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Research article

Nebulization of pharmacological solutions with an innovative medical device based on microvaporization

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ABSTRACT

The currently available nebulization devices have a slow aerosol flow and produce vapor with large microdrops. Improved devices that achieve higher airflow and produce smaller microdrops are needed to improve the clinical care of patients. To address this critical need, we developed a novel system for the molecular vaporization of liquids. This device vaporizes an active pharmacological substance dissolved in water, alcohol, or a mixture of water and alcohol using two energy sources at the same time: high-frequency ultrasound and thermal induction. Application of energy to a solution contained in the device's tank allows, within tens of seconds, for the vaporization of the solution itself, with the generation of a vapor consisting of microdrops of very small diameter (0.2–0.3 μm). In this article, we illustrate the technology used, the main verification tests performed, and the primary fields of application for this device. In particular, the advantages of both the aerosol delivery system and the administration system are highlighted.

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1. Introduction

Aerosolization techniques for delivering compounds to humans date back at least 4,000 years, originating, likely, in India, with smoking and datura preparation. The use of aerosols for medical therapy—in particular, for inhalation of air draws through infusion of plants and other ingredients—became popular in the eighteenth and nineteenth centuries [1]. There are three main types of devices used to administer inhaled medications: pressurized metered dose inhalers, dry powder inhalers, and light mist inhalers. Of course, each type of inhaler device has advantages and limitations that dictate its suitability for a particular patient [2]. Substantial technological advances have been made in the last 30 years to improve the nebulization of liquids into particles, including efforts to reduce the size of nebulized particles and avoid condensation of nebulized drops [3]. However, the aerosol flow in the currently available devices is often slow, and the vapor produced comprises large microdrops with a tendency to condense and be retained in the upper respiratory tract—therefore, new devices that overcome these limitations are needed. In particular, the care of unconscious or intubated patients would benefit greatly from the availability of better systems of delivery. Improved aerosolization of compounds into smaller particles would allow delivery of low concentrations of therapeutic agents directly to the alveoli, minimizing the incidence of blood stream–related systemic side effects. With the goal of increasing the speed of aerosol flow and reducing the size of particles to 0.2–0.3 μm in diameter, we developed a novel device for microvaporization of liquid media. The novel device described and used in this paper is proven to be innovative and particularly effective in improving the therapeutic success of inhalation therapy by promoting a correct inhalation technique and enhancing compliance and associated treatment outcomes.

2. Materials and methods

The device was designed by Bruno Brandimarte and built by Ginevri (Rome, Italy; https://www.ginevri.com) and DAV Electronics (Twickenham, UK; https://davelectronics.com). It has been granted an international patent (#11910772).

The device integrates high-frequency ultrasound energy (3 MHz) and energy density (12 W/cm^2) with low thermic energy and thus avoids the need for heat-induced vaporization of the solution [4]. The combination of these energy sources induces sublimation of the compound into microdrops 0.2–0.3 μm in diameter. The resultant compound is highly similar to a gas but does not undergo recondensation. Airflow delivered by a micro-turbopump mixes with the gas, producing a positive pressure. This process is much faster than that for common aerosols (in terms of seconds) and is electronically controlled by a microprocessor. The device is therefore (1) highly versatile, (2) able to interact with different platforms, and [5] capable of delivering medications at physiological temperature through a gas medium.

Models of three different sizes have been developed, with three reservoirs (5 cc, 10 cc, and 30 cc) for different experimental uses.

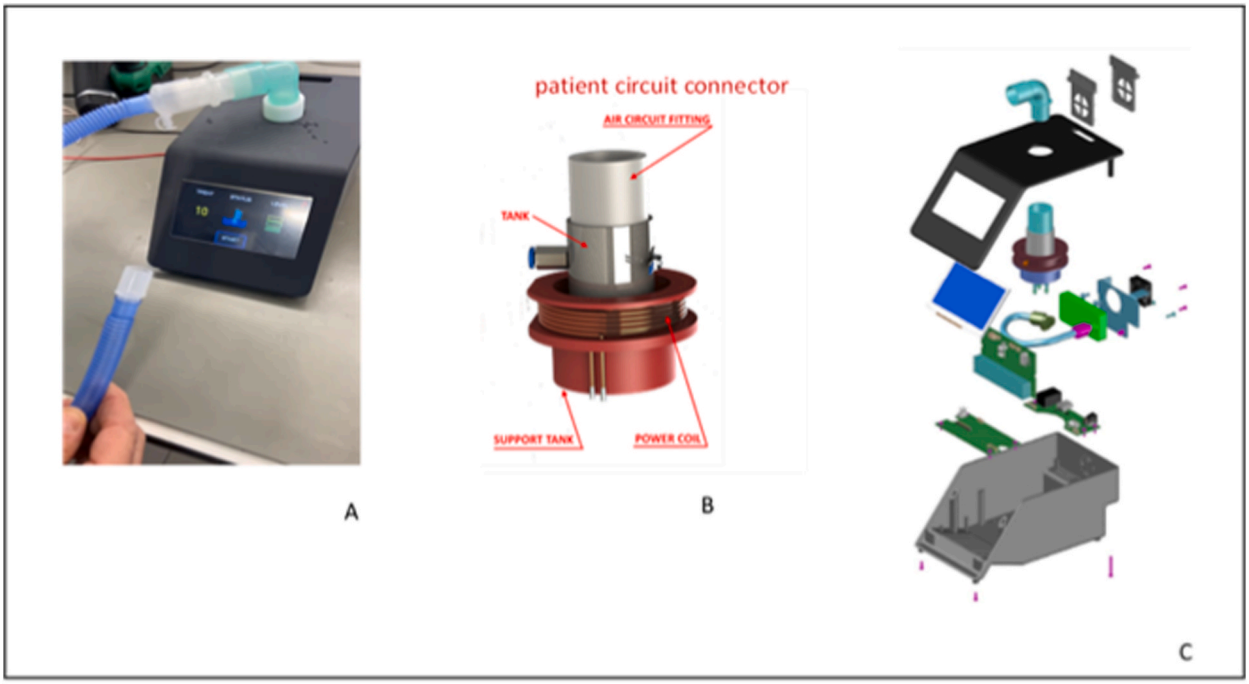

Fig. 1. (A) The new drug delivery device in operation. **(B)** The tank with inductor. **(C)** An expanded view of the device.

2.1. Operating principle

The operating principle of the device consists in the production of microdrops using of high-frequency ultrasound energy (3 MHz). Unlike the other devices, this device allows the microdrop productions using mechanical vibration at an ultrasonic frequency and not by boiling the solution through heat transfer. The vibratory cavitation approach leaves unaltered the solution vaporized, while boiling causes the separation of the solvent from the solute. Moreover it is possible to establish the drop diameter by varying the geometric dimensions of the vibratory turbulence, which in turn is connected to ultrasound wavelength used to vaporize the solution. For example using a frequency of 3 MHz, 12 W/cm^2 of energy provides a microdops diameter of 200–300 nm. The ratio between wavelength and frequency is inversely proportional, for example, reducing the frequency at 1.8 MHz causes an wavelength increases to 300–400 nm. The wavelength also increases with the density of the solution. This means that increasing the density of the solution will need to increase the wavelenght and therefore as a consequence the microdrops will have a greater diameter. Nebulized products were evaluated using the Spraytec instrument (Malvern Panalytical, Malvern, UK), which can assess the size of atomized or nebulized particles in a gaseous medium. The instrument uses laser diffraction to measure the intensity of light scattered as a laser beam passes through a dispersed particulate sample. The novel vaporization system is equipped with a suction device that directs the atomized particles and linearizes the stream, making the produced particles highly homogeneous. The solutions to be vaporized and used in the tests were selected on the basis of their different densities and viscosities: main device only, 5% glucose; main device + tube, 10% glucose; main device + tube, liposomes; main device only, liposomes. Tests were performed using the different colloidal solutions and dispersions. The methodology for assessment is able to detect both the mass of the product and the dimensions of the microbubbles produced to a limit of 0.1 μm; therefore, this methodology is not able to measure molecular particles, which the atomization product very likely contains. On the basis of recorded measurements, the vast majority of particles produced by the molecular vaporizer were <1 μm–0.1 μm (90 nm) in size, with some microdrops 4–5 μm in diameter.

2.2. Component parts

The core of the device consists of a tank (Fig. 1A–B), to which a ceramic ultrasonic transducer (3 MHz) is applied. A solenoid provides induction heat and is powered by an electric power source (Fig. 1B–C).

2.3. In vitro test

African green monkey kidney Vero E6 cells (Sigma-Aldrich, Merck, #CB_84113001) were maintained in Dulbecco's Modified Eagle Medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher

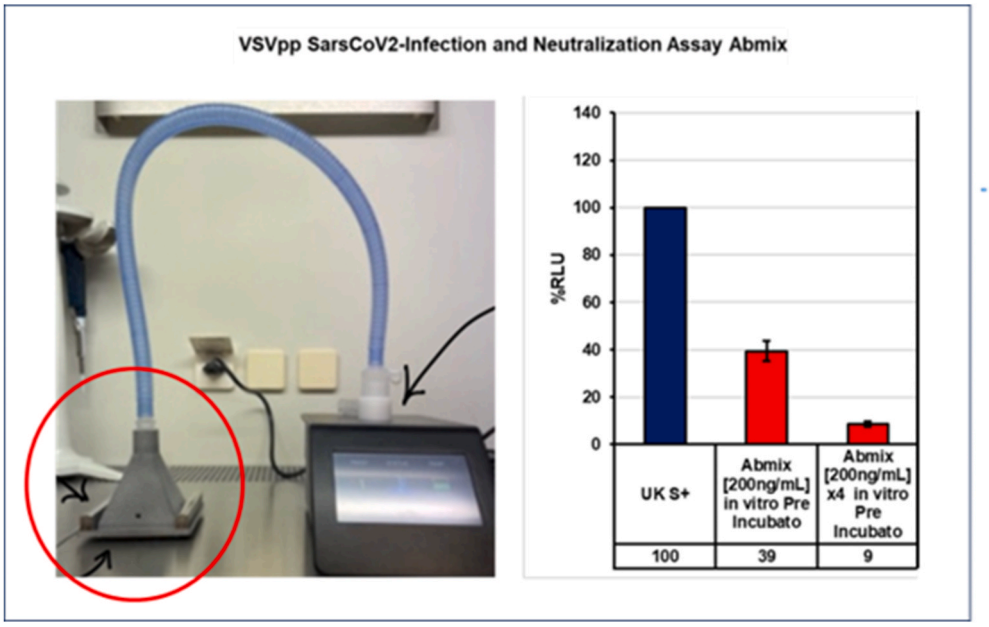

Fig. 2. (A) The device was adapted for use on a 4-well cell-culture plate, as indicated within the red circle. **(B)** A pseudovirus neutralizing assay was used to assess delivery of the neutralizing tetravalent Fab-IgG antibody 15033–7 at two different concentrations. VeroE6 cells were inoculated with pseudotype particles bearing the S proteins of the B.1.1.7 variant of SARS-CoV-2. Transduction efficiency was quantified by measuring virus-encoded luciferase activity in cell lysates 48 h after transduction and was expressed as a percentage. Data presented are the average from three biological replicates. UK S+ corresponds to VeroE6 cells infected with B.1.1.7 variant of SARS-CoV-2, while the other two samples are VeroE6 cells infected with the virus and treated by two different concentrations of neutralizing tetravalent antibody 15033–7.

Scientific) at 37 °C in a humidified atmosphere of 5% CO_2. We applied 200 ng/mL and 800 ng/mL of vaporized neutralizing tetravalent antibody (15033–7)—generated by protein engineering, as described previously [6]—to 1.2×10^5 cells/well in a 4-well plate that had been successively infected with a pseudovirus carrying the SARS-CoV-2 S protein variant (B.1.1.7). For pseudotyping, we used a replication-deficient vesicular stomatitis virus (VSV) vector that lacks the genetic information for VSV-G and instead codes for two reporter proteins, enhanced green florescent protein and firefly luciferase (VSV*DGFluc). This system accurately mimics key aspects of the entry of SARS-CoV-2 into cells. Vero E6 cells were transfected with a preincubated mixture of peptide and virus, as previously described [7]. The efficiency of virus entry for both protocols was specifically quantified by performing a luciferase assay. Each value was normalized relative to VSVpp.SARS-CoV2 pseudovirus alone (100%). For *in vitro* testing, we adapted the device as described in Fig. 2A.

2.4. In vivo test

A radiolabeled technetium-99 (^{99m}Tc)–based solution was vaporized by the device and aspirated by a 55-year-old healthy male volunteer without a history of pulmonary or upper airway diseases. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the institutional review board at Azienda Ospedaliera dell'Università di Palermo (IRB #3/22). The subject inhaled the aerosol through a mouthpiece for 20 s. Metastable ^{99m}Tc–labeled diethylene-triamino-pentacetic acid (DTPA) was used at a concentration of 4 mCi/mL (total amount, 24 mCi in 6 mL). Six milliliters of ^{99m}Tc (total amount, 24 mCi) was added to the DTPA vial and mixed until the contents were dissolved. After 30 min of incubation at 25 °C, radiolabeled DTPA was nebulized using the novel device and administered via nasal cannula. The total amount administered to the healthy volunteer was ~1 mCi. Images were obtained immediately after inhalation by planar acquisitions with a 256 × 256 matrix and 360° tomographic acquisition using a Millennium MG gamma camera (General Electric, Milwaukee, USA) equipped with low-energy high-resolution collimators.

3. Results

To study the delivery of the aerosolized solution into the human bronchial tree, the deposition of ^{99m}Tc administered via throat inhaler was imaged by autoradiography. ^{99m}Tc was not detected in the pharynx or in the trachea; rather, it was detected primarily in the pulmonary alveoli, as shown in Fig. 3. Gamma camera scans revealed tracer in the deep areas of the lungs, reaching the posterior costophrenic recesses (Fig. 4A–B). The tracer reached the kidneys via the blood.

To visualize the distribution of tracer *in vivo*, we acquired images of the tracer from the sinuses, with a large field of view that included the lungs as a secondary target (Fig. 5A–B). Tracer was deposited in a homogeneous and complete manner in the nasal cavities, including the rhino-sinusal meatus, paranasal sinuses, and nasopharynx. Interestingly, we observed symmetrical distribution of tracer in the deep areas of the lungs (alveoli), whereas in the intermediate respiratory tract, trachea, and bronchi, no deposition of ^{99m}Tc was observed (Fig. 5A).

To test the ability to deliver therapeutically effective biological products with this device, we conveyed vaporized SARS-CoV-2–neutralizing tetravalent antibody 15033–7 [6] on the Vero E6 cell line infected with a SASRS-CoV-2 pseudovirus. The vaporization device was adapted to operate on a 4-well plate in which cells had been cultivated (Fig. 2A). Antibody was vaporized onto the cells at two different concentrations and after the pseudovirus was added. Administration of the vaporized antibody resulted in neutralizing activity: the entry of pseudovirus into target cells (when compared with virus alone) was reduced by 61% when 200 ng/mL of antibody

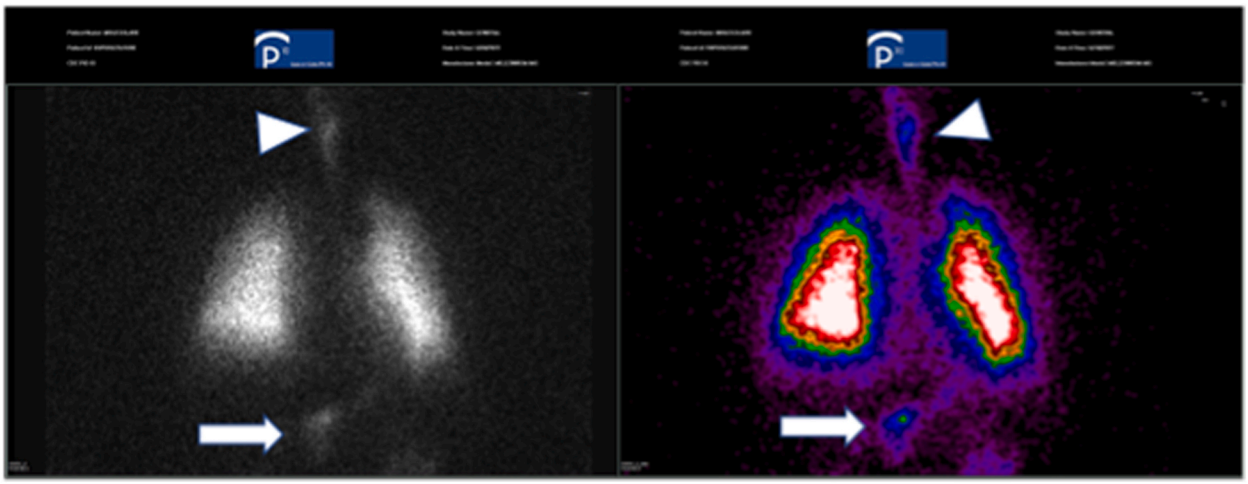

Fig. 3. Anteroposterior images showing symmetrical distribution of ^{99m}Tc-DTPA in the lungs. No significant deposition is seen in the upper respiratory tract (throat, larynx, and trachea), whereas some activity is seen in the esophagus (arrowhead) and in the stomach (arrow). The images were obtained after 5 min of deep breathing. Acquisition time was 10 min.

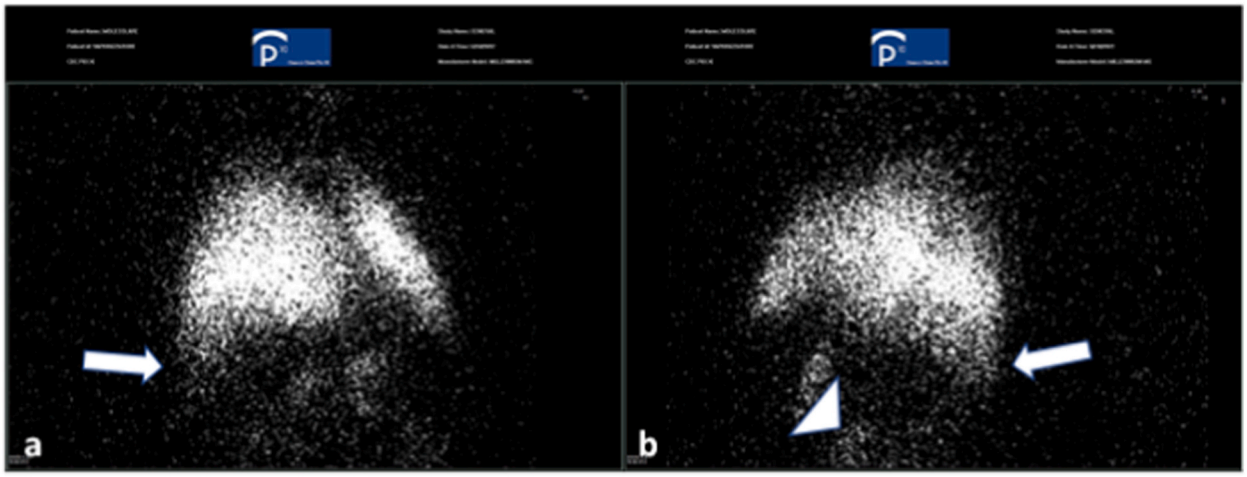

Fig. 4. Anterior right **(A)** and left **(B)** oblique images showing distribution of tracer in the deep areas of the lungs, reaching the posterior costophrenic recesses (arrows). The tracer reached the kidneys via the blood (arrowhead). Images were obtained after antero-posterior acquisition (after 15 min of deep breathing). Acquisition time was 5 min.

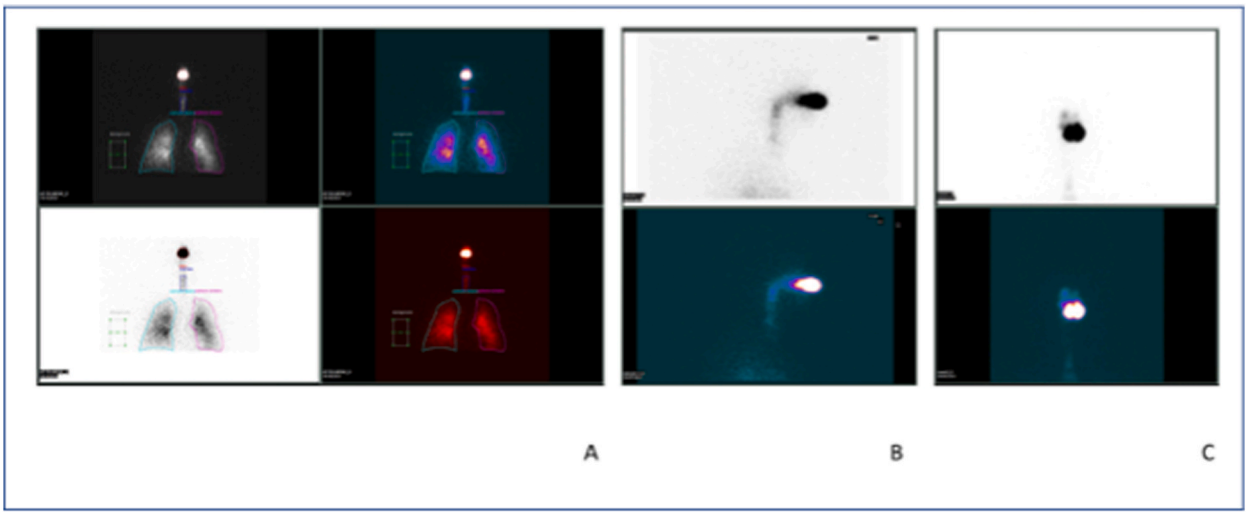

Fig. 5. (A) Acquisition was performed using a wide field of view. A greater concentration of particles was observed in the nasal cavities and nasopharynx than in the lungs, demonstrating the ability of the device to deliver targeted treatments via nasal or pulmonary administration. **(B)** Lateral acquisition. Concentration of the nebulized product was detected in the nasal cavity and nasopharynx. Homogeneous distribution of particles covering the entire internal mucosal surface of the nasal cavity, without missing any anatomical regions, is evident. **(C)** Anterior acquisition focused on the facial massif, highlighting the presence of tracer at the level of the paranasal sinuses and demonstrating that distribution overcame the anatomic-functional barriers of the rhino-sinus prechambers. The paranasal sinus cavities, rhino-pharynx, eustachian tubal ostium opening, and superior olfactory aspect of the nasal cavity were adequately reached and filled by nebulized particles.

was used and by 91% when 800 ng/mL of antibody was used (Fig. 2B).

4. Discussion

Aerosolized delivery of drugs is an important approach to treat pathologic conditions that primarily affect the lungs, including asthma, tuberculosis, emphysema, and bronchitis [1]. Pharmaceutical formulation, the inhalation device used, patient compliance, and particle size all play key roles in determining the performance of the aerosolized drugs. The mechanistic characteristics of the currently available aerosol-dispensing devices do not always allow adequate deposition of the therapeutic agent, for several reasons, including poor solubility of the drug, inadequate administration capabilities, and dispersion of the drug in the aerosol tank, the ambient air, and the oral cavity, resulting in lower efficacy and higher costs. Consequently, there is an urgent need for effective inhalation therapy devices that can overcome these limitations to produce a lasting therapeutic effect [8].

In recent years, various devices—such as nebulizers, dry powder inhalers, pressurized metered dose inhalers, and soft mist inhalers—have been developed in an attempt to improve performance [3]. However, the use of these devices can still result in sedimentation of inhalable particles in the airways, which compromises the bioavailability of the inhaled drug. Particle size plays an important role in determining the mechanism and the site of deposition of particles in the respiratory tract and can significantly affect drug delivery to the targeted site [8]. Therefore, a system that can quickly vaporize a solution into small particles would be of significant value in the treatment of a wide range of pulmonary and nonpulmonary diseases. The average delivery time for most of the current, commonly administered aerosols is approximately 10 min, and the droplets produced by these devices are typically 5–20 μm in size, which is ~100 times larger than the droplets produced by the novel device described here. The generation of particles in the range of <1 μm–0.1 μm (90 nm) would facilitate delivery of active drug ingredients deep into areas of the respiratory system in a more uniform fashion. Furthermore, small droplets are less inclined to condense in the upper airways, stopping at the throat, larynx, and nasal cavity and limiting the therapeutic potential of the agents. Of importance, the deposit of the solution onto the alveoli occurred rapidly, without evidence of condensation—an important consideration for intubated patients, who require effective delivery into the lower respiratory tract without condensation.

Another advantage of the use of a microvaporizer is the avoidance of damage to the oral cavity, throat, and larynx, which is frequently associated with aerosol inhalation of drugs. Aerosol inhalation can damage the delicate mucosal surfaces of these organs in the form of mycosis, infection, dysbiosis, dryness, and dysphonia. The homogenous diffusion of aerosol in the lung and nasal cavities achieved with our novel device validates our goal of producing small particles and a fast airflow. In addition, we demonstrated homogenous and complete deposition of tracer in the nasal cavities, rhino-sinusal meatus, paranasal sinuses, and nasopharynx. This has not been observed with other, currently available spray or aerosol devices. Such a capability could lead to new therapeutic approaches for pediatric patients with rhino-sinus-pharyngeal diseases (such as adenoid infection, rhinitis, and otitis) and for adult patients with rhino-sinusitis, otitis, and olfactory impairments, with the possibility of achieving better therapeutic action of many active ingredients. Last, *in vitro* experiments demonstrated that the low temperature required for vaporization (40 °C) leaves the antibody unaltered in its structure and, thus, function, even after exposure to ultrasound conditions—as was observed in our verification test with monoclonal antibodies described above. The documented inhibition of virus entry into host cells suggests that this device could be used to deliver active antiviral compounds directly into the nasal mucosa, up to the lung, as part of antiviral treatment.

There is a critical need for a therapeutically efficacious and safe means of delivering drugs into the airways. The possibility to administer small molecules that inhibit virus entry opens new possibilities for the prevention and treatment of infectious agents such as SARS-CoV-2 [9]. The administration of vaporized antibodies, and therefore of vaporized peptides or vaccines (such as those with mRNA), has numerous advantages over traditional methods of administration [5,10–13]. Pulmonary vaccination is a particularly useful strategy for respiratory tract diseases such as flu, pneumonia, whooping cough, and measles [14]. Our results suggest this approach has the potential to deliver targeted treatment to patients with severe COVID-19 via noninvasive selective lung-localizing administration. The device has a demonstrated ability to deliver tetravalent antibodies *in vitro* without the risk of denaturation. Based on this finding, the vaporizer could also be used *in vitro* for drug-screening programs using cell- and stem cell–derived organoid platforms for the development of personalized medicine approaches. In addition, small hydrophilic drugs, peptides, proteins, and nucleic acids can be delivered and tested for their efficacy. Peptide-based inhibitors represent an interesting therapeutic approach, as they are efficient, specific, and tolerable. Specifically, antiviral peptides conventionally target structures essential for viral attachment, fusion, replication, transcription, and maturation. We developed synthetic DPP4-derived peptides with a high binding affinity for the receptor-binding domain of SARS-CoV-2, these peptides were able to mask the virus to intercept DPP4 receptor and other known receptors on target cells, preventing virus entry into host cells [7,15]. In a previous study, the delivery of measles virus vaccine exclusively to the lower respiratory tract resulted in high levels of immunogenicity and protection [16].

This study has clearly demonstrated that the administration of vaporized drug allows for rapid access to the alveolar spaces and the nasal epithelium. This constitutes an advantage over other approaches—for instance, second-generation vaccines can be administered to the upper respiratory tract, which has a high potential to limit the spread of viruses such as SARS-CoV-2 more effectively than current vaccines [17,18]. Indeed, it is possible that the global implementation of messenger RNA (mRNA) vaccines will soon lead to the development of next-generation inhaled mRNA vaccines with the ability to drive long-lasting tissue-specific memory responses necessary for a quick recall and immediate local protection [19].

The device described here has several advantages and limitations, compared with the currently available options. The size of the microdrops produced is at least 50 times smaller than aerosols from current devices. The behavior of the vapor in this new device is similar to that of a gas, which creates the possibility of reaching the pulmonary alveoli and overcoming barriers such as the dermatological or blood-brain barrier and the mucosa that separates the pulmonary alveoli from the blood flow. However, this device is not suitable for vaporization of oily solutions, and for technical reasons it is necessary to have a residue of a few cc of solution, as ultrasound (for attainment of the device's vaporising effect) requires a certain amount of liquid to be present. Water-based or hydro-alcoholic solvents should preferentially be used.

It should be noted that the clinical feasibility of administration of vaporized drugs and therapeutic antibodies requires supporting evidence from carefully designed preclinical studies and clinical trials. We also believe that the availability of this innovative device can potentiate further studies on viral transmission, disease pathogenesis, and new therapeutic interventions, including for pharmacological treatment of COVID-19.

5. Conclusions

The results presented in this study demonstrate that biological therapeutic agents vaporized by a specifically designed device can

reach the expected targets while maintaining their fundamental properties. We believe that this innovative device could improve aerosol administration of therapeutic agents to the upper and lower respiratory tracts to treat a wide range of diseases. Furthermore, the availability of this device can result in the generation of interesting new hypotheses regarding direct administration of molecules to specific targets. However, further preclinical and clinical studies are needed to evaluate the efficacy and establish the pharmacokinetic and pharmacodynamic parameters of each product delivered using this approach.

Author contribution statement

Bruno Brandimarte conceived and designed the experiments; Lino Di Rienzo Businco analyzed and interpreted the data and wrote the paper; Francesco Cappello analyzed and interpreted the data; Roberto Fiore contributed reagents, materials, analysis tools or data; Giuseppe Bastone performed the experiments; Gianfranco Gualdi contributed reagents, materials, analysis tools or data; Saadi Sollaku contributed reagents, materials, analysis tools or data, Emanuele Casciani performed the experiments; Federica Tortorella performed the experiments; Pasquale Longo analyzed and interpreted the data, Eleonora Centanini performed the experiments; Silva Pavaci analyzed and interpreted the data; Federica Sangiuolo conceived and designed the experiments and wrote the paper; Maria Patrizia Patrizi contributed reagents, materials, analysis tools or data; Shane Miersch analyzed and interpreted the data, Sachdev S. Sidhu analyzed and interpreted the data, Virgilio Sacchini conceived and designed the experiments and wrote the paper; Giuseppe Novelli conceived and designed the experiments and wrote the paper.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Abbreviations

DTPA	diethylene-triamino-pentacetic acid
^{99m}Tc	technetium-99
VSV	vesicular stomatitis virus

References

[1] P.J. Anderson, History of aerosol therapy: liquid nebulization to MDIs to DPIs, Respir. Care 50 (2005) 1139–1150.
[2] J. Schreiber, T. Sonnenburg, E. Luecke, Inhaler devices in asthma and COPD patients – a prospective cross-sectional study on inhaler preferences and error rates, BMC Pulm. Med. 20 (2020) 222, https://doi.org/10.1186/s12890-020-01246-z.
[3] H. Jain, A. Bairagi, S. Srivastava, S.B. Singh, N.K. Mehra, Recent advances in the development of microparticles for pulmonary administration, Drug Discov. Today 25 (2020) 1865–1872, https://doi.org/10.1016/j.drudis.2020.07.018.
[4] R.H. Petrucci, R.H. Petrucci, General Chemistry : Principles and Modern Applications, 9th Edition (Pearson Education International), 2007.
[5] K.O. Saunders, E. Lee, R. Parks, D.R. Martinez, D. Li, H. Chen, R.J. Edwards, S. Gobeil, M. Barr, K. Mansouri, et al., Neutralizing antibody vaccine for pandemic and pre-emergent coronaviruses, Nature 594 (2021) 553–559, https://doi.org/10.1038/s41586-021-03594-0.
[6] S. Miersch, Z. Li, R. Saberianfar, M. Ustav, J. Brett Case, L. Blazer, C. Chen, W. Ye, A. Pavlenco, M. Gorelik, et al., Tetravalent SARS-CoV-2 neutralizing antibodies show enhanced potency and resistance to escape mutations, J. Mol. Biol. 433 (2021), 167177, https://doi.org/10.1016/j.jmb.2021.167177.
[7] M. Murdocca, G. Citro, I. Romeo, A. Lupia, S. Miersch, B. Amadio, A. Bonomo, A. Rossi, S.S. Sidhu, P.P. Pandolfi, et al., Peptide Platform as a Powerful Tool in the Fight against COVID-19, 2021, https://doi.org/10.3390/v13081667. Viruses *13*.
[8] A. Chandel, A.K. Goyal, G. Ghosh, G. Rath, Recent advances in aerosolised drug delivery, Biomed. Pharmacother. 112 (2019), 108601, https://doi.org/10.1016/j.biopha.2019.108601.
[9] V.S. Salian, J.A. Wright, P.T. Vedell, S. Nair, C. Li, M. Kandimalla, X. Tang, E.M. Carmona Porquera, K.R. Kalari, K.K. Kandimalla, COVID-19 transmission, current treatment, and future therapeutic strategies, Mol. Pharm. 18 (2021) 754–771, https://doi.org/10.1021/acs.molpharmaceut.0c00608.
[10] Y. Rong, F.J. Torres-Velez, D. Ehrbar, J. Doering, R. Song, N.J. Mantis, An intranasally administered monoclonal antibody cocktail abrogates ricin toxin-induced pulmonary tissue damage and inflammation, Hum. Vaccines Immunother. 16 (2020) 793–807, https://doi.org/10.1080/21645515.2019.1664243.
[11] H. Xu, L. Cai, S. Hufnagel, Z. Cui, Intranasal vaccine: factors to consider in research and development, Int. J. Pharm. 609 (2021), 121180, https://doi.org/10.1016/j.ijpharm.2021.121180.
[12] P.A. van der Ley, A. Zariri, E. van Riet, D. Oosterhoff, C.P. Kruiswijk, An intranasal OMV-based vaccine induces high mucosal and systemic protecting immunity against a SARS-CoV-2 infection, Front. Immunol. 12 (2021), 781280, https://doi.org/10.3389/fimmu.2021.781280.
[13] M. Sharma, S. Waghela, R. Mhatre, G.K. Saraogi, A recent update on intranasal delivery of high molecular weight proteins, peptides, and hormones, Curr. Pharmaceut. Des. 27 (2021) 4279–4299, https://doi.org/10.2174/1381612827666210820100723.

[14] E.C. Lavelle, R.W. Ward, Mucosal vaccines — fortifying the frontiers, Nat. Rev. Immunol. 22 (2022) 236–250, https://doi.org/10.1038/s41577-021-00583-2.
[15] P. Spitalieri, F. Centofanti, M. Murdocca, M.G. Scioli, A. Latini, S. Di Cesare, G. Citro, A. Rossi, A. Orlandi, S. Miersch, et al., Two different therapeutic approaches for SARS-CoV-2 in hiPSCs-derived lung organoids, Cells (11) (2022), https://doi.org/10.3390/cells11071235.
[16] R.L. de Swart, R.D. de Vries, L.J. Rennick, G. van Amerongen, S. McQuaid, R.J. Verburgh, S. Yüksel, A. de Jong, K. Lemon, D.T. Nguyen, et al., Needle-free delivery of measles virus vaccine to the lower respiratory tract of non-human primates elicits optimal immunity and protection, NPJ Vaccines 2 (2017) 22, https://doi.org/10.1038/s41541-017-0022-8.
[17] J.L. Americo, C.A. Cotter, P.L. Earl, R. Liu, B. Moss, Intranasal inoculation of an MVA-based vaccine induces IgA and protects the respiratory tract of hACE2 mice from SARS-CoV-2 infection, Proc. Natl. Acad. Sci. USA 119 (2022), e2202069119, https://doi.org/10.1073/pnas.2202069119.
[18] Y. Wang, A. Yan, D. Song, C. Dong, M. Rao, Y. Gao, R. Qi, X. Ma, Q. Wang, H. Xu, et al., Biparatopic antibody BA7208/7125 effectively neutralizes SARS-CoV-2 variants including Omicron BA.1-BA.5, Cell Discovery 9 (2023) 3, https://doi.org/10.1038/s41421-022-00509-9.
[19] E.H. Roh, C.A. Fromen, M.O. Sullivan, Inhalable mRNA vaccines for respiratory diseases: a roadmap, Curr. Opin. Biotechnol. 74 (2022) 104–109, https://doi.org/10.1016/j.copbio.2021.10.017.