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PUBTEXTO

Investigation the Effect of NA2SIO3/Sio2/Tio2 Nanocomposites against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) Measured Using Plaque Assay

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Abstract

COVID-19 is an ongoing pandemic that lacks effective therapeutic interventions. The causal agent of coronavirus disease 2019 has been discovered as the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). We now face a harsh reality: an exponential surge of infection means that we can go from 100 infected people to 10,000 infected people in a matter of days. There are urgent challenges facing us, as it has become crystal clear that achieving recovery on a large scale will not come without ending the health crisis, and obtaining a vaccine is the key to achieving both goals. Remarkable progress has been made on the vaccine front. However, the inequity in the production and distribution of vaccines not only leaves countless millions of people at the mercy of the virus, but also gives way to the emergence of deadly mutations with a deadly impact on the entire world. Due to the difficulties in creating vaccines in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic conditions, the development and testing of new antiviral medicines is urgently required. Given the practical importance of microbicides having efficacy against SARS-CoV-2 in home, community, and health care settings, we report evidence of the virucidal efficacy of a number of formulated microbicidal actives against SARS-CoV-2, as evaluated using Plaque assay standards.

Keywords: SARS-CoV-2; COVID-19

Introduction

Corona viruses are a large family of viruses known to cause illnesses ranging from the common cold to more severe diseases, such as Middle East Respiratory Syndrome (MERS) and severe acute respiratory syndrome (SARS) [1]. A new coronavirus was identified in 2019, in Wuhan, China [2]. This virus is a new strain that has not been previously identified in humans. Because many of the symptoms of COVID-19 are like those of the flu, colds, and other illnesses, getting tested for COVID-19 is critical [3]. Symptoms can emerge anywhere from two to fourteen days after being exposed to the virus, and they can range from mild to severe. Some patients have no symptoms at all. So far, evidence suggests that the virus is transferred mostly between persons who are very close together, frequently within one meter of each other [4]. Inhaling virus-carrying aerosols or droplets, or encountering the eyes, nose, or mouth, might cause infection. The virus can also spread in crowded, poorly ventilated, or bivalent environments where people spend prolonged periods of time. Indoor surroundings, particularly those with insufficient ventilation, are more hazardous than outside environments [5].

The substructural morphology of coronaviruses is depicted in figure 1 from the Centers for Disease Control and Prevention (CDC) [6]. When seen electron microscopically, the protrusions embellishing the outside of the virus give the appearance of a halo surrounding the virion. E, S, and M protein particles, which were also present on the particle's outer surface, were seen as a threat in this interpretation [7].

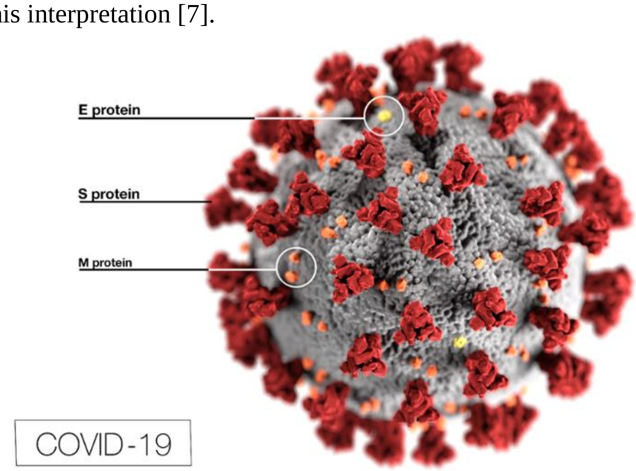

Figure 1: *Ultrastructural morphology exhibited by coronaviruses [6].*

The structural structure of the Corona virus consists of a protein membrane with a diameter of 50-200 nm, and encapsulating the DNA of the virus, RNA, and like other coronaviruses, the virus consists of four types of structural proteins that contribute to the formation of the structure of the virus body, including the protein (S), which forms the protrusions The spines on the surface of the virus give it the characteristic coronal shape. According to the sequencing of the new Corona virus genome, it was found that the RNA molecule consists of about 30,000 bases containing 15 genes, including the "S" gene, which codes for a protein found on the surface of the viral envelope [8].

The virus used the S protein to infect a cell, which binds to receptors on the membrane of the host cell - the lung cells - like a key to a lock, allowing the virus to enter the cell. This binding is a crucial phase in the infection. The virus's ability to infect different types of hosts is determined by the mechanism of that relationship [9].

The development of safe and effective vaccines against COVID-19 is a major step forward in our global effort to end the pandemic. Vaccines function by imitating a disease-causing agent, such as viruses, bacteria, or other microbes. Our immune system is 'taught' to respond rapidly and efficiently to the infectious pathogen in this simulation. If an intruder enters the body, the immune system is prompted to recognize it and make antibodies to learn how to combat it. Vaccines are unlikely to make you very sick, but a small percentage of people who take them may experience long-term side effects such as arm inflammation or a high fever [10].

Despite the great success in developing vaccines against the Corona virus, the world is facing a great challenge, which is the unfair distribution of vaccines between countries, which led to the emergence of successive waves of the emerging Corona virus [11]. On the other hand, many vaccines face wide criticism because of their cause of blood clots and very dangerous side effects [12]. This prospective study was designed to investigate a new method, where a substance consisting of NA2SIO3/SiO2/TiO2 nanocomposites to eliminate the Corona virus in a natural physical field without the need to inject the patient, and thus the Corona virus is eliminated within 30 minutes without any side effects, and this method has been tested using Plaque assay. This is the first study to eliminate the Corona virus naturally using NA2SIO3/SiO2/TiO2 nanocomposites.

Related Work

Using internationally recognized standards, Ijaz et al., (2020) assess the efficacy of virucidal for personal care and surface cleaning and disinfection products against SARS-CoV-2. Laboratory investigations looked at the active ingredients of well-known and widely used disinfectant liquids, hand sanitizers, hand washes, disinfectant sprays, personal care and surface wipes, bar soap, and surface cleaners (Dettol and Lysol) hand sanitizer, hand wash, disinfectant spray, personal care and surface wipes, bar soap, surface cleanser, and laundry sanitizer are some of the products available. The investigation discovered that all microbicidal actives were efficient at inactivating SARS-CoV-2, with a reduction of infectious virus within 1 to 5 minutes [13].

It is noticeable on Ijaz et al.'s study that it only took external sterilization tools, which eliminated the virus when infected, on the other hand, Assis et al.'s (2020) examined the efficacy of SiO2-Ag composite as a highly virucidal material to that rapidly eliminates SARS-CoV-2. The as-fabricated samples have strong antibacterial activity against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus), as well as SARS-CoV-2, according to the results. They offer a probable mechanism to explain the augmentation of biocidal activity based on the current findings and radical scavenger studies [14].

It is critical to guarantee that viruses in samples have been inactivated on site prior to processing in order to permit SARS-CoV-2 point-of-care (POC) testing. Van Bockel et al.'s (2020) tested the virucidal activity of commercially available viral transport medium (VTMs) to inactivate SARS-CoV-2. To test for viral inhibition, Van Bockel et al.'s used a variety of methodologies, including the previously reported WHO procedure and a buffer exchange method, in which the virus is physically separated from the VTM following exposure [15]. When incubated with a VTM, the latter approach allows for sensitive measurement of virus vitality at a greater viral titer. The combination of VTM, Primestore® Molecular Transport Medium (MTM), and COPAN eNATTM totally inactivates high titer SARS-CoV-2 viruses (>1 107 copies/ml) and is suitable with POC treatment, according to their findings [15]. They concluded that adding some VTM constructs as a first step after sample collection will render SARS-CoV-2 non-infectious for transfer or further POC molecular testing in the field using GeneXpert rapid-transformation platforms or equivalent will render SARS-CoV-2 non-infectious for transfer or further POC molecular testing in the field using GeneXpert rapid-transformation platforms or equivalent.

Therefore, the current research suggests the use of NA2SIO3/SiO2/TiO2 nanocomposites helps reduce the viral load of the SARS-CoV-2 disease. This treatment is designed to work against all beta coronaviruses such as the original SARS-CoV-1 as well as SARS-CoV-2 and any new variants that may emerge in the future because it targets highly protected regions of the virus genome.

Research Methods

This study uses Plaque infectivity assay [16] to evaluate NA2SIO3/SiO2/TiO2 nanocomposites against SARS-CoV-2. Briefly, six-well tissue culture plates were seeded with Vero-E6 cells (105cells/well). At 90–100% confluence (one day post-seeding), the cells were washed twice with 1X PBS. Control and treated HCoV-19/Egypt/NRC-1/2020 virus samples were diluted 10-fold in medium with 2% fetal bovine serum (FBS) (Lonza, USA), 2% Antibiotic-Antimycotic Mixture (Lonza, Walkersville. MD, USA) and 100 µl of from each dilution were mixed with 400 µl infection medium to inoculate into Vero-E6 cells. The plates were incubated at 37°C with 5% CO2 for 1 hr. The wells were then aspirated to remove residual inoculum. Each well was then immediately covered with 2 ml of overlay contain 2X medium and 2% agaros type 1 (Lonza, Basel, Switzerland) with 1:1. Plates were then incubated at 37°C with 5% CO2 for 2 days. The formation of the plaques was microscopically observed daily. Once clear plaques could be visualized, 1 ml of 10% formaldehyde was added to each well for 2 hrs at room temperature for cell fixation and virus inactivation. The formaldehyde was then discarded, and the plates rinsed with water and dried. For visualization of the plaques, 1 ml of the staining solution, consisting of 1% crystal violet and 20% methanol in distilled water, was added to each well and incubated at room temperature for 5 min, the dye was then discarded. Viral plaques were then counted, and virus titer was calculated through the following equation:

Plaque forming unit (PFU)/ml = Number of plaques × virus dilution × volume of inoculum x multiplicand number to complete the inoculum volume to one ml.

% inhibition= viral count (untreated) - viral count (treated)/viral count (untreated) x 100

Results

The results demonstrated a reduction of 98.2 percent in viral load at 0 cm distance after direct exposure of SARS-CoV2 virus stock to activated NA2SIO3/SiO2/TiO2 nanocomposites liquid form for 30 minutes. The antiviral impact gradually declines to 70% at 10 cm and 11.7 at 50 cm from the active NA2SIO3/SiO2/TiO2 nanocomposites liquid form as shown in table1.

Table1: *Antiviral activity against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) measured using Plaque assay.*

Sample	Contact Distance	Virus control (PFU/ml)	Virus after exposure to NA2SIO3/SiO2/TiO2 nanocomposites (PFU/ml)	Viral Inhibition %
NA2SIO3/SiO2/TiO2 nanocomposites	Direct (0 cm)	$1.7X10^6$	$3X10^3$	98.23
	10 cm		$5X10^5$	70.5
	50 cm		$1.5X10^6$	11.7

The results also showed that these nanoparticles are stable for more than six months at normal room temperature, implying that this agent can be used in low-resource settings to effectively treat infected patients, despite the fact that no human experiments have been conducted and the given results were obtained from the Vaccine Development and Virological Tests Unit appendix (A).

Discussion

It is known that nanomaterials that can be used require conditions, the most important of which is high purity, and the materials used for this purpose must be biocompatible. In the absence of this condition, we will need a mechanism to expel the used substance and exclude it from the human body shortly after eliminating the virus [17-18]. This process may cause serious damage. One of the current research's accomplishments is that the NA2SIO3/SiO2/TiO2 nanoparticles can eradicate Covid-19 in a natural physical field without requiring the patient to inject or inhale these compounds, ensuring that the patient is not exposed to any side effects as demonstrated by our previous research [19-20].

These findings suggest that in general that the resistance of these NA2SIO3/SiO2/TiO2 nanoparticles to types of bacteria and viruses, so it is possible to inoculate fabrics with these nanoparticles or use them as nanofibers such as those worn by health staff for protection, such as head coverings, body, shoes, and respirators, or use them as surface coatings, making them self-sterilization.

Conclusion

Covid-19 disease caused by infection with the emerging corona virus has claimed the lives of many people around the world. In the face of the high number of infections, efforts are intensifying in the manufacture of vaccines, and the use of antiviral drugs, and despite the success of these vaccines, there are still concerns about their side effects in the future. On the other hand, nanotechnology has contributed to confronting the emerging pandemic of the Corona virus (Covid 19), by limiting its epidemic spread in several areas, including treatment, diagnosis, and prevention. The main goal of the current study was to determine whether the NA2SIO3/SiO2/TiO2 nanocomposites are effective against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through a physical field. Plaque infectivity assay method was used at the Vaccine Development and Virological Tests Unit, Egypt appendix (A). One of the more significant findings to emerge from this study is that a reduction of 98.2 percent in viral load at 0 cm distance after direct exposure of SARS-CoV2 virus stock to activated NA2SIO3/SiO2/TiO2 nanocomposites liquid form for 30 minutes. The antiviral effect gradually decreases to 70% at 10 cm and 11.7 at 50 cm from the activated NA2SIO3/SiO2/TiO2 nanocomposites.

Appendix A

مركز التميز العلمي لفيروسات الأنفلونزا
Center of Scientific Excellence for Influenza Viruses

وحدة تطوير اللقاحات والاختبارات الفيروسية
Vaccine Development and Virological Tests Unit

المركز القومي للبحوث
National Research Centre

Name of Sample Owner: Hashem Yehya

Product Name: HST

Antiviral activity against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) measured using Plaque assay.

Sample	Contact Distance	Virus control (PFU/ml)	Virus after exposure to HST (PFU/ml)	Viral Inhibition %
HST	Direct (0 cm)	$1.7X10^6$	$3X10^3$	98.23
	10 cm		$5X10^5$	70.5
	50 cm		$1.5X10^6$	11.7

Conclusion

After direct exposure of SARS-CoV2 virus stock to activated HST liquid form at a contact time of 30 min, the results showed a reduction of 98.2% in viral load at 0 cm distance and the antiviral effect decreases gradually to 70% at 10 cm and 11.7 at 50 cm from the activated HST.

Signature

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