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Viruses and Bacteria Inactivation through Gas Plasma

M. Rasouli[a], N. Fallah[b] and A. Divsalar[b,*]

[a]*Institute for Plasma Research and Department of Physics, Kharazmi University, Tehran, Iran*

[b]*Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran*

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ABSTRACT

Due to bacteria and viruses are the pathogens of many living organisms, it is important to introduce a novel modality to inactivate them. Each alternative platform should bring about an appropriate platform to overcome ongoing challenges such as the COVID-19 pandemic. Gas plasma as an emerging technology has recently become a promising solution to a number of challenges due to its diverse applications in healthcare and medicine, environmental remediation and pollution control, materials processing, electrochemistry, nanotechnology, *etc*. Based on the physical and chemical effects, plasma creates a unique opportunity for specific applications. This article first provides an overview of how gas plasma works and then describes its use in effectively inactivating bacteria and viruses. With reviewing recent works in mentioned areas, we emphasize the application of plasma for SARS-CoV-2 inactivation. Furthermore, we summarize insights regarding the inactivation mechanism of gas plasma.

Keywords: Gas plasma, Plasma medicine, Virus inactivation, SARS-CoV-2 inactivation, Bacteria inactivation

INTRODUCTION

Plasma, after solid, liquid, and gas, is often referred to as the fourth state of matter. Plasma is the most abundant form of matter in the globe and makes up more than 99% of the visible matter in the world. They are synthesized of positive ions, electrons or negative ions and neutral particles [1]. When a solid (the first state of matter) receives energy from an external source such as heating, the particles in it obtain enough energy to loosen their structure and therefore melt to liquid form (the second state). After obtaining sufficient energy, the particles in the liquid change state and evaporate to the gas (the third state of matter). This occurs at a certain pressure at a constant temperature, leading to a phase transition. Then, when a significant amount of energy is applied to the gas through mechanisms such as electrical discharge, the electrons released from atoms or molecules not only allow the ions to move more freely, but also produce more electrons and ions by colliding after a rapid acceleration in an electric field. Eventually, more electrons and ions alter the electrical properties of the gas, which in turn become ionized gas or plasma. However, this transfer from a gas to plasma is not a phase transfer in the thermodynamic sense because it occurs gradually with increasing temperature [2,3].

Plasma medicine is a combination of plasma physics, chemistry, biochemistry, cell biology and medicine. The field is known as a new field that has emerged by combining several sciences in search of new solutions to address medical problems and issues. In this regard, plasma medicine in recent years has achieved many early and promising successes that are unique and have a benefit for patients [4].

One of the characteristics of cold atmospheric plasma, also called non-equilibrium plasma, is its low gas temperature. Gas plasma (GP), in which the temperature of the ions is close to room temperature, is produced by applying a noble gas to a pair of electrodes connected to kV sinusoidal waves or pulses or to a radio frequency.

As shown in Fig. 1, Plasma has many uses in medicine, including virus inactivation, wound healing, cancer treatment, biofilms, *etc*. and, in general, plasma containing charged particles, neutral particles and electron, thermal effects, ultraviolet radiation (UV), electromagnetic waves (EM), reactive oxygen species (ROS) and reactive nitrogen species (RNS). The possibility of using plasma in particular

*Corresponding author. E-mail: divsalar@khu.ac.ir

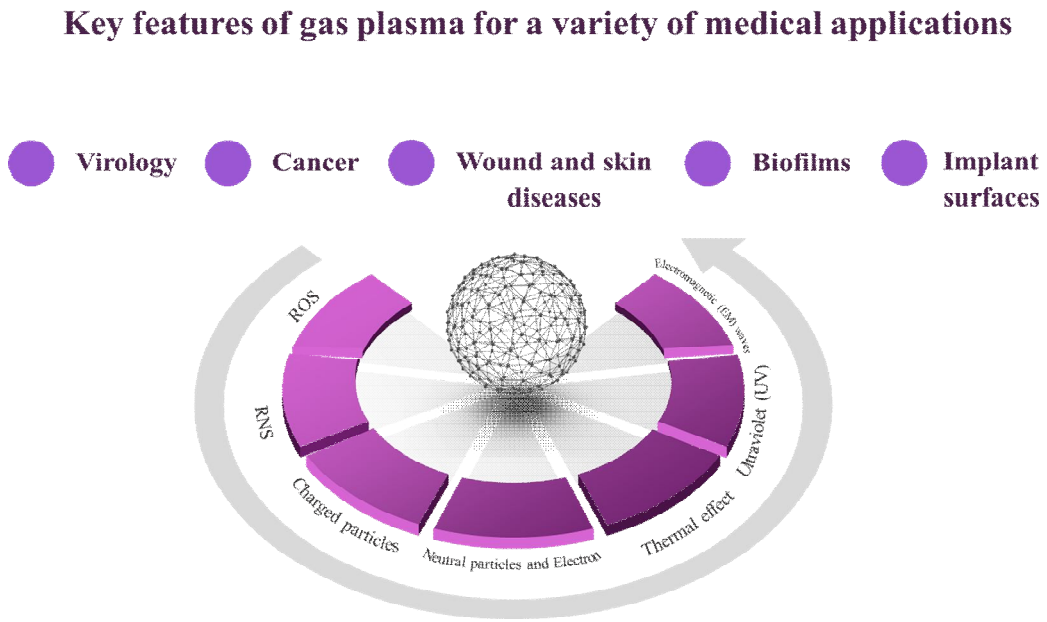

Fig. 1. Key features of gas plasma and its applications for diverse medical applications. Abbreviations: ROS, Reactive oxygen species. RNS, reactive nitrogen soecies.

or in combination with other therapies makes plasma a unique treatment [5-8].

PLASMA FOR BACTERIA INACTIVATION

Physical therapies for defeating microbial challenges have a long tradition, and many medical applications focus on extensive remote antimicrobial activity. However, no device with pure physical antimicrobial effect has entered the medical field, and potential treatments with strong antimicrobial properties such as UV photons are not excluded from the market for safety reasons [9,10]. In this review article, we focus on the inactivation of bacteria and viruses through gas plasma. The main interplay of plasma and the plausible mechanism of gas plasma for these mentioned areas has been shown in Fig. 2.

Unlike traditional physical therapy, GP now offers a wide range of effective species with strong antimicrobial properties. Advances in techniques for accurate detection and characterization of plasma compounds and quantitative measurement of its reactive species will lead to more information on the basic and complex mechanisms of GPJ-mediated microbial inactivation. Reactive oxygen species (ROS), such as ozone O_3, hydroxyl radical $OH^{\cdot}$, singlet oxygen $^1O_2$, superoxide $O_2^{-\bullet}$, atomic oxygen O, organic radicals $RO^{\bullet}$, $RO_2^{\bullet}$, reactive nitrogen species (RNS), such as atomic nitrogen N, nitric oxide $^{\bullet}NO$, nitrogen dioxide $^{\bullet}NO_2$, ultraviolet and electric field have been reported to contribute to plasma inactivation. Each of these has different levels of reaction, stability, and biological function, leading to a host of mechanisms that can act individually, synergistically for widespread antimicrobial activity [11-13].

These species justify GP-induced antimicrobial applications based on membrane oxidation, cell wall, and DNA breakdown. Compared to other methods such as UV, the "cocktail" provided by GP can be used with a much lower energy dose, which makes safer clinical use possible. None of the above types of reactions can be used alone without side effects because much higher doses are needed to achieve the desired result [14].

Among the GP devices that show a very similar antimicrobial effect in semi-solid media *in vitro* are: DBD (PlasmaDerm®, CINOGY, Duderstadt, Germany), DBD

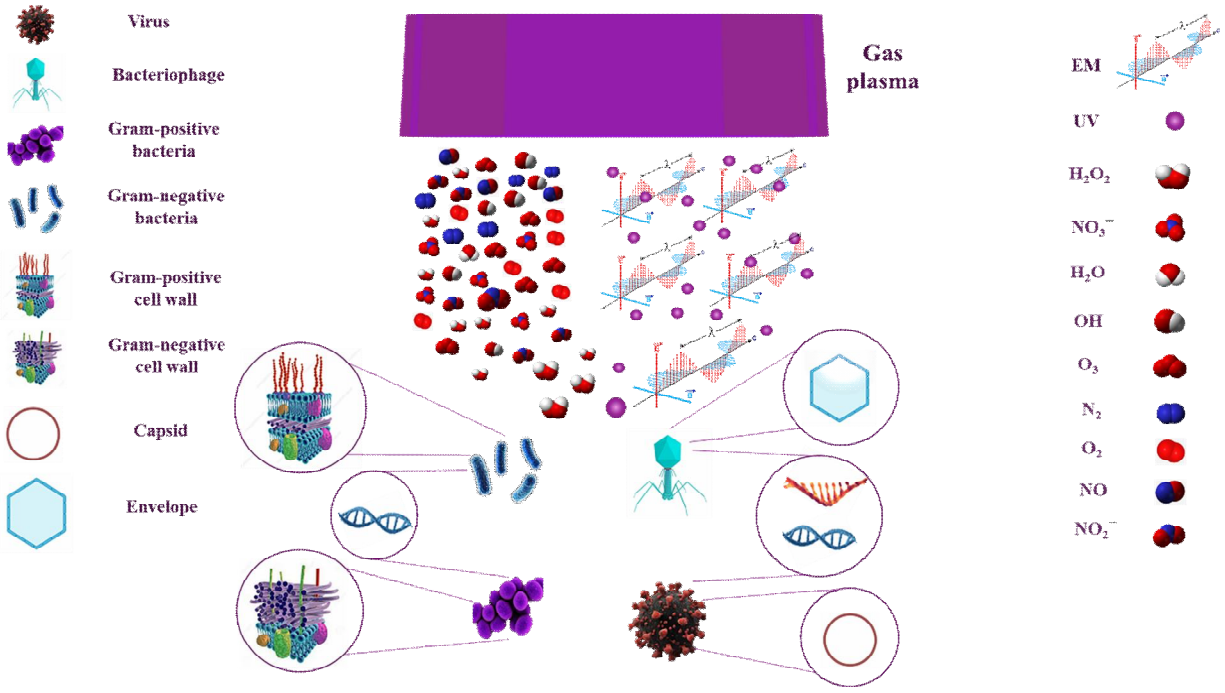

Fig. 2. Schematic illustration of reviewed targets and a possible mechanism under gas plasma irradiation.

with glass electrode (TEFRA, Berlin, Germany), DBD with glass electrodes (Medical Plasma Systems, Bad Ems, Germany), APPJ, gepulsed and non-pulsed modules (KINPen Med, INP Greifswald, Germany) and Argon Plasma-Jet (Maxium®-Beamer, KLS Martin, Tuttlingen, Germany) [15-17].

However, before planning treatment, options and potential side effects and costs must be weighed. For example, it does not make sense to use GP to disinfect surfaces where simple ethanolic disinfectants have similar applications. GP applications seem meaningful when routine methods are not (completely) successful.

There is a variety of opinions about the difference between GP sensitivity to gram-positive and gram-negative bacteria. Some believe that gram-negative bacteria are more sensitive, while others believe that the difference depends on the characteristics of the bacteria and the type of plasma device [18]. For example, gram-positive species are significantly more sensitive to DBD plasma than gram-negative species [19]. The plasma jet is another device with the best sensitivity to the gram-negative, followed by the gram-positive *Methicillin-resistant Staphylococcus aureus* (MSSA) to both sources [20].

The use of plasma directly on bacteria along with the use of GP to produce ozone in water treatment began in the 1850s. In 1996, the real use of GP as a potent disinfectant was first discovered. In this study, YEPG medium infected with Pseudomonas bacteria was treated with plasma for 10, 15 and 20 min. All samples were found to be completely sterile without damaging the environment itself [21].

In another study in 1998, several bacterial species (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* spores) under different discharges and an array of gases (air, pure argon, O_2, CO_2 and N_2/O_2, Ar/O_2) were compared. *B. subtilis* spores by N_2/O_2 GP showed that the survival curve included three stages of inactivation. UV radiation is the first phase agent due to its action on isolated spores, which may also be present in the first layer of accumulated spores. The process of slow erosion by active species continues, which is the cause of the second phase and the slowest stage. Finally, in the third stage, UV penetrates the genetic material of the spores after clearing the spores and debris.

These stages appear to be a function of the time of plasma exposure and are consistent with changes in the kinetics of the inactivation process [22].

Since then, the ability to produce plasma at atmospheric pressure has entered a new phase by making various configurations, and today we are witnessing tremendous progress in this field. The basis of the existing devices for producing cold plasma at atmospheric pressure is that by applying an electric field on noble gases, air, nitrogen and a combination of them, depending on the structure of the device, different physical and chemical factors are produced. In 2000, successful inactivation by GP of bacteria was performed both on the surface and in suspended water [23]. Since then, more research has been done to optimize the effect of bacterial inactivation using different plasma sources and operating conditions, as well as to identify the mechanisms of this inactivation.

In 2006, Lee *et al.* compared the susceptibility of *E. coli* and *S. aureus* as vegetative bacteria to plasma He/O_2 jets at dry surfaces. The results showed that the vegetative bacteria were inactivated after one minute of plasma exposure. Bacterial spores were slightly inactivated after one hour of treatment [24]. In 2010, the vulnerability of nine microbial species, including gram-positive and gram-negative bacteria, spores of *Deinococcus radiodurans* and *Geobacillus stearothermophilus*, and the yeast *Candida albicans* were determined. The susceptibility of the bacterial vegetative forms of all bacteria was analogous, while the inactivation of *Candida albicans* and *Geobacillus* spores took longer, which confirmed the previous results [25].

Then in 2011, Dobrinin *et al.* also studied the role of moisture and ions in various gaseous mixtures in the same year and concluded that the presence of oxygen and water plays an important role in the production of ROS and the inactivation of bacteria [26]. Another study was conducted in 2013 on the resistance and susceptibility of diverse strains of *Salmonella* at different levels. This study was important in the food industry to control the microbial load of fresh produce by applications of this method. Different microorganisms, as well as various plasma sources working on several parameters, were tested. The results showed that cases that used only one plasma source were more appropriate [27].

One study also showed that organic molecules in liquid-containing bacteria could reduce the antibacterial effect of plasma. This suggests that plasma-produced reactive species that have the strongest antibacterial effects also react with other organic molecules [28].

The antibacterial effect of GP has been evaluated against several types of bacteria associated with dental applications, including *Enterococcus faecalis*. Experimental results show that LTP can effectively kill *Enterococcus faecalis*, one of the main types of bacteria that prevents root canal healing, in a few minutes. Experiments were performed with the root canal model both in wet conditions (root canal full of bacterial suspension) and in dry conditions (infected and dried root canal). The direct treatment has been shown to be most effective in dry conditions and leads to a reduction in bacterial load [29].

PLASMA FOR VIRUSES INACTIVATION

Viruses are very small agents that only replicate inside living cells. Viruses have very diverse hosts, they possess the ability to infect animals, plants, microorganisms including bacteria and even humans. Although viruses are known to cause various diseases, but they have always been involved in the evolution of life on earth and can be useful for the preservation of the earth's important ecosystems and natural cycles, such as the carbon cycle at sea [30]. Among the most important pathogenic viruses are influenza, Ebola, AIDS (HIV) and coronavirus (SARS-CoV-2). The coronavirus, although less prevalent in the past, has caused serious human and economic damage in recent months, and the problem has not yet been resolved. Viruses have historically played a major role in countless epidemics and pandemics.

For viruses inactivation, there are a large number of physical and chemical agents that are common in the world. Bleach, chlorhexidine, povidone-iodine, ethanol, chloroxylenol and benzalkonium chloride are examples of chemical disinfectants, while UV light, high temperature, and pressure, are physical therapies [31]. For example, the traditional clearing method was used to disinfect water, but it has now been shown that it does not effectively inactivate some viruses and in the long term can be dangerous to human health by releasing toxic by-products [32]. In general, it has been determined that the technologies used

each have advantages and disadvantages. Several studies in the lab show that cold plasma has the ability to overcome these problems, but their validation requires focused and specific studies based on the needs of the day and on an industrial scale [33].

Viruses can be transmitted in a variety of ways, including surfaces, food, air, water, or even from one infected person to another. As in the COVID-19 pandemic of SARS-CoV-2, transmission from contaminated surfaces and airborne particles play a key role [34].

GP used at room temperature, is well-suited for treating a variety of biological conditions, including solids, liquids and airborne particles [35]. GP is usually associated with stable electrical discharge and the gas temperature is unaffected, but the chemical reaction is very extensive compared to the source gas due to the presence of reactive species. In most cases, atmospheric pressure plasma is used to inactivate the virus. Plasma has many uses in medicine so that it is used to kill microorganisms such as viruses, cancer treatment, wound healing, *etc*. [36,37].

GP is produced when an electric current enters a pure gas or gas mixture. UV, electrons, protons, neutrons, active free radicals and other compounds are the components of plasma. Both physical and chemical properties in interplay with the target sample cause interactions that play the most important role in studying viruses inactivation, but the mechanism has not yet been precisely identified. This new technology has many advantages because it is easy and cost-effective compared to other disinfection technologies, and it is a green technology [38,39]. In general, the effect of plasma on the inactivation of viruses can be attributed to its effects on the RNA or DNA genome of the virus, as well as their lipid and protein structures [40]. Furthermore, based on the unique nature of plasma, it is a multimodal antiviral agent with applications ranging from surface disinfection to the possible mixing with the vaccine media (Fig. 3) [41,42]. Plasma is very useful in inactivating respiratory viruses. At present, the treatment of respiratory viruses' influenza A and B [43] and respiratory syncytial virus (RSV) is performed only with a high voltage GP source.

RSV is the most common cause of lower respiratory tract infections in infants and is one of the most important viruses in pediatrics, especially because it spreads easily by contact with contaminated surfaces [44]. Active species of oxygen and nitrogen play an essential role in inactivating RSV virus. Plasma therapy alters viral genomic RNA. In general, neutral active species produced from nitrogen gas plasma cause this genomic damage [45].

More recent research on the effectiveness of gas plasma on SARS-CoV-2 indicates that not only can plasma inactivate SARS-CoV-2 infected surfaces, but also it can inhibit pseudovirus with the SARS-CoV-2 S protein infection. Chen *et al.* utilizing Ar and He as feeding gas evaluated the efficacy of GP on various SARS-CoV-2 infection surfaces including baseball leather, plastic, basketball composite leather, metal, cardboard and football leather. Regarding the carrier gas, Ar compared to He had a better inactivation performance. Also, the finding showed 30 s Ar-fed plasma exposure, exhibited decontamination for metal surfaces. Another significant finding revealed plasma in less than 180 s is able to inactivate six surfaces from all SARS-CoV-2. Inactivating leather football, cardboard, and basketball surfaces under 60 s treatments, plasma can inactivate plastic until 30 s treatment. Regarding the inactivation mechanism, they hypothesize that reactive species from plasma are able to change membrane functions [46].

To achievement a new disinfection strategy, Guo and colleagues by means of pseudovirus and the RBD examined the inactivation potential of plasma activated water (PAW). To this end, PAW-5 min and PAW-10 min were produced by 5-min and 10-min plasma irradiation on deionized water, respectively. In addition to long-lived reactive species (H_2O_2, NO_2^-, NO_3^-), short-lived reactive species concentration was measured to explore the chemical action mechanism of PAW. While PAW-10 min can inactivate viruses and triggers virus aggregation, PAW-5 min inactivates the viruses without fueling the morphological changes. These results suggested pseudoviruses S proteins are inactivated by PAW reactive species. The authors employing RBD tried to understand the mechanism of PAW on S protein. They observed that to prevent RBD protein binding with hACE2, PAW damaged the RBD. Contrary to the previous results long-lived reactive species have a major role in the inactivation mechanism, they conclude that short-lived reactive species, especially $ONOO^-$ play a crucial role in the inactivation process (Fig. 4) [47].

On the other hand, disabling adenovirus on a glass

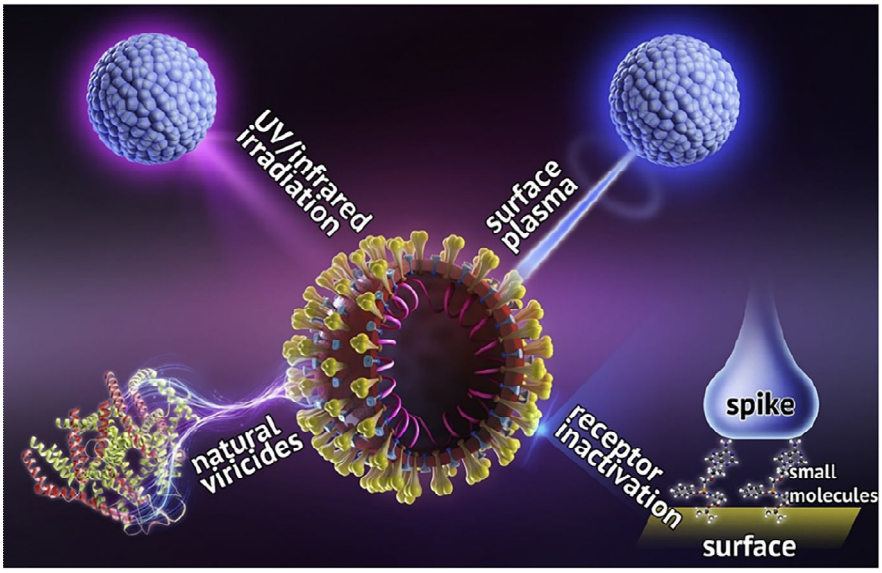

Fig. 3. Possible structural targets and effective reactive agents for coronavirus inactivation. Since the latest natural evolution leading to the novel coronavirus, SARS-CoV-2 was in the specific binding domain to human cells located on its spikes, these receptor-binding domains (RBDs) may be targeted as a potential "Achilles's heels" of the next, more evolved and potentially even deadlier viruses to cause future outbreaks. This figure was obtained with permission from [41] under the terms of the creative commons CC BY license.

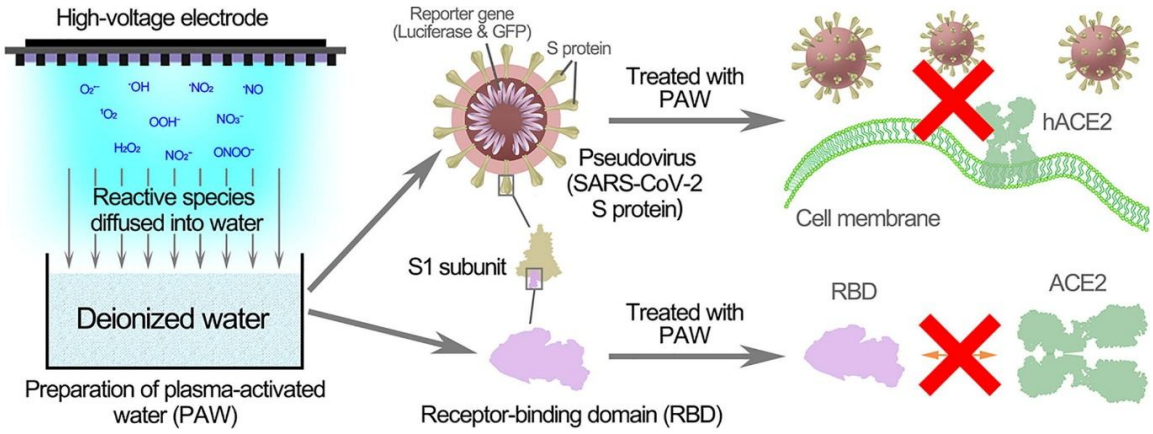

Fig. 4. Graphical abstract of the study from experimental setup to plausible inactivation mechanism. This figure was obtained with permission from [47] under the terms of the creative commons CC BY license.

surface with a high voltage pulse source has not been very successful and therefore the size of the DBD will not be suitable for this purpose [48]. One of the most successful inactivation events in a liquid environment is a 15 s FCV treatment performed using a plasma jet. This very short treatment time indicates that plasma jets can be an important

tool for inactivating the intestinal virus in fluids [49].

Lately, GP has been examined as a proper treatment for herpes simplex virus type 1 (HSV-1). HSV-1 is often referred to as herpes labialis, however, it is associated with cases of encephalitis, conjunctivitis, or neonatal herpes as a perinatal disease. In this study, HSV-1 was gradually exposed to GP for 0 to 150 s. The positive control was the antiviral drug acyclovir. After GP exposure, the virus suspension was transferred to a standard HSV (Vero) cell line and also to the SH-SY5Y neuroblastoma cell line as a model of neuro infection. The results showed that GP had a negligible antiviral effect on HSV-1 in both Vero and SH-SY5Y cells in high-dose [50].

In addition, GP therapy affects human adenoviruses (HAdV) have also been reported. Adenoviruses are pathogens that are rarely associated with severe clinical symptoms in healthy individuals. In contrast, immunodeficiency patients are life-threatening. There is currently no definitive antiviral treatment for these patients. Therefore, finding an effective and safe treatment is very important [51]. Due to the lack of antiviral therapy, GP was used on a variety of human adenoviruses from different species. The data showed that GP has a type-dependent effect on adenoviruses and according to the reported data, it can increase infection for some types of adenoviruses. Therefore, GP may be a suitable choice as an antiviral therapy in a virus-dependent manner, but these processes entail further study [52].

The effect of GP on HIV-1 replication in monocyte-derived macrophages (MDM) was also studied. The results showed that treatment of the virus suspension with GP significantly reduced the ability of the virus to cause infection in MDM. These results were evaluated with infected cells from 6 different donors. This effect of GP can be due to damage to the viral envelope, which prevents cell-virus fusion, or the destruction of the viral capsid, which leads to reverse transcription disorder [53].

Herpes zoster virus was also studied in conjunction with GP therapy. Herpes zoster is a painful infectious skin disease that occurs with an increasing prevalence in the elderly population due to reactivation of the varicella-zoster virus [54]. The effects of GP treatments for herpes were evaluated in terms of pain relief, recovery rate, and safety. 37 patients with herpes zoster were treated daily with 5 min of argon-based GP (active) or 5 min of argon gas (flow control, placebo), along with one standard. Examinations showed that there was a significant reduction in pain in patients treated with GP compared to the control group. Plasma therapy noted faster clinical recovery in the first 1-2 days. Therefore, 5-min daily treatment with argon-based GP was effective, painless, and safe, resulting in initial healing and severe pain relief in herpes zoster lesions [55].

Plasma is also used to inactivate plant viruses that were first discovered [56]. Members of the genus Tobamovirus, such as the tobacco mosaic virus (TMV), were inactivated after 10 min of treatment with DBD, despite their inherent stability [57]. Inactivation of the most important potato viral pathogen, potato Y virus (PVY), was performed in water samples using a plasma jet with 5 min and 1-min treatments. Given the results that GP can effectively inactivate important plant viral pathogens, it is believed that the use of plasma as an anti-pollution tool in agriculture has high potential and deserves special attention, especially in the future global warming scenario [58].

Newcastle disease virus (NDV), avian influenza virus (AIV) and swine reproductive syndrome (PRRSv) are three important animal pathogens that can be treated with plasma. Vaccines are one way to fight these viruses. GP has been used as a potential inactivation step in vaccine preparation [59]. Complete deactivation in NDV is achieved after a 2-min period of plasma jet treatment [60]. PRRSv is economically one of the most important pathogens in the pork industry and can be transported as airborne particles and remain infected after long distances [61]. GP can potentially provide immunity with a charge-driven filter and RONS, respectively, by stopping the spread of the virus and eliminating the virus infection [62]. Based on these results, it can be concluded that GP has a very good potential for direct air disinfection, which can also be used to combat the spread of COVID-19. However, issues such as high ozone production need to be addressed before such treatment becomes part of the routine.

CONCLUSIONS

Comprehensive research on the effects of plasma on bacteria and viruses has led to an increase in knowledge of

the mechanisms by which plasma interacts with living systems over the years. Although many details are not yet clear, some basic principles are known. As mentioned earlier, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are major players in the biological effects of plasma. Finding solutions for plasma devices that are somewhat compatible is one of the main requirements for physical and technical research and development in the field of plasma medicine. Such adaptability and flexibility are also required by plasma parameters such as gas composition to achieve different therapeutic goals.

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