Scientific Reports

https://doi.org/10.1038/s41598-026-37584-x

Article in Press

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Received: 6 November 2025

Accepted: 22 January 2026

Published online: 13 February 2026

Cite this article as: Veras F.P., Nakamura G., Pereira-da-Silva M.A. *et al.* Ultrasound effectively destabilizes and disrupts the structural integrity of enveloped respiratory viruses. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-37584-x

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Ultrasound effectively destabilizes and disrupts the structural integrity of enveloped respiratory viruses

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Abstract

This study demonstrates that high-frequency ultrasound (3–20 MHz) can effectively disrupt the structural integrity of both Influenza A virus (H1N1) and SARS-CoV-2 through a resonance-driven mechanism distinct from classical cavitation (kHz range). Under these conditions, viral particles undergo pronounced alterations (fragmentation, envelope rupture, and loss of morphological uniformity) consistent with direct mechanical destabilization rather

than thermal or bubble-mediated effects. Detailed structural analyses revealed significant disruption of the viral envelope, accompanied by measurable shifts in particle size distribution and reduced diameters, indicative of resonance-induced fragmentation. These structural modifications were paralleled by biological consequences: SARS-CoV-2 infectivity was markedly reduced *in vitro*, with infected cells exhibiting substantially lower viral loads. Importantly, this work provides the first experimental evidence that acoustic resonance can directly couple with viral structural components, inducing selective mechanical destabilization of the envelope. The convergence of structural and functional data supports the view that this represents a previously undescribed biophysical phenomenon, fundamentally distinct from cavitation. This resonance-mediated destabilization highlights a novel, non-invasive, and broad-spectrum antiviral strategy that differs from cavitation, more suited to asepsis and sterilization, and offers a therapeutic approach with potential applications against enveloped respiratory viruses and other clinically relevant pathogens.

Keywords: Ultrasound; Resonances; SARS-CoV-2; H1N1; Antiviral

1. Introduction

Viruses are submicroscopic, obligate intracellular parasites that replicate exclusively within host cells by commandeering their molecular machinery to propagate their genomes. They constitute a persistent public-health challenge, causing diseases ranging from mild seasonal infections to severe, life-threatening conditions. Rapid mutation rates, zoonotic spillover, and drug-resistant variants undermine existing therapies. The limited availability of effective antivirals heightens global vulnerability and underscores the need for innovative therapeutic strategies, accelerated vaccine development, and comprehensive public-health measures to mitigate the evolving threat of viral pathogens.

While biological and chemical approaches dominate research and control strategies, physics-based options, have also gained attention. Approaches traditionally used for *in vitro* or environmental disinfection, such as ionizing radiation or cavitation-based ultrasonic sterilization, operate under high-intensity conditions that are not compatible with therapeutic applications in living tissues.

Ultraviolet and higher-energy ionizing radiation interact strongly with electron-rich regions, including nucleic acids, enabling rapid virus inactivation[1], but their potential for tissue damage limits their applicability in clinical settings[2]. Non-ionizing radiation may also harm biological structures[3].

In acoustics, ultrasound bioeffects can broadly be categorized into thermal and mechanical mechanisms, and the present study focuses exclusively on mechanical interactions. Ultrasonic waves can induce oscillatory motion in gas bubbles dispersed within the medium; however, such oscillations do not inherently constitute cavitation. Cavitation encompasses two distinct regimes: stable (non-inertial) cavitation, characterized by reversible bubble oscillations, and inertial cavitation, defined by bubble collapse, shock generation, and radical formation occurring only above a specific acoustic pressure threshold. The high-frequency, low-pressure ultrasound conditions employed in this study do not support inertial cavitation, and cavitation-associated bioeffects are therefore not expected under our experimental parameters.

In contrast to cavitation-driven inactivation, high-frequency ultrasound can produce non-cavitational, frequency-dependent mechanical interactions at the viral scale that are compatible with therapeutic contexts**.** A promising strategy is to use acoustic waves to induce resonances directly in viral structural components, such as the envelope and surface proteins. Theoretical insights suggest that acoustic resonances depend primarily on viral geometry, such as size and shape, rather than biochemical composition[4-7], indicating a targeted mechanical response at the viral scale.

Here, we evaluated the structural response of Influenza A virus (H1N1) and SARS-CoV-2 to ultrasound-induced acoustic resonances within the 3–20 MHz range. Both enveloped viruses exhibited pronounced alterations upon exposure, including fragmentation and envelope disruption, consistent with a direct mechanical effect distinct from cavitation or thermal damage. These findings highlight a shared vulnerability among spherical, lipid-enveloped respiratory viruses subjected to high-frequency acoustic energy. Taken together, our results suggest resonance-mediated destabilization as a potential therapeutic mechanism**,** distinct from sterilization approaches**,** and provide evidence for a

non-invasive antiviral strategy relevant to current and emerging respiratory pathogens.

2. Results

2.1. Ultrasound-induced nanoscale changes in viral particle size

To test whether ultrasound effects generalize beyond a single viral model, we analyzed SARS-CoV-2 and Influenza A (H1N1), two clinically relevant respiratory pathogens with distinct structural features. In SARS-CoV-2, ultrasound exposure led to a clear reduction in average particle size and an increase in polydispersity, suggesting envelope disruption and partial disassembly. Ultrasound exposure was performed under resonance conditions using a clinical diagnostic system operating at 7.5 MHz for 5 minutes. DLS measurements of untreated samples showed a single, sharp peak centered around 107 nm, consistent with intact virions. In contrast, ultrasound-treated samples displayed additional smaller peaks (~1.5 nm and ~4.9 nm), indicative of fragmentation or formation of small aggregates (**Fig. 1a** and **b**). These low-diameter components reflect the presence of subviral fragments rather than intact particles, consistent with heterogeneous structural breakdown.

H1N1 exhibited a distinct response. While untreated H1N1 samples showed a well-defined peak at ~129 nm, ultrasound-treated samples displayed no detectable signal across the measurable range (**Fig. 1c** and **d**). The absence of residual peaks indicates extensive structural degradation rather than fragmentation into resolvable small components, in contrast to the profile observed for SARS-CoV-2. Hydrodynamic diameters and polydispersity indices (PdI) increased in both viruses after treatment, supporting mechanical destabilization.

2.2. Ultrasound-mediated structural damage to viral surface architecture

We next focused on SARS-CoV-2 to examine how structural alterations translate into biological consequences. To evaluate physical changes, we used scanning electron microscopy (SEM) and atomic force microscopy (AFM), which

together characterize topographical and mechanical features before and after ultrasound treatment (7.5 MHz, 5 minutes).

SEM of untreated Wuhan-strain samples revealed well-defined spherical particles (80–100 nm), isolated or in small aggregates (**Fig. 2a**). In contrast, ultrasound-exposed samples showed prominent morphological alterations (**Fig. 2b**): irregular surfaces, reduced diameters, disrupted contours, envelope indentations and collapse, and surface fragmentation. Line-profile analyses confirmed significant loss of envelope integrity.

AFM imaging in tapping mode (**Fig. 2c–f**) provided nanoscale detail. Untreated particles retained spherical shape with smooth curvature and discrete elastic protrusions (likely spike proteins), giving a mildly irregular topography (**Fig. 2d** and **e**). After treatment, AFM revealed collapsed and fragmented envelopes with irregular boundaries and ruptured surfaces (**Fig. 2d**). A digital color correction emphasizing mid-height features highlighted discontinuities and a distinct “popcorn-like” pattern (**Fig. 2f**), indicative of structural breakdown. Although the precise disruption mechanism remains to be fully resolved, our imaging findings demonstrate clear morphological alterations following ultrasound exposure.

2.3. Ultrasound exposure impairs SARS-CoV-2 antigen expression and replication

We evaluated virucidal effects using clinical ultrasound imaging devices (3–20 MHz). Beyond safety features, these devices display the mechanical index (MI $\propto$ p/f), indicating cavitation likelihood. As a guideline, MI < 0.3 is essentially cavitation-free; 0.7–1 supports non-inertial cavitation with moderate probability; MI > 1 supports inertial and non-inertial cavitation with high probability. In our experiments, MI was 0.3–0.5, below the 0.7 threshold.

To establish the experimental workflow for testing ultrasound-mediated viral inactivation, SARS-CoV-2 stock suspensions were prepared and transferred into Petri dishes (**Fig. 3a**). Samples were then exposed to insonation using diagnostic linear-array transducers for 30 minutes. Immediately after treatment,

the viral suspensions were applied to infect Vero-E6 cells. At 24 hpi, infected cultures were examined by immunofluorescence/confocal microscopy to evaluate viral replication and cytopathic effects. Control conditions included untreated virus, which consistently induced robust infection, and mock-infected cells (DMEM), which showed no detectable viral antigens. This workflow provided the basis for comparing the susceptibility of different SARS-CoV-2 lineages to ultrasound treatment in subsequent experiments.

To investigate whether ultrasound sensitivity varied among SARS-CoV-2 lineages, we analyzed the Wuhan reference strain (WT), the Gamma variant (P.1), and the Delta variant. Viral suspensions were insonated for 30 minutes using three distinct diagnostic linear-array transducers: Equipment A (Philips Envisor HD, 3–12 MHz), Equipment B (Esaote MyLab 60, 5–10 MHz), and Equipment C (Esaote MyLab 60, 6–18 MHz). For each system, results were consolidated across available imaging modes, while frequency-dependent outcomes are detailed below. Negative controls included mock-infected cells (DMEM only), whereas untreated virus served as the infection control.

Vero-E6 cells were infected with treated or untreated samples and analyzed at 24 hpi by immunofluorescence/confocal microscopy, staining for spike protein (green) and viral dsRNA (red), with nuclei counterstained with DAPI (blue) (**Fig. 3b**). For the Wuhan strain (**Fig. 3b**), all three devices reduced infection to varying degrees, with Equipment B (centered at ~7.5 MHz) showing the strongest virucidal effect, almost completely abolishing viral antigen and dsRNA signals. Equipments A and C also markedly suppressed infectivity in the WT strain. When extended to variants, however, Gamma (**Supplementary Fig. 1**) and Delta (**Supplementary Fig. 2**) displayed increased resistance. In these cases, Equipment B remained the most effective, whereas A and C displayed incomplete activity, with residual spike and dsRNA signals detectable. Taken together, these results demonstrate that while all three ultrasound devices reduce infectivity, the Wuhan strain is more susceptible (**Fig. 3b**), whereas Gamma and Delta require the acoustic conditions provided by Equipment B for optimal inactivation.

To quantify frequency dependence, we performed TCID50 assays on WT after exposure using the Philips L3-12 (3–12 MHz, 38-mm aperture) for 1, 5, or 10 minutes at different modes (**Fig. 4**). Modes centered near 7.5 MHz markedly reduced infectivity regardless of duration, suggesting irreversible alterations in proteins critical for entry. Lower frequencies were significantly less effective (**Fig. 4a**), arguing against cavitation (more likely at lower frequencies, $\propto 1/\sqrt{f}$) as the primary mechanism. Longer exposures progressively reduced TCID50 across profiles, consistent with cumulative effects. We hypothesize that ~7.5 MHz better matches viral resonant absorption, enabling efficient energy deposition.

These findings suggest that the antiviral efficacy of ultrasound may not only depend on the acoustic parameters but could also vary according to the viral lineage. Such differences might reflect strain-specific protein modifications that alter viral stability, thereby creating a possible frequency–strain dependent effect.

2.4. Resonance mechanisms underlying viral structural destabilization

To clarify the physical basis of the observations, we propose a model describing how acoustic energy interacts with viral particles across frequency ranges. Unlike kHz cavitation (bubble collapse, heating, radicals), MHz resonance involves direct coupling between the acoustic wave and viral structures, driven by impedance mismatch at the virus–medium interface.

In cavitation, low-frequency ultrasound promotes bubble nucleation, oscillation, and collapse, producing mechanical stress, heat, shock waves, microjets, and reactive species that fragment biological structures non-selectively, useful for sterilization but unsuitable therapeutically (**Fig. 5a**).

In our resonance-based model, MHz ultrasound induces periodic compression/rarefaction within virions. These oscillations generate alternating potential and kinetic energy, accumulating mechanical stress in the envelope and structural proteins. Cyclic radial vibrations excite resonance modes, deforming the envelope, reducing morphological uniformity, and destabilizing architecture. Efficiency depends on geometry (size, shape, elastic properties) rather than biochemical composition, introducing a previously undescribed biophysical

phenomenon (**Fig. 5b**). **Table 1** summarizes differences between cavitation and resonance.

We next evaluated whether resonance-driven ultrasound operates via thermal or chemical alterations of the medium. Using a 7.5-MHz linear transducer (as above), we continuously monitored temperature and pH of DMEM during exposure. Temperature remained below protein-denaturation thresholds (42–45 °C) (**Fig. 6a**), and pH was unchanged (**Fig. 6b**), ruling out acidification/alkalinization. Thus, 7.5-MHz resonance conditions did not cause thermal or chemical disruption; observed effects arise from non-thermal mechanical interactions.

Conversely, a 42 kHz ultrasonic bath, favoring cavitation, caused medium heating (**Fig. 6c**) and acidification (**Fig. 6d**), consistent with bubble dynamics and aqueous chemistry generating H^+. Together, these results support two mechanistically distinct ultrasound–virus interaction pathways: (1) a non-thermal, resonance-driven mechanism at MHz frequencies (stable temperature and pH; selective energy absorption by virions), and (2) a cavitation-driven mechanism at kHz frequencies (temperature elevation, medium acidification, non-selective damage). This establishes resonance-mediated destabilization as a novel biophysical phenomenon distinct from cavitation and supports targeted, safe ultrasound-based antiviral strategies.

3. Discussion

Viral diseases continue to exert a profound burden on global health, not only due to their direct morbidity and mortality but also because of their ability to disrupt healthcare systems and economies. Vaccination campaigns and antiviral therapies remain central strategies of prevention and treatment, yet they are constantly undermined by viral evolution, antigenic variation, and the emergence of resistant variants[12,13]. These biological dynamics lead both approaches reactive, requiring continuous updates and re-investment.

Despite significant scientific advances, there is still no universally applicable antiviral therapy capable of acting across multiple families of viruses[14-

[16]. Antivirals typically demand high specificity, while vaccines are limited by the time required for design, testing, and distribution. The recent COVID-19 pandemic highlighted both the potential and the shortcomings of these interventions: rapid vaccine development provided unprecedented relief, but mutations compromised long-term efficacy[17,18].

In this context, physical strategies emerge as an underexplored alternative. While methods such as ultraviolet irradiation, ionizing radiation, or thermal inactivation can neutralize viruses outside the host, they are unsuitable for therapeutic use because they damage surrounding tissues[1-3]. The absence of a safe, inert, and non-invasive physical method for treating viral infections *in vivo* represents a critical gap in the antiviral arsenal. Our work addresses this gap by exploring ultrasound as a candidate capable of selectively destabilizing viral structures without collateral tissue injury.

Our findings demonstrate that ultrasound exposure within diagnostic frequency ranges produces pronounced structural alterations in enveloped viruses. SARS-CoV-2 and Influenza A (H1N1) both exhibited fragmentation and envelope rupture following treatment. These morphological disruptions were accompanied by functional consequences, with viral infectivity significantly reduced across different strains. This establishes ultrasound as more than a simple perturbation, instead acting as a direct antiviral mechanism.

High-resolution imaging provided critical insights into this process. Scanning electron and atomic force microscopy revealed clear signs of envelope collapse and surface deformation, including the striking “popcorn effect”, which suggests the release of nucleoproteins from within the viral core. Such observations indicate that ultrasound generates stresses that propagate through the viral structure, producing failure at both the membrane and internal organizational levels. Besides, particles analyzed by DLS displayed significant reductions in average hydrodynamic diameter and increased polydispersity, with the emergence of smaller peaks consistent with viral fragmentation and aggregation. These findings further corroborate the notion that ultrasound destabilizes the viral envelope, leading to heterogeneous particle populations rather than intact, monodisperse virions.

Crucially, these effects occurred in the absence of measurable changes in temperature or pH, eliminating indirect thermal or chemical contributions as explanations[19]. The specificity of the response points to mechanical resonance as the driver, whereby acoustic energy is absorbed and redistributed within the virus until structural breakdown occurs[20,21]. This mechanistic understanding reinforces the plausibility of ultrasound as a broad-spectrum antiviral modality. The frequency-dependent nature of the antiviral effect supports the hypothesis that internal acoustic resonances are central to viral destabilization[21-23]. Viruses behave as viscoelastic particles, where the lipid envelope and protein core form a composite structure capable of deformation and energy absorption. At resonant frequencies, ultrasound couples efficiently to this architecture, amplifying mechanical stress and leading to envelope rupture.

Importantly, vibrational modes are determined by physical parameters such as particle diameter, shell thickness, elastic modulus of the capsid or envelope, and surface viscoelasticity. Theoretical and computational studies have demonstrated that these mechanical factors strongly modulate nanoscale vibrational behavior, with distinct resonance frequencies emerging from differences in viral size, geometry, and stiffness[29-31]. Normal-mode and continuum-elasticity analyses of viral capsids have shown that even small variations in shell thickness or elastic modulus can shift resonance modes significantly[7,32]. Likewise, AFM-based nanoindentation studies reveal lineage-specific differences in capsid rigidity and viscoelastic response, supporting the idea that viruses with similar biochemical composition may nevertheless differ mechanically[33]. These structural determinants provide a mechanistic explanation for the lineage-specific differences observed in our dataset, reinforcing that acoustic susceptibility is governed primarily by physical rather than biochemical properties.

This mechanistic framework also explains why all three ultrasound systems used in this study, despite differences in manufacturer, transducer geometry, and operational bandwidth, produced comparable reductions in infectivity. Acoustic scattering theory predicts that MHz-frequency ultrasound couples efficiently to nanoscale spherical particles according to their intrinsic

vibrational modes, largely independent of device architecture. Because all three devices emit within the diagnostic MHz range, their output overlaps with the vibrational frequencies predicted for enveloped virions, enabling similar resonance-driven mechanical failure across systems. This convergence strongly supports a shared, frequency-dependent mechanism of viral destabilization rather than device-specific artifacts.

This mechanism stands in clear contrast to cavitation, the process by which bubbles form and collapse under low-frequency ultrasound. Cavitation, commonly employed in aseptic procedures to eliminate microorganisms, is nevertheless known to generate heat, free radicals, and pressure shocks, all of which can indiscriminately damage biological material[4-6]. When we tested cavitation-prone conditions at 42 kHz, we observed increases in both temperature and medium acidification[24,25], phenomena that were absent at the higher ultrasound frequencies typically used in medical applications.

The divergence between these two regimes underscores the safety and selectivity of resonance-based ultrasound. Cavitation is unpredictable and potentially harmful, whereas resonance is tunable, reproducible, and free of thermal or chemical side effects. This distinction not only clarifies the mechanism of action but also highlights why ultrasound in the diagnostic frequency range is suitable for translational therapeutic applications.

The translational potential of ultrasound as an antiviral tool is particularly compelling given its established role in medicine. Ultrasound devices are already widely distributed, safe, and regulated, which lowers barriers to clinical adaptation[26]. The ability of acoustic waves to penetrate tissues without invasive procedures positions ultrasound as a promising candidate for targeting viral reservoirs in anatomically challenging sites, including lung parenchyma and the central nervous system[27,28].

Moreover, ultrasound could be deployed not only as a stand-alone antiviral intervention but also as a synergistic adjuvant. By mechanically destabilizing viral envelopes, ultrasound may enhance viral susceptibility to conventional drugs or immune system clearance. This opens opportunities for

reducing drug dosages, mitigating resistance development, and improving therapeutic outcomes through multimodal strategies.

Future investigations should expand toward *in vivo* validation, exploring the biodistribution, safety, and efficacy of ultrasound-mediated antiviral therapy in animal or organoid models. Parallel development of dedicated transducers optimized for resonance frequencies of different viral families could accelerate clinical translation. At the interface of physics and virology, this approach creates a new frontier where existing medical technologies are repurposed to meet urgent antiviral needs.

In conclusion, our study demonstrates that ultrasound at safe diagnostic frequencies can disrupt the structural and functional integrity of enveloped viruses through resonance-driven mechanical destabilization. Unlike traditional physical methods, this approach avoids collateral thermal or chemical damage, positioning ultrasound as a safe, inert, and non-invasive antiviral strategy. By revealing both structural mechanisms and translational perspectives, we provide a foundation for future studies aiming to harness ultrasound as a therapeutic or adjuvant platform against viral diseases.

4. Materials and methods

4.1. Virus stock production

For *in vitro* assays, parental SARS-CoV-2 Wuhan strain (wild type, WT), Gamma (P1) and Delta variants were used. All SARS-CoV-2 procedures were conducted under BSL-3 at Ribeirão Preto Medical School, University of São Paulo (Brazil). Viral stocks were authenticated by genomic sequencing. To assess broader applicability, Influenza A virus (IAV, H1N1) was included (BSL-2). Each virus was propagated in a permissive cell line: SARS-CoV-2 in Vero E6 (ATCC® CRL-1586™); H1N1 in MDCK (ATCC® CCL-34™). Viral inoculum (1:100) was added to confluent monolayers and incubated 48 h at 37 °C, 5% CO_2, in DMEM (Sigma-Aldrich D5796) without FBS, supplemented with Penicillin-Streptomycin-Antimycotic (Penicillin 10,000 U/mL; Streptomycin 10,000 µg/mL; Sigma-Aldrich P4333). Cytopathic effects (CPE) were monitored.

Upon extensive CPE, cells were scraped, harvested, centrifuged (10,000 × g, 10 min, RT). Supernatants were aliquoted and stored at −80 °C. Titers were determined by TCID50 (Reed–Muench), in quadruplicate on the same cell lines, and expressed as TCID50/mL.

4.2. Ultrasound exposure, temperature and pH measure

Viral stocks (100 µL) were exposed using clinical diagnostic ultrasound systems (Esaote MyLab 60; Philips Envisor HD) equipped with linear-array transducers operating at 3–12, 5–10, or 6–18 MHz. Manufacturer-specified acoustic outputs were recorded for each probe, including mechanical index (MI = 0.4–1.0), thermal index (TI < 1.0), acoustic power (0.1–1.2 W depending on frequency), and spatial-average temporal-average intensity (I_SATA = 20–120 mW/cm²). These values fall within standard diagnostic-imaging safety limits. Acoustic parameters were optimized with short preliminary trials, and final exposure durations tested were 1, 5, 10, and 30 minutes, with similar qualitative outcomes observed at shorter times. Exposure was performed in sterile Petri dishes filled with sodium phosphate buffer to maintain aseptic conditions and stable acoustic coupling. Temperature and pH were continuously monitored using a digital LCD thermometer (Contec TPM-10; NTC thermistor) and a pH meter (Kasvi K39-0014PA), respectively. The temperature probe and pH electrode were immersed directly in the liquid medium containing the viral suspension, positioned at the same vertical level as the sample and at a fixed distance from the ultrasound transducer. Measurements were performed at a single representative point within the solution, which was maintained under gentle mixing to ensure homogeneity throughout ultrasound exposure. For cavitation-positive controls, samples were exposed to a commercial ultrasonic bath (Yaxun 3060, 42 kHz) for up to 30 min, a condition known to induce inertial cavitation and therefore used only as a mechanistic contrast.

4.3. Dynamic Light Scattering (DLS)

To evaluate whether ultrasound induces nanoscale structural disruption in viral particles, we employed DLS as a non-invasive and highly sensitive method to quantify changes in particle size distribution. Due to biosafety requirements

and the morphological fragility of enveloped virions, DLS was selected because it allows the analysis of native, hydrated viral suspensions without the preparation-related artifacts associated with high-resolution imaging techniques such as SEM or AFM[10]. By maintaining particles in suspension, DLS provides real-time, population-level measurements of hydrodynamic diameter and polydispersity under physiologically relevant conditions[11], enabling the detection of subtle ultrasound-mediated structural alterations.

Samples, both before and after ultrasound exposure, were suspended in sodium phosphate buffer (10 mM, pH 7.4). Measurements were performed using a Zetasizer Nano ZS90 instrument (Malvern) equipped with a 633 nm laser, at a constant temperature of 25 °C. The hydrodynamic diameter of viral particles was determined at a fixed scattering angle of 90°, and results were expressed as the Z-average, considering both intensity- and volume-weighted size distributions.

4.4. AFM and SEM imaging

SARS-CoV-2 stock samples were UV-inactivated, frozen at −80 °C, and processed outside BSL-3. Poly-L-lysine was incubated 5–10 min on clean Si substrates (5 × 5 mm). Inactivated samples were placed on coated substrates and fixed with 4% formaldehyde. After washing, samples were dehydrated through graded ethanol (40–100%). For SEM, samples were sputtered with ~10 nm graphite and imaged (Sigma Zeiss FE-SEM, 2 kV). Line-profile analyses were performed on SEM micrographs using Fiji by ImageJ. Calibrated transects were drawn across viral envelope boundaries, generating intensity–distance profiles. Abrupt signal drops, irregular edge transitions, and reduced amplitude were quantified as indicators of envelope discontinuity and structural disruption. For AFM, uncoated samples were imaged (Bruker Icon-Dimension, tapping mode).

4.5. *In vitro* SARS-CoV-2 infection and ultrasound exposure

Vero E6 cells were infected (MOI = 1.0; SARS-CoV-2 infectious clone) or mock-infected and then exposed for 30 min to linear-array transducers (3–12, 5–10, or 6–18 MHz) on MyLab 60 or Envisor HD systems. After 24 h, infection and replication were assessed by immunofluorescence/confocal microscopy. TCID50

assays were performed after exposure at 3, 5, 7.5, 10, or 12 MHz for 1, 5, 10, or 30 min. Experiments were in technical triplicate.

4.6. TCID50 assay

Virus stock was diluted 1:100 in DMEM and/or ultrasound-exposed preparations, incubated 1 min at RT, serially diluted 10-fold, and 100 µL of each dilution inoculated in quadruplicate monolayers to determine TCID50 in Vero cells (96-well plates) by standard limiting dilution.

4.7. Immunostaining and confocal microscopy

Vero-E6 cells grown on glass coverslips were fixed with 4% paraformaldehyde (PFA) for 10 min at room temperature (RT), followed by blocking with 1% bovine serum albumin (BSA) and 22.52 mg/mL glycine in PBST (0.1% Tween-20 in PBS) for 2 h at RT. Cells were then incubated with primary antibodies: rabbit anti-spike (Invitrogen, 703959; 1:500) and mouse anti-dsRNA (SCICONS, clone J2-1909; 1:1,000). After washing, secondary antibodies were applied: alpaca anti-mouse IgG Alexa Fluor 488 (Jackson, 615-545-214; 1:1,000) and alpaca anti-rabbit IgG Alexa Fluor 594 (Jackson, 611-585-215; 1:1,000). Nuclei were counterstained using Vectashield mounting medium with DAPI (H-1200-10). Images were acquired on an Axio Observer/LSM 780 confocal microscope (Carl Zeiss) at 630× magnification under identical settings for all samples, and processed using Fiji/ImageJ software.

4.8. Statistical analysis

Significance was assessed by one-way ANOVA with Tukey's post hoc test ($p < 0.05$). For two-factor comparisons, two-way ANOVA with Šídák's multiple-comparisons test was used. Analyses and graphs were generated in GraphPad Prism 9.5.1.

Acknowledgments

O.M.B thanks Daniela Gravina Stamato for discussions on clinical ultrasonography and early suggestions.

Author contributions

F.P.V, G.N, and O.M.B designed the study. F.P.V performed immunofluorescence and confocal analyses. R.M performed TCID50 assays. F.P.V and G.N performed ultrasound exposures. G.N and M.A.P.S performed SEM and AFM analyses. G.C.M.R and C.J.L.C performed, analyzed, and discussed DLS experiments. F.Q.C, E.A, and O.M.B provided critical materials and comments. F.P.V, G.N, and O.M.B wrote the manuscript. O.M.B coordinated and supervised the project. All authors approved the manuscript.

Conflict of interest statement

The authors declare no competing interests.

Financial support

FAPESP: F.P.V 2020/07645-0; G.N 2023/07241-5; F.Q.C 2013/08216-2 and 2020/05601-6; E.A 2019/26119-0; O.M.B 2018/22214-6, 2021/08325-2, and 2023/07241-5. O.M.B also acknowledges CNPq 05610/2022-8. F.P.V also acknowledges and Institut Merrieux Grant.

Data availability

Data supporting this study are available from the corresponding author upon reasonable request. When applicable, processed datasets and code are available in public repositories as described in Methods.

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Figures captions

Figure 1 – Dynamic Light Scattering (DLS) analysis of respiratory virus particle size distribution before and after ultrasound treatment. Measurements focused on (**a,b**) SARS-CoV-2 (wild type) and (**c,d**) Influenza A (H1N1). Untreated samples showed monodisperse distributions consistent with intact virions. Ultrasound-treated samples exhibited broadened, shifted profiles, reduced average diameters, emergence of smaller peaks, and increased PdI, indicative of disruption, fragmentation, and aggregation. Ultrasound was delivered under resonance conditions using a clinical diagnostic system at 7.5 MHz for 5 minutes. Data represent at least two independent experiments.

Figure 2 – Ultrasound-induced morphostructural alterations in SARS-CoV-2 particles by SEM and AFM. Viral suspensions were exposed to ultrasound (7.5 MHz, 5 min). Untreated particles (**a**) display spherical morphology with smooth and intact surfaces, whereas ultrasound-treated particles (**b**) exhibit pronounced surface disruption and loss of structural integrity. Atomic force microscopy (AFM) height profiles, shown at full scale (**c, d**) and half scale (**e, f**), further confirm envelope destabilization and compromised viral integrity. Data are representative of at least two independent experiments.

Figure 3 – US treatment inhibits SARS-CoV-2 infection and replication. (**a**) Experimental schematic illustrating ultrasound exposure of viral suspensions using linear-array diagnostic transducers operating at Equip.1 (3–12 MHz), Equip.2 (5–10 MHz), or Equip.3 (6–18 MHz) for 30 min. Treated and untreated viral samples were subsequently used to infect Vero-E6 cells. (**b**) Immunofluorescence analysis of infected Vero-E6 cells after ultrasound treatment. Viral spike protein was detected in green, while viral double-stranded RNA (dsRNA), a replication intermediate, was detected in red. Nuclei were counterstained with DAPI (blue). Scale bar: 50 µm.

Figure 4 – Frequency-dependent efficiency of ultrasound inactivating SARS-CoV-2. Viral infectivity of SARS-CoV-2 wild-type (WT) was quantified by $TCID_{50}$ assay following ultrasound exposure for 1, 5, or 10 min at different frequency modes. Data are presented as mean ± SEM from at least two

independent experiments. Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test.

Figure 5 – Ultrasound-mediated physical mechanisms at biological interfaces. Panel a illustrates the classical cavitation pathway. When ultrasound is applied in the kHz range (**Step 1**), acoustic waves promote the nucleation and growth of bubbles within the medium. As these bubbles oscillate, they generate local heating and reactive chemical species such as H+ and OH− (**Step 2**). Ultimately, violent bubble collapse produces shock waves, microjets, and intense mechanical stress (**Step 3**), leading to indiscriminate fragmentation of both host cells and viral particles (**Step 4**). Panel B depicts the resonance pathway, which operates in the MHz range and reveals a distinct physical mechanism. When highfrequency ultrasound impinges on a viral particle (**Step 1**), part of the acoustic energy is transferred to its surface (**Step 2**). Because of impedance mismatch at the virus–medium interface (**Step 3**), energy is efficiently absorbed by the virion, exciting its natural vibrational modes (**Step 4**). These vibrations drive cyclic compression and rarefaction (**Step 5**), creating alternating states of potential and kinetic energy that accumulate within the viral envelope (**Step 6**). As the energy builds up, the viral shell becomes mechanically destabilized and loses structural integrity. In parallel, host cells exposed to the same acoustic field (**Step 7**) do not absorb significant energy (**Step 8**), and therefore maintain their normal morphology and structural integrity (**Step 9**).

Figure 6 – Temperature and pH during ultrasound exposure. (**a, b**) Under resonance conditions (7.5 MHz), both temperature and pH remained stable over 30 min, as measured with a digital thermistor and pH meter, respectively. (**c, d**) In contrast, exposure in a low-frequency ultrasonic bath (42 kHz, cavitation-prone conditions) resulted in progressive temperature increase and medium acidification. Data are presented as mean ± SEM from at least two independent experiments. Statistical analysis was performed using two-way ANOVA followed by Šídák's post hoc test.