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RESEARCH ARTICLE

Endemic Respiratory Viruses Inactivation in Aerosol by Means of Radiated Microwaves

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Abstract:
Background. Airborne transmission of endemic respiratory viruses, such as SARS-CoV-2 and influenza viruses, poses significant public health challenges.
Aims. This manuscript investigates the efficacy of electromagnetic waves as a novel approach for airborne viruses inactivation in bioaerosol suspension, that is their natural route of transmission.
Methods. Using a bioaerosol system in a controlled laboratory environment, different variants of SARS-CoV-2 and the human influenza virus were exposed to resonant radiated microwaves within safe power levels.
Results. Radiated microwaves exposure led to a substantial reduction in the infectivity of highly transmissible SARS-CoV-2 variants, including the *delta* and *omicron* variants, achieving 80-90% reduction in infectivity. These variants exhibited susceptibility to the resonant radiated microwaves similar to the original Wuhan variant of SARS-CoV-2, confirming the effectiveness of this approach against a range of SARS-CoV-2 strains. Furthermore, the H1N1 human influenza virus displayed a 90% reduction in infectivity when exposed to microwave waves. However, the influenza virus exhibited distinctive response patterns, being susceptible to higher frequencies (up to 16 GHz) compared to SARS-CoV-2. Additionally, longer exposure times (5 minutes) were required to achieve the same level of inactivation observed in SARS-CoV-2.
Conclusions: These findings highlight the potential of radiated microwaves as a strategy for inactivating SARS-CoV-2 and influenza viruses. Further, they contribute to determining the optimal frequencies, exposure times, and power levels required for effective virus inactivation. This innovative approach could provide valuable insights for developing sanitization strategies and public health interventions to mitigate the airborne transmission of respiratory viruses.

Keywords: SARS-CoV-2; Influenza Virus, airborne pathogens; air transmission; microwave inactivation

1. Introduction

Airborne pathogens encompass a broad range of microorganisms that are responsible for transmitting various bacterial, viral, and fungal infections.[1,2] Among these pathogens, of note is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the global COVID-19 pandemic.[3] In late 2019, this coronavirus emerged in Wuhan, China, causing a rapid onset of severe respiratory syndrome and deadly pneumonia. This pandemic has had a profound impact on healthcare systems, economic growth, and social cohesion worldwide. The original Wuhan strain of SARS-CoV-2 has undergone progressive mutations, resulting in the emergence of a number of variants. Among these, the *delta* and *omicron* variants have gained global prevalence.[4] Remarkable research efforts have yielded several safe and effective COVID-19 vaccines.[5–7] In response to the COVID-19 public health emergency, the Food and Drug Administration (FDA) granted Emergency Use Authorization (EUA) for various new drugs and medical products, bypassing the need for full FDA approval. Currently, the primary treatment options for the disease include antiviral drugs, immunomodulators, neutralizing antibodies, as well as cell and gene therapies.[8,9]

In addition to SARS-CoV-2, there are numerous other viruses that can be transmitted through the air, including the influenza virus. Influenza A viruses circulate within the human population as an epidemic disease that resurfaces annually from a vast reservoir of zoonotic sources, causing recurrent respiratory illnesses in humans and significantly impacting both human health and the economy.[10] The progression of the disease can vary, ranging from symptom-free cases or a mild infection affecting the upper respiratory system to a severe illness characterized by high fever, chills, muscle pain, pneumonia, and even death.[11] Ongoing research aims to prevent and treat the influenza virus. In addition to the widespread use of vaccines, which are appropriately modified annually,[11] several drugs have been developed. Currently, there are three approved drug categories specifically targeting the influenza virus: M2 proton channel antagonists, neuraminidase inhibitors, and a polymerase acidic endonuclease inhibitor.[12]

Notwithstanding the advancements made, both SARS-CoV-2 and the influenza virus continue to persist as ongoing challenges. According to a recent editorial on The Lancet, the COVID-19 pandemic in 2023 "is far from over", due to several factors [13]. These include the occurrence of new waves of infection, such as the recent one in China, incomplete vaccination coverage, especially among older individuals, a global increase in travel aspirations, and a relaxation of preventive measures such as physical distancing and mask-wearing. As per the influenza virus, according to the World Health Organization (WHO), the yearly worldwide incidence of influenza affects around 20% to 30% of children and up to 10% of adults.[14] The estimated annual death toll from influenza is approximately 290,000 to 650,000.[15] These factors contribute significantly to the global burden on health, medicine, and the economy.[16,17]

If vaccination and drug treatment efforts are not sufficient, implementing a strategy to reduce the transmission of respiratory viruses becomes extremely valuable. Influenza virus is well recognized to be airborne transmitted.[18] More recently, world health authorities have also recognized the airborne transmission of SARS-CoV-2 [4,19] based on compelling experimental and epidemiological evidence.[20–22] Virus-laden airway fluid (greater than 5μ m in diameter) or aerosol (smaller than 5 μm) can travel several meters and can remain for hours.[23] Tidal breathing has been shown to generate particles predominantly sized between 0.300–0.499 μM.[24] The primary challenge lies in effectively controlling the airborne transmission of respiratory viruses to prevent further infections.

In recent years, the use of electromagnetism for pathogenic virus-inactivation has gained increasing attention.[25–27] Compared to traditional methods of pathogen inactivation, electromagnetic wave radiation can rapidly and uniformly penetrate microorganisms, causing resonance. We recently described the inactivation of the Wuhan variant of SARS-CoV-2 [28] by the use of the confined acoustic dipolar mode of resonance using microwaves of the same frequency.[26,29] Through an experimental *in vitro* model, the resonant energy transfer effect from microwaves to the virus was demonstrated, effectively inactivating the Wuhan variant of SARS-CoV-2 in aerosols. Importantly, the microwave power density used in this technique remains well within the safety limits set by worldwide regulatory agencies.

The mechanisms underlying virus inactivation through electromagnetic waves is tied to the virus type, frequency, time of exposure to the waves used. Therefore, we investigated whether the technology would work on different SARS-CoV-2 strains and other respiratory viruses, such as the human influenza virus. We selected the most common variants of SARS-CoV-2, namely the *delta*

and the *omicron* variants, and the most common influenza virus, namely H1N1.

2. Methods

2.1. CELLS AND VIRUS CULTURE PRODUCTION

SARS-CoV-2 cultures were handled in a BSL-3 laboratory at ViroStatics facilities located at the Scientific and Technological Park Porto Conte Ricerche srl (Alghero, Italy). Vero E6 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Biowest, Nuaillé, France), 1% antibiotic solution penicillin/streptomycin (Biowest, Nuaillé, France), 1% L-glutamine (Biowest, Nuaillé, France), i.e., complete medium, at 37 °C with 5% CO2. The human 2019-nCoV strain 2019-nCoV/Italy-INMI1, isolated in Italy (ex-China) from a sample collected on 29 January 2020, was provided by the Istituto Lazzaro Spallanzani, Rome, Italy.[30] *Delta* variant B.1.167.2 and *omicron* variant BA.1 were kindly provided by the Department of Molecular and Translational Medicine, Section of Microbiology and Virology, University of Brescia Medical School, Brescia, Italy.[31–33] Each virus was propagated in Vero E6 cells as described above to obtain high titer virus (>1 × 10^6 $TCID_{50}$/mL) and was stored at −80 °C until use. Tissue culture infectious dose ($TCID_{50}$) is defined as the dilution of a virus required to infect 50% of a given cell culture. Similarly, influenza virus (Influenza A 1 H1N1 PR/8/34, ZeptoMetrix) was cultured in complete medium in MDCK (Madin-Darby canine kidney) cells. Experiments were considered valid if the titer of the virus in the absence of treatment was >1 × 10^3 $TCID_{50}$/mL for the Wuhan and the H1N1 strains and >1 × 10^4 $TCID_{50}$/mL for the *delta* and *omicron* strains, due to the fact that the latter strains did not propagate as efficiently in the Vero E6 cells as the former ones.

2.2. BIOAEROSOL TESTS

The virucidal activity of microwaves produced by a Radio Frequency (RF) generator was evaluated against the SARS-CoV-2 Wuhan, *delta* and *omicron* strains in an *in vitro* bioaerosol experimental system by measuring the replicative capacity of the virus treated with microwaves compared to untreated virus. An aerosol containing the virus was introduced in a plastic, air-proof container (0.5 L volume) and particles with a size up to 10 µm were generated with a commercially available aerosol generator (Omron, Kyoto, Japan). A viral suspension of the infectious SARS-CoV-2 was used to generate the aerosol. High titer virus (1 × 10^6 $TCID_{50}$/mL for Wuhan variant, 1 x 10^5 $TCID_{50}$/mL for *delta* and *omicron* variants) was obtained from infected cultures of Vero E6 cells. Similarly, a viral suspension of influenza virus was used to generate aerosol. High titer virus (1 x 10^5 $TCID_{50}$/mL) was obtained from infected cultures of MDCK cells. During the test, the chamber containing the infected aerosol was subjected to irradiation by an electromagnetic signal at the appropriate frequency (that is, the speed of the wave or the distance between the start and end of each wave) and amplitude (represented by the height of the wave) to maximize the reduction of the viral load. The RF generator was controlled with a software application installed in a mobile device. The treated aerosol was then recovered by means of active impingement directly in complete medium with 2% serum contained in a special glass collector with an inlet and tangential nozzles, through which air from the plastic chamber was sucked when vacuum was applied (at 12 L/min). Viral particles were absorbed in this medium and then cultured in the laboratory for viral titer determination. The use of this system allowed us to consistently recover 5% of the aerosolized virus. The control virus was processed in the same experimental conditions with the device turned off. The replicative capacity (i.e., viral titer) of the virus treated with microwaves (compared to untreated virus) was determined in an *in vitro* system. Vero E6 cells (for SARS-CoV-2 tests) or MDCK cells (for influenza virus tests), were seeded at a density of 20,000 cells/well into a 96-well plate in complete medium at 37 °C and 5% CO2. Twenty-four hours after seeding the cells reached ~90% confluency; the fetal bovine serum concentration was decreased to 2% to avoid interference with the viral infection. The cells were then infected with 10 fold serial dilutions (8 replicates for each dilution) of the viral suspensions obtained after the impingement procedures for aerosol collection and control. The cells were then cultured for seventy-two hours, and infection was determined by observing the cytopathic effect under an inverted microscope. The viral titer of the recovered virus was determined according to the Reed and Muench method and expressed as $TCID_{50}$/mL.[34]

2.3. BIOAEROSOL APPARATUS

The analytical formulation and RF Generator Block (and its use) have been described in Manna *et al.*[28] Unless otherwise indicated, viruses were exposed to the following conditions: frequency = 8 to 10 GHz; electromagnetic field amplitude = 6 V/m; step = 10 MHz; dwell = 3.2 seconds; time = 10 minutes.

3. Results

3.1. BIOAEROSOL TESTS SETUP

The aerosol system apparatus was set as shown in Figure 1. Under a class II biosafety cabinet in a Biosafety Level 3 laboratory, an aerosolized virus

sample was generated by the bioaerosol generator and transferred into the aerosol treatment chamber. Aerosol sample was the subjected to irradiation through the RF device and the treated aerosol sample was then collected by the impinger, connected to the vacuum pump.

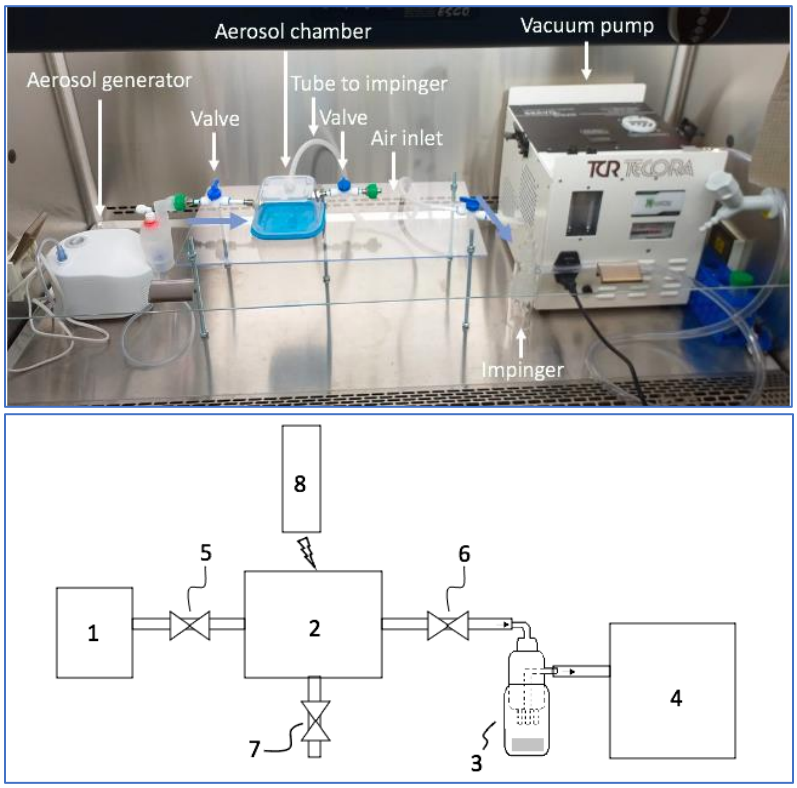

Figure 1. Upper part: assembled bioaerosol apparatus. Lower part: block diagram of the bioaerosol apparatus. *1: Aerosol generator; 2: aerosol treatment chamber; 3: sample collector; 4: vacuum pump; 5, 6, 7: valves; 8: radio-frequency device emitting electromagnetic signal*

To perform the inactivation tests on pathogens, an RF Generator Block was developed capable of generating and irradiating the different waveforms required by aerosol treatments.

The main element of the unit is the Analog Devices ADF4371 [35] integrated Ultra-Wideband (UWB) frequency synthesizer that includes a fractional-N and integer-N phase-locked loop (PLL), an external loop filter, an external reference frequency and a wideband microwave voltage oscillator (VCO) with a fast automatic center frequency calibration. The integrated circuit includes a x 2 frequency multiplier at the VCO output to generate the higher portion of the frequency band. To suppress the unwanted products of frequency multiplication, a harmonic filter was inserted between the multiplier and the output stage.

The frequency tuning of the component is set by a SPI interface. The output signal from the synthesiser is modulated in amplitude by UWB preamplifier, a UWB programmable digital attenuator and a High-Power GaN Amplifier (Figure 2). The final power amplifier of the demonstrator was developed using the latest 0.15 µm GaN on SiC solid-state High-Electron-Mobility Transistor technology and can deliver up to 10 W in a UWB.

The RF output of the transmitter was connected via an RF cable to a horn antenna capable of generating an appropriate electromagnetic field near the area to be sanitized.

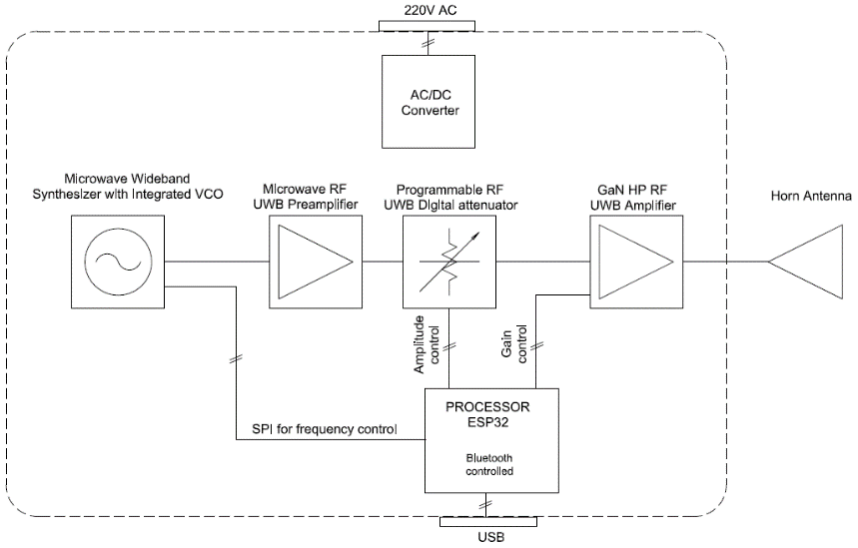

Figure 2. Block diagram of the RF UWB synthesiser

To set all the possible configurations of the RF components, an embedded software written in C++ was developed on the ESP32 platform using Visual Studio Code (https://code.visualstudio.com/).

For the correct operation of the demonstrator, an automatic test equipment was implemented using a Vivaldi UWB test antenna, placed in proximity of the area to be treated and connected to a spectrum analyzer.[28]

3.2. BIOAEROSOL TESTS OUTCOME

To evaluate the antiviral efficacy of the resonant effect, we first measured the residual viral infectivity of different variants SARS-CoV-2 after illumination with microwaves (Figure 3). Each viral sample was placed below the horn antenna and irradiated with a fixed frequency, electromagnetic field amplitude, step, dwell and time, as these conditions were already optimized.[28] The inactivation ratio was similar among the different SARS-CoV-2 variants, within a range of 80 to 90%

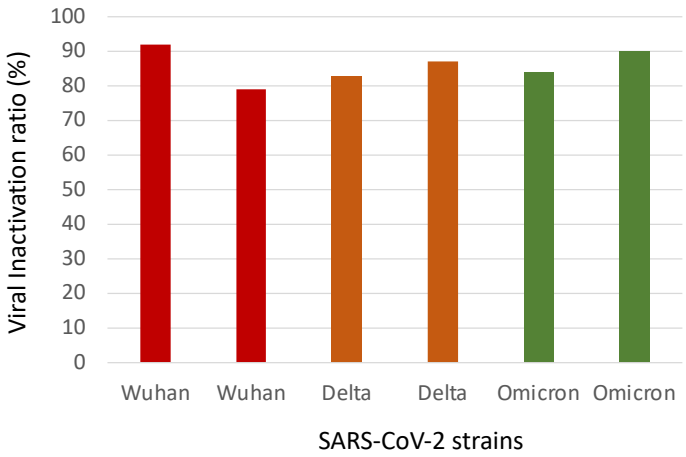

Figure 3. Inactivation ratio of different strains of SARS-CoV-2 exposed to microwaves. Similar inactivation ratios were observed with different SARS-CoV-2 strains, namely Wuhan (original strain), *delta* and *omicron*.

We then measured the residual viral infectivity of different virus, namely human influenza virus SARS-CoV-2, after illumination with microwaves. Unlike SARS-CoV-2 we had no previous reference regarding the optimal frequency to be used. We initially applied the same conditions used with SARS-CoV-2, that is 8 to 10 GHz (Figure 4, left), obtaining a viral inactivation ratio (around 90%, a 10 fold ratio), similar to SARS-CoV-2. Then, we gradually increased the frequency up to the 14 to 16 GHz range (Figure 4, right part).

The optimal inhibition range was between 8 and 16 GhZ, whereas the range previously observed with SARS-CoV-2 was 8 to 12 GHz.[28]

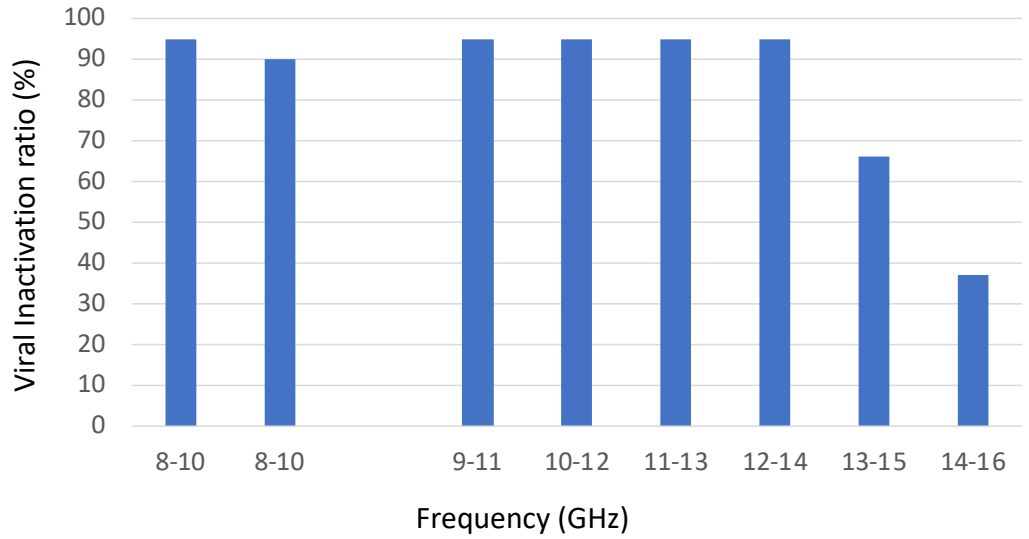

Figure 4 Inactivation ratio of human influenza virus exposed to microwaves at different frequencies.

We finally checked whether we could reduce the total time exposure. No loss in antiviral activity was observed when reducing the total time exposure to 5 minutes, whereas a slight yet progressive loss of activity was observed when the total time exposure was reduced to 3 and 1 minute, respectively (Figure 5).

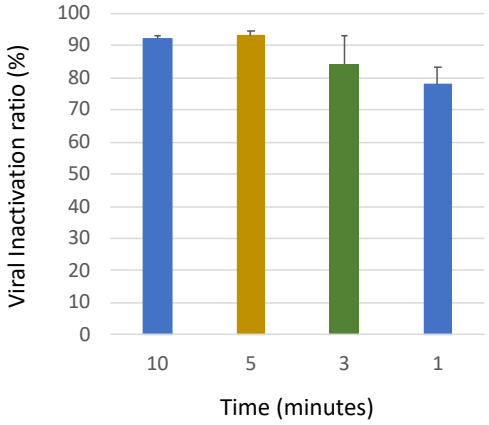

Figure 5. Effect of different time exposures on antiviral activity in the bioaerosol system. Virucidal activity was maintained when exposure time was reduced to 5 minutes

4. Discussion

The virus inactivation technology reported in this manuscript is based on the confined acoustic dipolar mode of resonance with radiated microwaves of the same frequency.[26] Radiation microwaves bear the potential to inactivate airborne viruses,[29] however, our understanding of the intricate mechanisms involved in the inactivation of viruses when exposed to electromagnetic waves remains to be fully investigated, particularly regarding the interplay of the virus type, the specific frequency and the power of the waves. These factors are crucial in determining the effectiveness of electromagnetic wave-based inactivation strategies. In a previous manuscript, we began shedding light on this intricate relationship. By focusing on the Wuhan variant of SARS-CoV-2, the initial causative agent of the global COVID-19 pandemic, we identified the microwave resonant frequencies and other critical conditions that exhibit potent antiviral effects.[28]

The inactivation of viruses through electromagnetic waves, however, is a complex process that may vary depending on factors such as the specific virus type, the frequency of the waves employed, and the duration of exposure. To further explore the potential of this phenomenon, we conducted additional tests involving different viruses. In this study we utilized an RF generator to investigate the effects of electromagnetic waves on different variants of SARS-CoV-2, namely the *delta* and *omicron* variants, which have gained significant attention due to their increased transmissibility. By the use of RF waves, we achieved a reduction of

around 80-90% (that is approximately 10 fold) in the infectivity of these highly transmissible variants. This result was achieved by conducting experiments under similar conditions to those used in our initial investigations,[28] thus ensuring the reliability and consistency of our results. The confirmation of the virucidal effect of RF waves on the *delta* and *omicron* variants reinforces the potential of this approach as a viable solution in mitigating the spread of these highly contagious strains of SARS-CoV-2.

Furthermore, we expanded our investigation to include another prevalent airborne virus, the H1N1 human influenza virus. By subjecting this virus to the same RF wave exposure, we wanted to determine if the observed virucidal effect could be extended beyond SARS-CoV-2. We discovered that the rate of reduction in infectivity for the influenza virus was comparable to the one observed with SARS-CoV-2 under similar experimental conditions. However, the influenza virus also displayed distinctive response patterns in comparison to SARS-CoV-2. For example, the influenza virus was susceptible to higher frequencies, up to 16 GHz, whereas SARS-CoV-2 exhibited sensitivity up to 12 GHz.[28] Moreover, our investigation revealed that the duration of exposure to radiated microwaves plays a crucial role in achieving viral inactivation for the influenza virus. Unlike SARS-CoV-2, which demonstrated a 10 fold reduction in infectivity within a shorter exposure period (down to 1 minute), the influenza virus required a longer exposure time of 5 minutes to attain the same level of inactivation. This result highlights the importance of considering exposure time and frequencies as crucial parameters when designing and implementing microwave-based inactivation strategies against different viral strains.

To optimize the effectiveness of radiated microwaves in viral inactivation additional fine-tuning studies are warranted. These future investigations will enable us to gain a more comprehensive understanding of the optimal frequencies and duration of exposure required to achieve maximal virucidal effects for both SARS-CoV-2 and the human influenza virus. Through systematic exploration of these parameters, we aim to establish a precise framework for utilizing radiated microwaves as a tool for combating a wide range of airborne viruses, thereby contributing to the development of effective sanitization strategies and public health interventions.

The primary mode of transmitting respiratory viruses is through the inhalation of aerosol and droplets released by infected individuals. The dispersion of these droplets is influenced by their size and the surrounding environment. The findings of our study were derived from experiments conducted within a controlled laboratory environment, specifically designed to replicate the conditions encountered during natural transmission of airborne viruses. By introducing aerosol particles with diameters of up to 10 µm, we aimed to simulate the realistic scenarios in which these viruses are typically dispersed.

It is important to note that this virucidal effect was achieved using an RF-wave emission system that operates within safe power levels according to the Specific Absorption Rate (SAR) standards and specifications set by the United States, Canada, Europe, and Japan. This means that the method we propose for human environment sanitization is considered safe for people. This presents the opportunity to evaluate the application of electromagnetic wave-based devices in confined spaces, either through the installation of a stationary device capable of covering a substantial area, such as a 50 m2 room, or by utilizing a portable device with a shorter coverage range, typically around 3 meters, in any given space.

Our results provide valuable insights into the applicability of electromagnetic wave-based inactivation techniques across a range of viral strains. By targeting multiple variants of SARS-CoV-2 and extending our investigation to encompass other common airborne viruses like H1N1, we contribute to the growing body of knowledge surrounding the effectiveness of electromagnetic waves as antiviral strategy. Understanding how different virus types respond to varying frequencies and power levels, as well as how the composition of the viral growth medium influences the efficacy of electromagnetic wave-based inactivation, will be pivotal in developing comprehensive and targeted antiviral strategies. By delving deeper into these issues, we can unravel the underlying mechanisms behind electromagnetic wave-induced viral inactivation. This will enable us to optimize the parameters involved, such as the specific frequencies and power levels required, and develop a more nuanced understanding of the factors that contribute to the successful inactivation of viruses.

5. Conclusions

Respiratory viruses pose an ongoing healthcare challenge and a persistent risk to the global economy. Among these viruses, SARS-CoV-2 and the influenza virus have established themselves as endemic, recurring at regular intervals. We have

devised a methodology that enables the inactivation of these viruses suspended in aerosols using radiated microwaves. Given that the radio frequency wave emission system we employed adheres to safe power levels in accordance with the specific absorption rate standards and regulations established by the majority of countries globally, it is justifiable to conduct testing in both confined and open environments. This exploration aims to identify devices suitable for daily use that can complement the safety precautions currently employed, with the objective of further enhancing safety measures.

Conflicts of Interest Statement

A. Manna, M. Bartocci, N. Pasculli are employees of Elettronica S.p.A., sponsoring the study
A. Sangiovanni-Vincentelli receives consulting fees from Elettronica S.p.A., sponsoring the study
D. De Forni, B. Poddesu, F. Lori are employees of ViroStatics S.r.l., retained for services by Elettronica S.p.A., sponsoring the study

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