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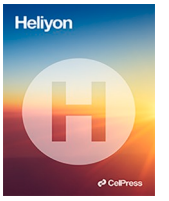

Review article

Recent development in antiviral surfaces: Impact of topography and environmental conditions

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ABSTRACT

The transmission of viruses is largely dependent on contact with contaminated virus-laden communal surfaces. While frequent surface disinfection and antiviral coating techniques are put forth by researchers as a plan of action to tackle transmission in dire situations like the Covid-19 pandemic caused by SARS-CoV-2 virus, these procedures are often laborious, time-consuming, cost-intensive, and toxic. Hence, surface topography-mediated antiviral surfaces have been gaining more attention in recent times. Although bioinspired hydrophobic antibacterial nano-patterned surfaces mimicking the natural sources is a very prevalent and successful strategy, the antiviral prospect of these surfaces is yet to be explored. Few recent studies have explored the potential of nanopatterned antiviral surfaces. In this review, we highlighted surface properties that have an impact on virus attachment and persistence, particularly focusing and emphasizing on the prospect of the nanotextured surface with enhanced properties to be used as antiviral surface. In addition, recent developments in surface nanopatterning techniques depending on the nano-scaled dimensions have been discussed. The impacts of environments and surface topology on virus inactivation have also been reviewed.

1. Introduction

Human civilization has been susceptible to respiratory viruses for ages, and factors like globalization and intensive urbanization have fostered viral transmission leading to catastrophes like the Covid-19 pandemic. Respiratory viruses primarily transmit via three main routes. Virus-laden droplets or aerosols formed in the respiratory tract of a diseased person can eject via talking, sneezing, breathing, or coughing to directly enter the recipient [1–3]. Physical contact with the surface with a previously deposited droplet or fomite can serve as a source of infection spreading. The third route of transmission is particularly the airborne transmission. These three routes in general, are referred to as the 'droplet', 'contact', and 'airborne' transmission routes [1,4]. However, it is not always very straightforward to control this transmission. Virus structure and virus inactivation mechanisms are very crucial in developing the strategy to constrain viral transmission.

Viruses cannot survive without a host, and after a certain time, they will undergo degradation, failing to access a host. However, when they have access, they undergo the replication process starting with absorption or attachment to the host cell followed by penetration. Inside the host, virus uncoating, replication, and assembly will take place with the cycle ending with the release of virions [5]. It is crucial to understand the physical structure of a virus because virus-specific morphology has a role to play in the transmission and survival of viruses in environmental conditions. Virus can be categorized as enveloped and nonenveloped depending on whether it

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has the outermost lipid bilayer. In many viruses, there is an additional inner coating termed 'matrix protein' right next to the inner side of the lipid bilayer. There is a protective protein shell, capsid, inside the envelope that contains the genome of the virus which can either be DNA or RNA. Three different types of capsid shapes can be found in general; spherical, filamentous, and complex [6]. Capsid shape helps virus identification and additional information on virus-specific life cycles [7]. Enveloped viruses are equipped with outer glycoprotein spikes that can facilitate virus entry into the host cell [8]. Lipid bilayer in enveloped viruses can act as an additional protective layer, but outside the host cell it is susceptible to harsh chemical and environmental conditions leading to degradation and viral inactivation [9]. Envelope offers protection during virus replication phase, protects capsid spike antigens from host's antibodies, and imparts structural flexibility to the virus [10]. Since the envelope is the weakest part of the virus, nonenveloped viruses can survive under extreme conditions either due to lack of lipid bilayer or due to evolving a stronger capsid layer. These are more resistant to heat, detergent, and harsh environment that nonenveloped viruses [11]. Researchers reported that surface protein adsorption of the virus on surfaces is entropy driven implying that protein can undergo a conformational change in response to surface properties alteration leading to irreversible damage and inactivation of the virus [12,13]. Therefore, surface modification is expected to be an effective strategy to curb the spread of contagious viral diseases like SARS-CoV-2 [13]. In an extreme environment, the presence of non-enveloped viruses is quite common as they can adapt to harsh conditions. Temperature has a negative impact on SARS-CoV-2 since the increase in temperature causes a reduction in the virus's lifetime [14]. Hence, higher surface temperature can effectively improve antiviral properties. However, the relationship between inactivation and humidity is not just as simple [15]. Even though enveloped viruses are more susceptible to environmental and chemical-induced stress, over the past few decades, enveloped viruses have been responsible for causing viral epidemics such as Ebola, Measles, Zika, Avian influenzas, severe acute respiratory syndrome (SARS), Middle-East respiratory syndrome (MERS), and the most recent, Covid-19 [23,24]. The plausible explanation would be that the primary transmission route of the majority of respiratory viruses is through direct inhalation; not surfaces or environmental transmission. Even so, surface transmission control limits the extent of transmission to some degree. Further, other than developing vaccines and drugs to control viral epidemics, controlling surface transmission is the only way for science to actively make its contribution.

Antiviral mechanism circles around two main inhibition methods: inhibition before virus entry and inhibition after entering other steps of the replication cycle. Previous studies extensively reviewed metals (Cu, Ag, Zn), inorganic nanomaterials (silver, copper, gold, MgO, CuO, ZnO, TiO_2 based nanoparticles) and nanocomposites [16–18], organic nanomaterials (lipid-based nanoparticles, polymer nanoparticles, dendrimers, micelles) and polymer-based surfaces (polymer-antimicrobial agent composite, cationic polymers, polyzewitterions [16, 19–21], as antiviral functional materials, and studies on the development of such materials as an antiviral coating to surfaces has been an active research area over the recent years. For surface coatings with these antiviral agents, methods like drop casting, dip coatings, cast-coating, doctor-blading, spraying method and spin coating can be used. Erkoc and Ulucan-Karnak explored these coating strategies for the fabrication of antiviral surfaces [20]. Despite the number of investigations on antiviral coating and materials, many challenges lie ahead in the path of commercializing, *i.e.*, efficacy level optimization, toxicity, virus specificity, and cost of such surfaces. The lack of data on toxicity and environmental impact associated with the leaching of coating materials from the substrate limits their widespread adaptation in healthcare setting. Hence, researchers are putting effort into improving the adhesion of antiviral agents to the substrates. In a recent study, it has been demonstrated that laser texturing coupled with coating technology can enhance the adhesion of coating material to the surface [22]. In contrast, tailoring surface physical properties and surface topography suited for antiviral surface application can avoid the problem of leaching of antiviral coating materials altogether. This review casts new light on the development of topography-mediated antiviral surfaces as well as suitable surface properties for virus inactivation. The persistence and behavior of viruses in aerosol and surfaces under different environmental conditions may introduce some additional variables to consider while designing antiviral surfaces. To facilitate the better understanding regarding the persistence of viruses, this review highlights the potential influence of some environmental conditions on the survival of viruses on surfaces and aerosol.

2. Surface properties influencing antiviral activity

2.1. Sorption

Droplet dynamics play a vital role in the propagation of viruses. Enveloped virus transmission and propagation are mainly ascribed to the aqueous phase of a respiratory droplet. Therefore, evaporation in particular is related to the virus on a surface [4,25,26]. Chatterjee et al. demonstrated that coronavirus survives longer on impermeable surfaces than on porous surfaces. After initial bulk droplet evaporation from both surfaces, residual thin liquid film persists which evaporates faster on a porous surface than a nonporous one. Faster evaporation of residual film is attributed to capillary imbibition and adjusted wetted area of porous structure [27,28]. Absorption mechanism through porous surface may enhance virucidal efficiency of the antiviral surface. Lai et al. studied that cotton gown loses virus drop infectivity after 1 h compared to 24 h inactivation time required for an impermeable disposable gown. This study trailed off with the suggestion of tailored fluid-repelling disposable garments coupled with an outer fluid-absorbing surface [29]. A recent study tested the viability of SARS-CoV-2 and SARS-CoV-1 on five different surfaces - aerosol, copper, plastic, stainless steel, and cardboard and found that both viruses had the longest viability on plastic and stainless steel; persistence on copper and cardboard was less. Both viruses remained in the aerosol throughout the entire runtime of the experiment [30]. Although copper is nonporous, copper both in metal and nanomaterial form, has good antiviral properties that contributed to the lower viral persistence on this surface [31]. The synergistic effect of absorption and porous surface structure on the viability of viruses was evident from the result. The outermost glycoprotein spike of SARS-CoV-2 is naturally hydrophobic; therefore, the polypropylene (hydrophobic) surface of the mask is electrostatically treated to impart hydrophilic property to impede surface adsorption of SARS-CoV-2. Protein can bind to the surface due to

both positive charge and hydrophobic interactions [13]. Protein adsorption to surfaces by hydrophilic-hydrophilic interaction is weaker than hydrophobic-hydrophobic binding [32]. Surface adsorption of protein might be reversible or irreversible depending on surface properties. Irreversible adsorption of protein typically occurs on hydrophobic surfaces and causes a conformational change of the protein, leading to viral surface protein damage and inactivation [33]. In contrast, a hydrophilic surface is required to capture and inactivate aerosols [13]. Therefore, a balance in surface wettability is desired to capture both aqueous and protein molecules.

Hosseini et al. introduced the concept of virus adsorption or trapping mechanism to reduce viral transmission. They divided viral infectivity into two stages – virus recovery from the surface and the effect of the recovered virus on cells. If no virus is recovered from the surface due to adsorption inside cavities or inactivation in the surface, it can postpone infectivity in the first stage before it can infect individuals. They further emphasized that, unlike bacteria, viruses cannot reproduce without host cells; therefore, will naturally undergo decay when trapped inside the surface [34]. However, the studies related to virus inactivation mechanism via absorption still lack evidence with actual viruses.

2.2. Porosity

A porous surface or film provides greater surface area and a smaller diffusion path beneficial for contact inactivation. It also aids faster drying of droplets and reduces the required pulling time of the suspended virus into close contact with the active surface. Drying enhancement provides a significant advantage by reducing viral transmission to users of communal objects. In addition, porous film coupled with active pore structure is protected against mechanical abrasion due to such morphology [35]. Hosseini et al. indicated that an active antiviral agent on the porous structure might not be even necessary as long as the pores trap the virus and dry under environmental conditions with subsequent inactivation in time. According to this mechanism, virus propagation through communal objects becomes unlikely [34].

A study demonstrated that influenza A and influenza B viruses can survive for a prolonged period on non-porous surfaces like stainless steel and plastic than porous surfaces of paper facial tissue, handkerchiefs, pajamas, and magazines. Shorter survival was attributed to faster drying in porous surfaces. The study also found that influenza A can survive longer [36]. In a study concerning the stability of SARS-CoV-2 on surfaces, no virus was detected from porous surfaces (printed and tissue paper) after 3 h of incubation, from glass and banknote on day 4, and from stainless steel and plastic on day 7[30]. Another study reported the survival of two respiratory viruses, avian metapneumovirus, and avian influenza virus, on twelve varieties of porous and non-porous surfaces which revealed longer persistence (up to 6 days) on non-porous surfaces [37]. In contrast poliovirus and adenovirus demonstrated higher persistence on paper and cotton cloth compared to non-porous aluminum, China glazed tile, latex, and polystyrene [38]. This contrast might have to do less with surface porosity but more with the virucidal property of surface material. In porosity assisted inactivation mechanism, the pore diameter is also an important factor that cannot be overlooked. While porous materials can inactivate or trap viruses to some extent, antiviral protection targeting only porosity as the surface property may not suffice. Moreover, this proposed mechanism is still relatively new, and still lacks empirical evidence through experimentation with actual viruses.

2.3. Hydrophobicity and wetting behavior

Liquids in contact with the surface can wet (Wenzel state) or sit atop air pockets on the cavities thus not wetting the surface (Cassie–Baxter state) [39,40] or form a metastable state as depicted in Fig. 1. Hydrophilicity or wettability is characterized by the contact angle of water droplets to the surface. Contact angle (CA) ranges between 0° to 90° when the droplet wets the surface, often referred to as hydrophilic surface whereas CA ranges between 90° to 180° for hydrophobic surface. Super hydrophobic surface has CA$>150^{\circ}$ [41]. Hydrophobicity of a surface is critical to tackling virus transmission. Siddiquie et al. recommended the use of micro-/nano-textured hydrophobic surfaces to minimize virus and surface contact area by entrapping air in the nanocavities [42]. Hydrophobicity can affect viral persistence on any surface [43]. Hydrophobic viruses with lipid outer layer (envelope), are conveniently adsorbed by hydrophobic surfaces while hydrophilic virus (nonenveloped viruses) shows affinity to hydrophilic surfaces [44]. The proposed concepts for reduced virus persistence are not absolute and still need rigorous verification with a wide range of actual viruses in the laboratory setting. Various fabrication methods exist to create a superhydrophobic surface, such as sol-gel method, lithography, chemical vapor deposition, anodization, electrodeposition, and wet and dry etching, to mention a few. These techniques decrease surface energy and increase micro/nano-scaled surface roughness by forming impart hydrophobicity to the surface [45].

Xiao et al. established a thermodynamic model to understand a surface-wetting transition of nanopatterned surfaces between Wenzel and Cassie-Baster states and found that shape and size of surface patterns can modulate surface-wetting behavior [40]. According to them, the deep and thin nanoholes facilitate the Cassie-Baxter condition while the shallow and thick patterns lead to the

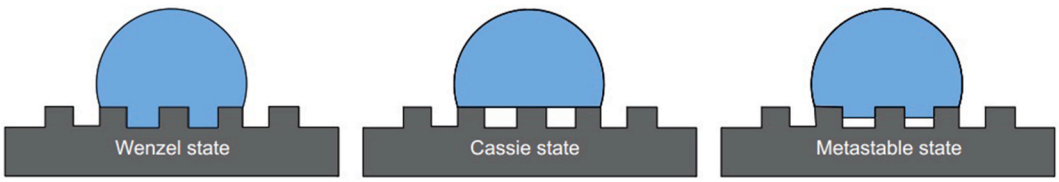

Fig. 1. Representative diagrams of Wenzel, Cassie, and Metastable state; adapted from ref [46].

Wenzel state. In contrast, on nano-pillared surfaces, shallow and narrow nanopillars have Wenzel state, and deep and wide pillars have Cassie-Baxter state. Laser texturing method offers to impart precisely tailored morphology and hydrophobic characteristics. Storage in ambient atmosphere, silanization, oxygen plasma treatment, or low-temperature annealing can turn typically hydrophilic surfaces superhydrophobic, however, conditions vary for different metals and substrates [46–48]. Scanning electron microscopic images of lotus leaves, a natural hydrophobic structure, has revealed dual roughness where microstructures are equipped with nano-scaled hairlike structures. Recent studies are focusing on developing bioinspired dual-scale hierarchical roughness or double-structured roughness to achieve superhydrophobicity [49–51]. Huerta-Murillo et al. used nanosecond pulsed direct laser and femtosecond laser to develop dual-scale hierarchical squared micro-pillars on aerospace-grade Titanium alloy with 810 nm-periodic distance. Hierarchical topography achieved a greater contact angle ($>150^{o}$) than non-hierarchical structures. The study concluded that storage in polyethylene bags could hasten the hydrophilic to hydrophobic transition than that in the air, although reasons for this behavior remain unclear [47]. Transition of wettability for laser textured surfaces has been reviewed by Ijaola et al. detailing laser texturing techniques to form superhydrophobic surfaces and their application. In addition to the prospective application to fabricate an antiviral surface, laser-textured hydrophobic surface has portrayed self-cleaning, anti-corrosion, anti-biofouling, and deicing applications [45]. Heckenthaler et al. studied that nanotexturing can enhance the self-cleaning efficiency of dust particles which may prove to be an additional benefit of antiviral surfaces [52].

Hydrophobicity enhancement on rigid surfaces is typically done by the combination of surface texturing and chemistry. Vasileiou et al. explored the synergistic interaction of surface flexibility with micro and nano-texturing of surfaces, as inspired by natural and technical surfaces, to improve superhydrophobic performance [53].

2.4. Surface topography

Over the past decades, topography-mediated bacteriostatic and bactericidal surfaces have been extensively studied. In contrast, the concept of surface engineering to develop antiviral surfaces is relatively new. Surface texturing causes the modification of surface properties like friction and wettability by creating nanoscale or microscale level of roughness [54]. Chatterjee et al. established that irrespective of surface geometry, tailoring both surface wettability and roughness can help achieve reduced residual thin film lifetime. The study recommended chemical modification to impart hydrophilic properties to hydrophobic surfaces to enhance droplets of residual thin film evaporation rate. They concluded that taller and closely packed pillar surfaces can return the shortest drying time and thereby the strongest antiviral effect [28]. It has been hypothesized that organisms will have stronger adhesion if the surface dimension is larger than the organism itself [55]. Furthermore, Siddiquie et al. recommended height and spacing of nanopatterns be less than the size of the virus so that it can pierce the envelope and/or decrease contact area; thus minimizing viral adhesion [42]. Although contact killing mechanism by sharp nanofeatures penetrating cell membranes with nanoscale level of surface roughness is an established and verified mechanism for bacterial inactivation, similar hypothesis for virus inactivation on nanostructured surfaces still needs verification and confirmation through wet bench research with actual viruses [56,57]. Surface engineering is receiving tremendous attention now, in the wake of the Covid-19 pandemic. The diameter of the virus typically ranges between 20 and 300 nm except for filoviruses with sizes up to 1400 nm [58]. Table 1 summarizes a list of prospective nanopatterned surfaces along with morphological characteristics in the abovementioned region. There are multiple ways to categorize nano-fabrication process. Nano-fabrication process mainly falls under two categories; one is top-down multiway etching, and another is bottom-up chemical synthesis. While top-down multiway etching generally refers to the removal of materials from a surface to form textures, the latter refers to the build-up of patterns or agglomeration of nano-materials on a surface [59]. These techniques can form either randomly organized nanopatterned roughness by plasma etching (based on reactive-ion etching), acid-mediated etching, anodic oxidation, and controlled polymer coatings; or well-controlled and defined nanopatterns surfaces by photolithography, nanoimprint lithography, colloidal and E-beam lithography, anodic oxidation of metallic surfaces, micro-contact printing and dip-pen nanolithography [60]. Fig. 2 summarizes possible nanofabrication pathways for both top-down and bottom-up approaches.

Guo et al. reported that surface nanofabrication with a silicon chip substrate can minimize the number of viruses attached and the strength of adhesion. Diameter, depth, and pitch distance of the nanohole array were 50 nm, 22 nm, and 100 nm, respectively. They compared male-specific coliphage (MS2) adsorption on smooth and nanostructured surfaces via AFM imaging after 2 h of MS2 incubation followed by three rinses and drying. Randomly distributed viruses were observed on smooth surface, whereas for nanostructured surface, particles were mostly present in between adjacent nanoholes. After 2 h and 24 h incubation, MS2 were found in 0.3% and 2% nanoholes respectively that is surprisingly low. They attributed reduced viral adhesion to pseudo-Cassie–Baxter state of surface wettability, in which nanoholes are preoccupied with air pockets thus preventing entry of MS2 virus in the hole. Moreover, the mentioned dimension of 50 nm diameter, and 100 nm pitch distance has limited space between adjacent pores for virions to land on and aggregate. Isolated MS2 virions are less stable than aggregates [77]. In contrast, MS2 adhesion and retention in randomly distributed nano-pores (60–80 nm wide and 20–30 nm deep) of PVC surface were much higher and virions were observed in every pore of the surface. Surface roughness of nano-patterned silicon was 1.7 nm compared to 10.8 nm for the porous PVC surface. Larger pore size, increased surface roughness, and overall heterogeneity of PVC favored MS2 retention by increasing contact surface area [88].

Hasan et al. tested the viability of *Pseudomonas aeruginosa* (Gram-negative bacteria) and *Staphylococcus aureus (*gram-positive bacteria) bacteria, and respiratory syncytial virus (RSV) and rhinovirus (RV) on nanostructured surfaces. 23 nm wide and randomly spread ridged nanostructure (see Fig. 3) was fabricated on aluminum 6303 alloy surface by wet etching technique with 2 M NaOH for 30 min, 60 min, and 3 h. Surface roughness increased significantly with increasing time. This insect wing-inspired nanostructure inactivated 92% and 87% of *P. aeruginosa* and *S. aureus* attached cells respectively. Viable RSV virus recovered from the etched surface was significantly lower than the control surface. This topography-mediation technique demonstrated better performance against

Table 1
List of prospective nanopatterned surfaces, their morphological characteristics, and antiviral performance.

Surface material	Texturing method	Morphological characteristics (pitch, structure)	Antiviral performance	Property alteration and/or remarks	Reference
Al 6063 alloy	Wet etching	Width 23 nm, randomly spread nanostructures	More efficient against rhinovirus (RV) than respiratory syncytial virus (RSV)	Either entrapment or envelop penetration inactivated virus particles after 6 h and 24 h exposure	[61]
			Complete inactivation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) after 6 h of exposure	Probable nanostructure entrapment or viral envelope or capsid rupture inactivation mechanism	[62]
SS	Femtosecond laser pulse	76–426 nm, nanogroove, nanospike, nanoripple	–	Superhydrophobic LIPSS (ripples) with CA: 154°	[63]
		100–200 nm, micro/nano hierarchical texture	–	CA > 150°	[64]
PMMA (Polymethylmethacrylate)	Electron-beam lithography	Diameter: 60 nm Pitch: 300 nm, nanopillars	–	–	[65]
	Nanoimprint lithography	Lowest value: Cap: 70 nm, Periodicity: 170 nm, Spacing: 100 nm, Height: 210 nm	–	–	[66]
Ti	Femtosecond laser pulse	70–90 nm, nanogroove	–	–	[67]
		200–400 nm,			[68]
		100–400 nm,			[69]
	Magnetic sputtering deposition	Height: 478 nm Width: 33 nm Spacing: 158 nm, Biomimetic nanocolumnar	–	–	[70]
Cu	Femtosecond laser pulse	270 nm, periodic grating	–	–	[71]
Carbon Nanotubes	Chemical vapor deposition (CVD)	Width: 0.9–30 nm Height: 2–70 μm Aspect ratio: 2000	–	–	[72]
Al	Femtosecond laser pulse	200–220 nm, nanoripples,	–	–	[73]
TiN	Femtosecond ultrashort laser pulse	170 nm, ripple and mound	–	–	[74]
SiO_2 and TiO_2 on Si	spin coating of silica, dry etching, Cr deposition, SiO_2/TiO_2 deposition	layer thicknesses: SiO_2 -73 nm; TiO_2 -38 nm, random bioinspired structure	–	–	[75]
Si	Femtosecond laser pulse	200 nm, fine ripples	–	–	[76]
	Electron-beam lithography and reactive ion etching	Diameter: 50 nm in, depth: 22 nm, and pitch: 100 nm, nanoholes	Reduction in number of attached MS2 coliphage virus and its adhesive strength to the surface	Air pockets in nanoholes preventing entry of MS2 to reduce MS2 adsorption and limited space between the adjacent holes for virus landing and aggregate formation CA: 68.1°	[77]
	Deep reactive ion etching	height: 4 μm deep diameter: 220 nm random interpillar spacing (biomimetic)	–	CA: 154°	[78]

(*continued on next page*)

Table 1 (*continued*)

Surface material	Texturing method	Morphological characteristics (pitch, structure)	Antiviral performance	Property alteration and/or remarks	Reference
	Reactive ion etching	diameter: 20−80 nm, height: 500 nm, nanopillars (biomimetic)	–	CA: 80°	[79]
Austenitic stainless steel as mother punch and aluminum as coin	Plasma nitride to form micro-patterned surface and femtosecond lasers to form nanotextures	300 nm micro/nano hierarchical texture	–	–	[80]
Polyimide sheets	Electron-beam lithography, step-flash imprint lithography and directed self-assembly	10 nm grooves and 25 nm metal lift-off	–	–	[81]
TiO_2	Hydrothermal synthesis	Nanofibers: length 298 ± 33 nm, diameter 52 ± 12 nm Nanostructures: length 251 ± 30 nm, diameter 22 ± 3 nm	–	–	[82]
	Femtosecond laser pulse	Pitch: 170 nm and 90 nm nanoripples Depth: 100–200 nm	–	–	[83]
	Sol−gel process	height: 230 ± 42 nm, spacing: 250 ± 18 nm, diameter: 75 ± 4 nm (top), 175 ± 10 nm (bottom), Biomimetic nanopillars	–	–	[84]
ZnO	Femtosecond laser pulse	Pitch: 200–280 nm	–	–	[85]
Ti	Electron-beam lithography	116–282 nm height and 93–214 nm diameter nanopillars	–	Prospective biomedical applications due to cytocompatibility	[86]
Polystyrene	Electroless plating, electroplating, and micro-injection compression molding	156 nm diameter and 180 nm pitched biomimetic nanopillars	–	CA: 143° ± 2°	[87]

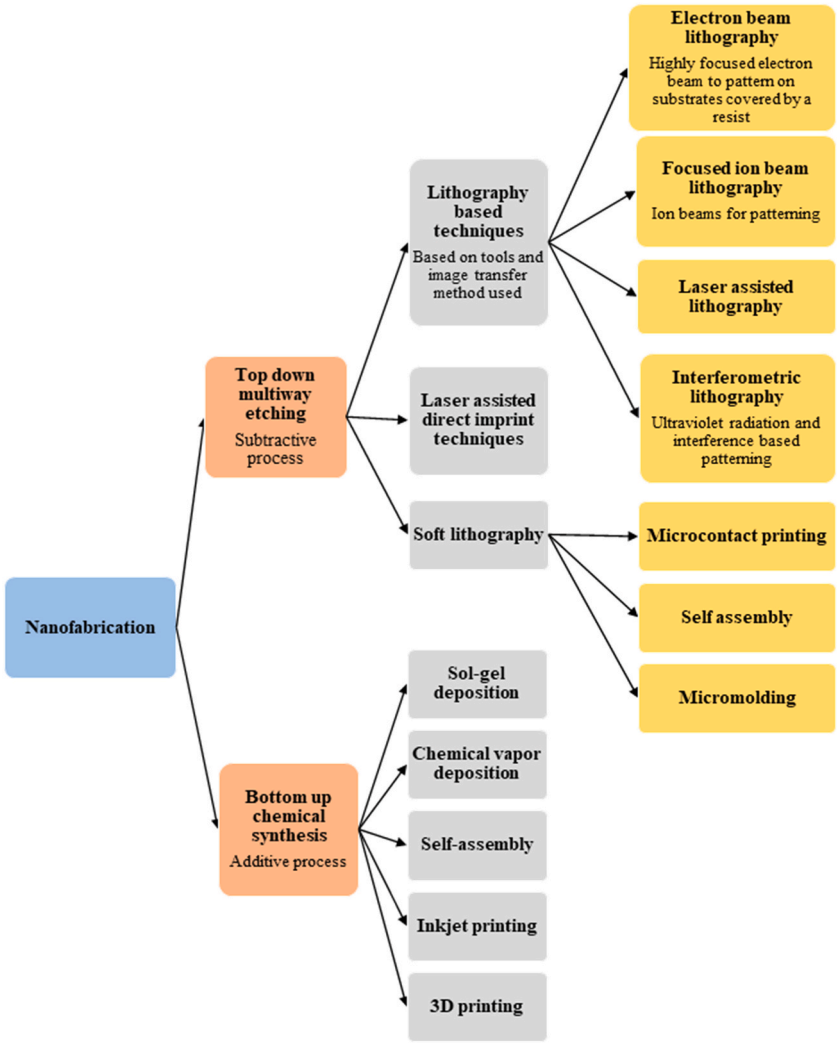

Fig. 2. A summary of frequently used nanopatterning methods.

nonenveloped RV than enveloped RSV virus [61]. Another study led by this researcher revealed that 6 h exposure to the same nano-patterned surface leads to complete inactivation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This time surface was fabricated by wet etching technique with 2 M NaOH for 3 h. However, the inactivation mechanism was not verified and can

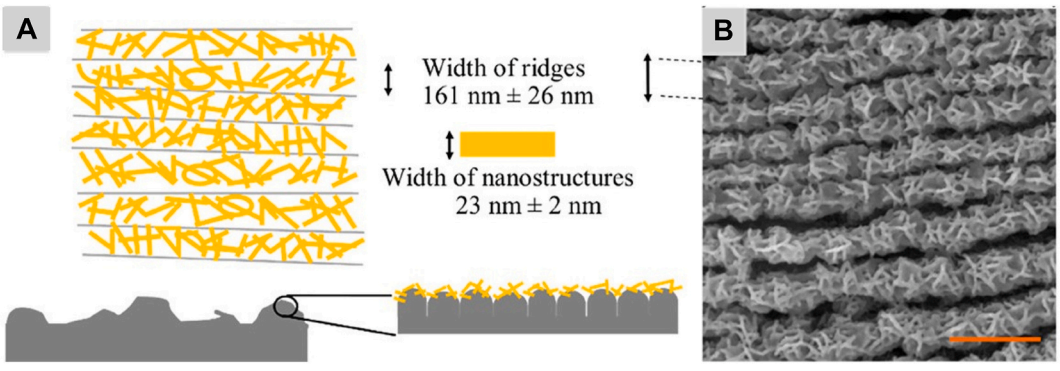

Fig. 3. (A) Schematic representations of etched samples with magnification from top and sides; (B) 3 h etched surface revealing the presence of random nanostructures (scale bar = 500 nm). Reprinted with permission from Ref. [61]. Copyright 2020 American Chemical Society.

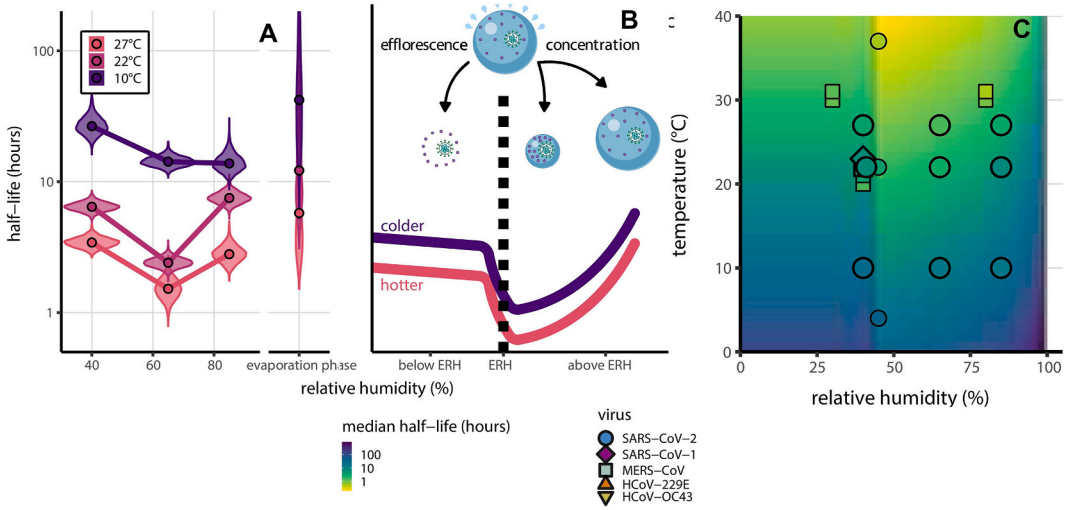

Fig. 4. (A) Measured half–life; (B) Estimated half-life from model as function of temperature and relative humidity; (C) Heat map depicting combined effect temperature and humidity on different viruses [119] (Made available under CC0 1.0 license).

only be speculated. This study suggests that susceptibility to nanostructured surfaces depends on the type of the virus [62].

Yang et al. designed "daylight-driven rechargeable nanofibrous membranes" for antimicrobial applications. They deposited PVA-*co*-PE solution on a copper grid-covered roller through a high voltage electrically charged needle and dried to form the substrate. They incorporated photoactive components in the rechargeable nanofibrous membrane (RNM) that can store biocidal activity under light irradiation and readily release reactive oxygen species (ROS) even under low or no light conditions. Briefly, the nanofibrous membranes intercept the pathogens and the grafted photoactive components or photobiocides in contact with the pathogens produce ROS, like hydroxyl radical (•OH), superoxide radical ($\bullet O_2^-$), and hydrogen peroxide (H_2O_2) in the presence of oxygen irrespective of the light condition. ROS disintegrates DNA, RNA, lipids, and proteins causing bacterial cell death and virus inactivation [89]. The proposed mechanism in this study operates in an integrated dual cycle mode, i.e., photoactive and photostorable cycle. The photoactive cycle initiates with the conversion of the RNM to the excited state (^{3}RNM*) under light irradiation that can remove and receive a hydrogen atom from a hydrogen donor to form free radical RNMH•. When oxygen availability is sufficient, the RNMH• radical reacts with oxygen molecules to revert back to RNM and produce ROS. Insufficient oxygen can lead to a side reaction including structural rearrangement and second hydrogen abstraction forming metastable structure and entering photostorable cycle that can produce ROS later with the presence of oxygen. Thus, the storage of photoactivity can ensure ROS production under dark conditions as well. Further, they coupled the rechargeable nanofibrous membrane with personal protective equipment (PPE) such as lab coats and N100 masks and demonstrated that the surface caused an approximately 6-log reduction in T7 phage plaque-forming units and 7-log reduction in *E. coli* colony-forming units compared to both covered area and control area section on the PPE [90]. Free radicals and singlet oxygen primarily target saturated lipids and cause lipid peroxidation and change in surrounding proteins, nucleic acids (mainly guanine), and other molecules. The interaction of free radicals with mentioned biomolecules led to the hypothesis that ROS damages viral envelop eventually causing virus inactivation. Considering this, enveloped viruses should be more vulnerable to photodynamic inactivation compared to nonenveloped viruses. Nevertheless, viral proteins of nonenveloped viruses undergo photodynamic damage causing the inactivation of the virus [91]. While the handful of existing studies of virus inactivation on nanostructured surfaces are remarkable, it is necessary to understand specific virus inactivation mechanisms for nanopatterned or nanostructured surfaces to pinpoint the topographical parameters that may work well against viruses.

To form a hydrophobic surface, laser texturing offers several advantages over other techniques including limited thermal distortion of the material, simplicity, environmental friendliness, material flexibility, and creation of complex patterns [12]. Aizawa et al. demonstrated that AISI316 austenitic stainless steel was polished, plasma nitrided to form micropatterned surface, and laser machined with femtosecond lasers to form nanotextures in those micropatterns. This substrate was used as a mother punch to coin this micro-nano-texturing on the pure aluminum surface [80]. Interestingly enough, this laser-assisted printing process facilitates the replication of micro/nano hierarchical texture onto any material substrates via a simple stamping process [92]. There are numerous studies with micro-and/or nano-textured surfaces with both hydrophilic and hydrophobic characteristics. The wettability property of nano-structured surfaces gained momentum alongside surface morphology in the recent years. It has been reported that even though laser-textured surfaces demonstrated hydrophilicity at earlier stages of fabrication, contact angle (CA) increases with storage time thereby achieving stable hydrophobicity [93–95]. Nonetheless, the period of wettability transition is different for different substrates. Yang et al. proposed the use of a low-temperature annealing process for a faster transition to hydrophobic surface, which allows the change of hydroxyl groups on the surface. They were able to fabricate micro/nano hierarchical textured surface with the maximum CA of 144.58° and 1100 nm periodic distance [46].

While the bactericidal property of bioinspired nano-structured surfaces has been extensively studied, the virucidal property of such

surfaces remains unexplored. However, many biomimetic nano-structured surfaces with dimensions matching that of viruses might have the potential for virus trapping and/or subsequent inactivation. Sub-100 nm scale might be of importance to tackle viruses below 100 nm size. Laser texturing works well to create nanostructures above 100 nm scale. Dry etching method is typically used to fabricate nanostructures in sub-100 nm scale. Despite the promising features, till date no antiviral surface in a sub-100 nm scale has been fabricated using dry etching method. However, it is troublesome to obtain a high aspect ratio (height: width >1) with ion-beam etching, a particular dry etching method. While with conventional one-step developing process, faulty nano-patterns are obtained, with the multi-step developing process proposed by Zong et al. defined and uniform nano-patterns were obtained. They incorporated a ~4 nm thick metal film on the resist to protect the fine resist nano-patterns in the developing solution [96].

DNA-based nanofabrication is quite similar to the bottom-up nanofabrication approach and can be considered novel compared to other nanopatterning techniques. While most bottom-up techniques focus on creating patterned surfaces, it is possible to form almost any arbitrary shapes with approximately 5 nm resolution by the DNA nanostructuring method. DNA primarily self-assembles to produce a template, which is then used to produce high-resolution patterns on a substrate. Surface engineering for large objects requires patterning with cheap, scalable, and non-flat surface-compatible patterning methods, to which DNA nanofabrication can offer a solution. Deposition, etching, and imprinting-these three approaches can be adapted for DNA nanostructure patterning applications in surface engineering [97]. Earlier work in DNA pattern transfer focused on metal deposition and coating on DNA structure for plasma etching pattern transferring [98–100]. However, for plasma-based etching, metal coating on the DNA template is often necessary to ensure template chemical stability [100]. Chemical vapor deposition for gas phase SiO_2 deposition and growth has been used in nanopatterning applications [101]. Mechanical stability of the DNA nanostructure can be improved with Al_2O_3 thin coating which additionally ensures multiple uses of the template [102]. Imprinting application of DNA nanostructures was recently studied by spin coating a thin polymer substrate film onto the DNA nanostructure, followed by peeling off the polymer film [103]. Currently, research on DNA nanopatterning is focused on creating guided or well-controlled pattern templates.

3. Environmental conditions influencing antiviral activity

3.1. Temperature and relative humidity

Virus-laden aerosols, ejected from coughing, sneezing or simply breathing, can dry under ambient conditions, and may or may not survive depending on relative humidity (RH), salt concentration in the droplet, etc. [104]. The effect of temperature and RH is closely intertwined. Increased temperature will increase aerosol drying, resulting in higher solute concentration in the droplet. Many airborne viruses have shown sensitivity to ambient humidity, but efforts to explain the relationship led to contradictions among existing literature [105]. Aerosols of vaccinia, influenza, and Venezuelan equine encephalomyelitis viruses showed better survival at low RH, whereas poliovirus showed better survival at high RH [106]. It is imperative to identify virus-decaying behavior at different humidity and temperature conditions. Tailored environmental conditions in hospitals, although cumbersome, need to be explored for emerging viruses to increase the performance of antiviral surfaces before another global viral epidemic hits the world.

A study on poliovirus, influenza, vaccinia, and Venezuelan equine encephalomyelitis (VEE) viruses observed the poorest viability at the highest temperature for each level of RH; RH induces greater influence at the higher temperature. RH had negligible influence on influenza, vaccinia, and Venezuelan equine encephalomyelitis (VEE) viruses at 10 °C (lowest temperature tested) [107]. Atmospheric aerosols have water significant water content and assist the transportation of microbes, particulates, and pollutants [108]. With favorable humidity, dry hydrophilic aerosols attract water and grow while absorbing viruses on their air-water interface and simultaneously dissolving gases and vapor from the environment into the liquid phase [109]. Increased RH in the atmosphere may incur hygroscopic growth of particles [110]and provide a greater surface for adsorption [111]. The relationship between humidity and ambient temperature with the inactivation of enveloped viruses is not completely understood yet. Viruses with high lipid content thrive at low RH, and viruses with low lipid content persist at high RH [112]. Valsaraj et al. reported that when water reacts with aerosol, it can provide a surface area to serve as a competitive adsorbate or a substrate [111,113]. The water film surrounding aerosol droplets can attract hydrophilic RNA-rich viruses [111]. SARS-CoV-2 is thermally sensitive and the rate of inactivation is enhanced at higher temperatures. It is stable at 37 °C for a day but gets inactivated at 56 °C and 70 °C within 30 min and 5 min, respectively [14]. Wang et al. suggested that high temperature and high humidity may modulate and slow down the transmission of SARS-CoV-2 [114]. Studies on the nonenveloped common cold virus human rhinovirus type 16 (HRV-16) [115] and influenza virus A (enveloped virus) [116] revealed that some viruses undergo faster inactivation at intermediate levels of RH (about 40–60%), than at high or very low RH [105]. In both studies, the virus was inactivated faster at 60% RH when it had been dried from higher RH than when it had been hydrated from lower RH [115,116]. This behavior was elaborated by "Efflorescence-deliquescence differential inactivation" model. Salt particles are solid at low RH and as RH is gradually increased, the particle abruptly takes up a large amount of water to dissolve and form supersaturated droplets. This transition at a particular RH is referred to as deliquescence RH (DRH). In contrast, water gradually evaporates from droplets as RH decreases, and nucleation starts below DRH at efflorescence relative humidity (ERH) [117]. Salt concentration in the hysteresis loop between ERH and DRH (typically 35%–65% RH) [118], can go beyond the prediction from equilibrium solubility. The studies attributed the high concentration of salt in the droplet or aerosol to the inactivation of both viruses [115,116].

In a recent study, Morris et al. tested the combined effect of temperature and humidity on the stability of SARS-CoV-2 on an inert surface to develop a mechanistic prediction model to explain and predict the effect of environmental conditions in virus inactivation [119]. They suggested a hybrid mechanism to understand the stability of many viruses. Their model is based on the premise that virus inactivation in the atmosphere is a chemical reaction and hence follows typical reaction kinetics. High temperature and solute

concentration speed up the inactivation reaction. Faster evaporation at lower RH results in higher solute concentrations. Nonetheless, when relative humidity is below a threshold, droplet crystallization may occur resulting in a change in reaction kinetics. These typical reaction principles are adopted in these mechanistic models. As observed in Fig. 4 from the mechanistic model, half-life is predicted for different temperature and humidity conditions. As inactivation proceeds slower at lower temperatures, virus half-lives are longer irrespective of humidity. Inactivation at quasi-equilibrium is split into two humidity regimes according to whether the ambient RH is below the equilibrium RH (effloresced regime) or above (solution regime). In the solution regime, solutes are concentrated by evaporation; at lower ambient humidity more water evaporation results in more solute concentration and thereby, faster inactivation. In effloresced regime, solutes effloresce and forms crystals; half-lives are not particularly affected by changes in relative humidity [119]. A similar U-shaped effect of RH on virus inactivation rate has been reported for enveloped RNA viruses such as animal coronaviruses, influenza viruses, paramyxoviruses, rhabdoviruses, and retroviruses [15,120–124]. Enveloped DNA viruses such as Herpesviruses and Poxviruses [121,122] and nonenveloped viruses like polioviruses [122,125] exhibit similar empirical RH dependency. It has been established that RH dependency for virus inactivation is similar for droplets on surface and suspended aerosols [126,127].

3.2. Sunlight and UV radiation

Solar radiation consists of UV-A, UV-B, and UV-C photons; among these UV-C has stronger germicidal property but cannot reach the earth's surface. On the contrary, UV-A and UV-B reach the earth surface in abundance. UV radiation can trigger antiviral activity by altering virus nucleic acid and capsid proteins [111]. UV inactivation affects single-stranded nucleic acid (ssDNA and ssRNA) viruses more than double-stranded (dsDNA and dsRNA) viruses [112]. Recently, a study used worldwide Covid-19 mortality data in their "Solar-Pump" model of epidemics to demonstrate the strong virucidal effect of UV-B/A on single-stranded RNA virus Covid-19. According to their study, solar radiation inactivated 63% of virions in 1.5×10^3 $TCID_{50}$/mL concentration, higher than typical aerosol concentration, in less than 2 min at noon during summers in temperate regions of the earth. Results showed that solar UV-A and UV-B can efficiently inactivate SASR-CoV-2 in both aerosols and surfaces [128]. However, dust and aerosol loading, and photolysis of pollutant gases in the atmosphere can cause UV flux scattering, resulting in reduced UV exposure to the surface [111]. Another study on virus inactivation with solar radiation suggests that environmental inactivation of the virus by solar UV radiation contributes to seasonal occurrence of influenza pandemics. They came forward with a doubt that the virucidal impact of solar UV radiation may be several degrees greater than temperature and relative humidity for surface and/or aerosol environmental virus inactivation [129]. Weber et al. nullified this doubt pointing to an error in their meta-analysis. They further claim that while influenza A virus is sensitive to a wide range of environmental factors, influenza outbreak's occurrence with heavy dependency on outdoor transmission is an unlikely hypothesis. In general, nonenveloped viruses are more environmentally stable than enveloped viruses; but not universally [130].

4. Conclusions and perspective

A number of studies are prevalent regarding virus inactivation in the environment. Although these studies are important to understand the persistence of respiratory viruses in the environment, it is equally imperative not to draw too many conclusions from isolated findings. Investigation on surface modification to impart antiviral properties targeting varying inactivation mechanisms is in the ripe now. This review has highlighted surface properties and environmental conditions which can impart and/or help enhance antiviral properties to communal objects. Recent advances in nanofabrication have made the possible fabrication of up to sub-10 nm structures with high aspect ratios, such as carbon nanotubes, black silicon, aluminum nanostructures, nanopillar polymers, titanium nanowires, and other nanostructured surfaces. We urge researchers to maintain a systematic approach while testing antiviral properties based on surface topographies targeting each category of viruses- DNA virus, RNA virus, enveloped or nonenveloped viruses, and rigorous documentation for future reference. Further works are necessary to compare the effective inactivation of viruses on antiviral surfaces fabricated with diverse surface modification approaches. Surface nanotexturing is more stable than antiviral coating. Antiviral coating strategy is limited because silver and other heavy metal ions tend to leach out into surroundings leading to exhaustion of embedded coating thereby making this an inefficient approach [131,132]. Large-scale coating also seems like a far-fetched option to cover places like hospitals which is frequently riddled with pathogen attack. It is safe to anticipate that nanofabricated surfaces with tailored topography to provide favorable wettability, dimension smaller than the virus, and shape-promoting adhesion resistance can resist, repel and kill pathogens effectively. While studies on nanostructuring are prevalent, the effect of surface topography in virus inactivation-related research is only a handful. Therefore, more attention must be paid to determining the mechanisms of virucidal activity of these surfaces and assessing their compatibility, feasibility as well as large-scale applicability for suitability in future biomedical devices, communal objects, and overall hospital surfaces.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

Data included in article/supp. material/referenced in article.

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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