学 位 論 文 の 要 旨

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学　位　論　文　名	Effects of Strain Differences, Humidity Changes, and Saliva Contamination on the Inactivation of SARS-CoV-2 by Ion Irradiation
発　表　雑　誌　名	Viruses
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論 文 内 容 の 要 旨

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for COVID-19, has a positive-sense, single-stranded RNA genome of ~30,000 nucleotides and contains structural proteins such as spike, envelope, membrane, and nucleocapsid. The spike protein facilitates viral entry via angiotensin converting enzyme 2 (ACE2) receptors. Although SARS-CoV-2 possesses RNA proofreading via NSP14, its high replication rate leads to frequent mutations and the emergence of variants of concern (VOCs), which differ in transmissibility, pathogenicity, and vaccine response.

While vaccines and antiviral agents have been developed, frequent S gene mutations challenge their long-term efficacy. Thus, physical and chemical inactivation methods, such as disinfectants and ion irradiation, offer alternative virus control strategies. Ion generation, producing ions and ozone from air molecules, can inactivate viral RNA and proteins.

Environmental factors like humidity and saliva may influence ion effectiveness. Moderate humidity is linked to COVID-19 outbreaks, and saliva may both protect and degrade viral particles. However, their combined impact on SARS-CoV-2 inactivation remains unclear. This study examines how humidity and saliva affect the ion-mediated inactivation of SARS-CoV-2 VOCs on surfaces.

MATERIALS AND METHODS

Cell Cultures

VeroE6/transmembrane protease serine 2 cells were cultured at 37◦C in 5% CO_2 in Dulbecco's modified Eagle medium/Ham's F-12 medium supplemented with 5% fetal bovine serum and 1% penicillin–streptomycin mixed solution.

Viruses

The SARS-CoV-2 Wuhan strain (Pango lineage: A, hCoV-19/Japan/TY/WK-521/2019), as well as the Alpha (Pango lineage: B.1.1.7, hCoV-19/Japan/QK002/2020), Delta (Pango lineage: B.1.617.2, hCoV-19/Japan/TY11-927/2021), and Omicron (Pango lineage: BA.1, hCoV-19/Japan/TY38-873/2021) variants were provided by the national institute of infectious diseases (NIID, Tokyo, Japan).

Virus titration

The 50% tissue culture infectious dose was determined by the Behrens–Karber method.

Treatment of viruses with ions

The experiments were conducted in a sealed humidity-controlled container (50 x 31 x 30 cm^3) with a built-in ion-generating electrode. In addition, the inactivating effect of ions on the virus was studied under different humidity conditions (30%, 60%, and 80%) using viral solutions containing 90% mixed saliva.

Humidity conditions

Humidity was controlled using a Humiai BLE-SD12-010 (Saginomiya, Tokyo, Japan). Ion irradiation was performed in containers maintained at humidity levels of 30%, 60%, and 80%.

Statistical analysis

Student's t-test was used to analyze the statistical difference between the two independent groups. An ANOVA test was used to analyze the statistical differences among more than three independent groups. Moreover, statistically significant differences in the reduction in viral infectivity between each strain were evaluated using Tukey's multiple comparison test. Data are expressed as the mean ±standard deviation. Statistical significance was set at $p < 0.05$.

Saliva

Saliva was obtained from individuals who provided informed consent, the study protocol was approved by the Research Ethics Committee of Shimane University (20210426-3).

RESULTS AND DISCUSSION

SARS-CoV-2 primarily spreads through airborne and contact transmission. While high humidity generally increases virus stability, seasonal data show that COVID-19 cases decline in high humidity and rise during dry winters. Temperature plays a minimal role in virus attenuation, making indoor humidity a key factor in viral persistence. At 30% humidity, SARS-CoV-2 showed natural inactivation over time, but the Omicron strain was more resistant than other strains. Ion irradiation enhanced inactivation, especially for Wuhan, Alpha, and Delta strains at 60 min. Omicron required 90–120 min for a comparable reduction. At 60% humidity, infectivity decreased over time, and ion irradiation became more effective after 90–120 min. Omicron showed minimal response to ion irradiation at 60 and 90 min but had a >100-fold reduction at 120 min. At 80% humidity, viral inactivation by ion irradiation was slower. The Wuhan strain needed up to 240 min for significant reduction. Omicron was less responsive at 80% humidity than at 30% over shorter durations.

Saliva reduced the inactivation effect of ion irradiation. For Wuhan, saliva inhibited inactivation at 30% and 60% humidity but allowed gradual reduction at 80% humidity. For Omicron, inactivation began earlier (60–90 min) at 30% and 60% humidity, even in the presence of saliva. When Omicron was mixed with saliva, more than 120 min of ion irradiation was required to achieve a >1000-fold reduction at all humidity levels. Saliva slightly enhanced natural decay but protected the virus from ion-based inactivation.

This study evaluated ion irradiation as a safer alternative to UV light for virus inactivation. When integrated into air systems, ion irradiation can deactivate airborne and surface-bound viruses. Across all VOCs, natural decay occurred over time, but ion irradiation significantly accelerated inactivation, especially at 30% humidity within 60–90 minutes. Higher humidity (60–80%) required longer irradiation for similar effects.

Saliva was found to slow viral inactivation, particularly for the Omicron strain, likely due to protective effects against drying. Nonetheless, ion irradiation still reduced infectivity, although to a lesser extent at higher humidity levels.

The differences in properties that lead to the resistance of the Omicron variant to inactivation compared to other strains can be speculated to arise from mutations in the structural protein genes. The Omicron variant carries significant mutations in the spike protein, envelope protein, membrane protein, and nucleocapsid protein, which makes the Omicron strain markedly different from the other strains. Particularly, amino acid mutations in the spike protein differ by as many as 30 sites from the Wuhan strain. Moreover, the receptor-binding domain of the spike protein, which in the Wuhan strain binds to ACE2 only partially, can bind to ACE2 entirely in the Omicron variant.

Our data support ion irradiation as a promising strategy to reduce indoor viral loads, especially under controlled humidity.

CONCLUSION

In conclusion, ion irradiation effectively reduced the number of infectious viruses compared to the control at all humidity levels. However, the Omicron variant required longer (120 min) irradiation at 60% humidity compared to the other VOCs. Irradiation is extremely effective, even if the reduction effect is only 50%, highlighting its relevance in preventing the rapid spread of SARS-CoV-2.