV2 [104]. Recent investigations using a genius amalgamation of strategies targeting various aspects of viral biology, as well as their mode of action has resulted in the development of novel therapeutic approaches that have helped in better management of viral infections [55,81,82,87,89]. As mentioned earlier, technologies like photodynamic inhibition, although were known to humankind since the last century, have only been recently used in developing PDI based antiviral technologies that have proved to be highly effective [42]. Numerous suggestions and investigations are going on throughout the World for the use of nanomaterials as a coating of masks, fabrics, doorknobs, medical instruments, etc. For example, we have also noticed a surge of investigations on the development of enhanced textile composite materials by incorporating nanomaterials (like graphene and its derivatives) in traditional textile manufacturing. These improved materials would provide a multifunctional alternative to conventional textile fabrics by combining properties like antimicrobial properties, fire retardant properties, or electricity resistivity [77]. Moreover, not only just the chemical properties of the nanomaterials are being exploited for the development of antimicrobial materials. Nanostructured surfaces having nanopillar like architecture, commonly observed upon dragonfly (cicada) wings [105] or gecko skins [106], are being investigated extensively to develop biomimetic nanostructured antimicrobial surfaces [107]. These nano-morphologies can

provide a drug or chemical-free alternative to traditional antimicrobial strategies. As being reported by Ishak et al. these nanostructured surfaces are already being utilized in the development of titanium-based medical implants [108]. Many more such broad-spectrum antimicrobial technologies will be investigated further in the near future. As we gain more insights into the field of microbial biology, many new innovative therapeutic strategies will be developed.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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