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Instability of nucleic acids in airborne microorganisms under far infrared radiation

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Abstract

Emergence of zoonotic-human pathogens is proven to be a lethal threat to public health, and RNA virus including influenza viruses, severe acute respiratory syndrome coronavirus, middle east respiratory syndrome coronavirus, Wuhan coronavirus (COVID-19), plays a pivotal role. As those viruses as airborne microorganisms spread mainly by tiny airborne particles, it is important to de-active those airborne particles before their entry into human bodies. In this study, we investigated the effect of far infrared (FIR) radiation on inhibition of airborne microorganisms. The result confirmed that double stand DNA from airborne microorganisms were stable under mild FIR radiation. However, single strand RNA from them was found to be sensitive to FIR radiation, indicating that RNA virus in airborne particles is instable under FIR radiation. Based on this observation, two models on usage of FIR radiation to prevent RNA virus transmission and cure RNA virus infection were proposed, implying that FIR radiation might be a cheap, convenient, and efficient method in clinic to treat RNA virus.

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Introduction

RNA virus, such as influenza virus and COVID-19 virus, has been inflicting human being for a long period. In history, “Spanish” influenza pandemic occurred worldwide and this influenza virus had killed 50-100 million people in 1918-1919 (Taubenberger et al. 2019; Okland et al., 2019). Today, influenza virus still affects 9-35 million people each year, and the annual rate of influenza-associated death in the United States overall ranged from 1.4 to 16.7 deaths per 100,000 persons (de St Maurice et al., 2020; Kandula et al., 2019; Thompson et al., 2009). Till February 19, 2020, COVID-19 virus has been spreading to over 27 countries with more than 2,000 deaths and 70,000 confirmed cases (Wang et al., 2020; Zhu et al., 2020). As traditional immune methods take a long period to prevent and cure RNA virus infection, new convenient ways that can be used immediately in clinic are eagerly needed.

In winter, far infrared (FIR) heating fan is always used to warm homes in China, which in fact makes FIR fan everywhere. The FIR fan generates FIR light to heat the air, which is a type of electromagnetic radiation. Generally, FIR wavelength ranges from 5.6 – 1000 μm, which is above those of microwaves and longer than those of visible light (Shui et al., 2015). Thus, FIR is invisible to the human eye, and in fact the human itself emits FIR (Li et al., 2017). FIR can penetrate up to about 4 centimeters beneath human skin, and can stimulate proliferation of cells and tissues, which makes FIR a promising treatment for cardiovascular malfunction, pain, inflammation, skin itches and some chronic health problems (Fujita et al., 2011; Beever et al., 2010; Oosterveld and Rasker, 2009; Hausswirth et al., 2011). As

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modern FIR fans have eliminated the near and middle infrared radiation, thus the fans efficiently deliver almost pure FIR radiation, which makes FIR fans safe and effective for physical health, stress, fatigue and ordinary usage (Vatansever and Hamblin, 2012). As FIR radiation is a non-ionizing form of radiation, thus it does not affect double strand DNA under normal conditions (Fischer et al., 2002; Young et al., 1989; Wittlin, et al., 1986). However, currently if FIR radiation could induce degeneration of RNA in airborne microorganisms is largely elusive. In this study, we measured the stability of RNA in airborne microorganisms under different FIR radiation dosages. The result showed that single strand RNA from them was found to be sensitive to FIR radiation, indicating that RNA virus in airborne particles is instable under FIR radiation. Then, this study theoretically implies that FIR radiation is a convenient way that can be used immediately in clinic to prevent and cure RNA virus infection.

Results

FIR could efficiently induce degradation of RNA, but not double strand DNA, in airborne microorganisms

We carefully chose two rooms for this experiment, which mainly depends on their air-tight status and contains just one door and one air-tight window each room. Further, the two rooms have same size (width x length x height: 2.5 meter x 8.5 meter x 2.4 meter) with same volume, 51 m^3. Before this experiment, we intentionally opened the windows to let environmental air enter into the room (Figure 1). The FIR fan was purchased from a local supermarket, which used FIR generating tubes to specifically emit FIR radiation. We putted

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one FIR heating fan into one room, and powered it on at the 400 wattage for different time. After those treatments, we closed the door air-tightly and collected the air-particles in the room by a simplified air-particle collector (Figure 1C).

After same amount volume of air was filtered, the collected particles in air were subjected for nucleic acid extraction. The result was shown in Figure 2. For double strand DNA, we could not detect its degradation significantly after FIR radiation (Fig 2A). However, for RNA, we readily found that RNA was sensitive to FIR radiation (Fig 2B). In a room with total volume 51 m^3, after FIR radiation at 400 wattage for 30 minutes, RNA degradation was slightly shown. In the same room, after FIR radiation at 400 wattage for 4 hours RNA degradation was significant. Taken together, this result indicates that FIR radiation under proper dosage is capable to induce degradation of RNA efficiently, but not double strand DNA, in airborne microorganisms.

The proposed models for clinic treatments on RNA virus infection by FIR radiation

RNA viruses, such as influenza virus and COVID-19 virus, rely on their genetic materials, RNAs, for survival (Shafiuddin and Boon, 2019; Lu et al., 2020; Zumla et al., 2020; Li et al., 2020). As the data from in this study shows that FIR radiation is capable to induce RNA degradation in total airborne microorganisms, in theory FIR radiation has the potential to destroy RNA viruses in air. Based on this scientific deduction, we proposed two models for clinic treatments to RNA virus infection. First, in the RNA virus infected patient's home and infected open areas, FIR fan could be used to eliminate them in air. For non-infected homes and open areas, such as supermarkets and streets, FIR also could be applied to prevent RNA virus contamination and infection. The first model is shown in figure 3A.

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Second, RNA virus infected patients themselves could be treated by FIR radiation. FIR radiation could be applied directly into patient's mouth to diminish large amounts of RNA viruses on surface of cells, which could shut off the circulation of them and improve lung activation by absorbing fresh and clean air. As FIR radiation could improve cell proliferation (Jeong et al., 2017), it might improve highly injured lung to recover. Further, as FIR radiation could penetrate about 4 cm tissue (Mero et al., 2015), it would be very helpful to apply FIR radiation to RNA virus infected patients' skin to improve cell activity and inhibit RNA virus in blood. The second model is shown in Figure 3B.

Discussion

In fact, Beijing is a big city with considerable air contamination and high PM2.5 value. In current experiment, we could not rule out the possibility that air pollution might play a role. In future, more sites and cities should be tested.

The proposed two models on treatments for RNA virus infection, were sheer based on the fact that FIR radiation can induce degradation of RNA in airborne microorganisms; however, we could not completely rule out the possibility that some of them could survive by unknown mechanisms. Further, as the genomic DNA after FIR radiation was examined by agarose gel electrophoresis, it is possible that minor breaks in the genomic DNA were not detected largely by this method. Furthermore, FIR is considered to be quite safe, but excessive exposure to FIR is harmful to the skin and the eyes through photo-aging (Holzer AM, Athar M, Elmets CA. 2010; Chiang C, Romero L. 2009). Thus, usage of FIR radiation to prevent RNA virus contamination and cure RNA virus infection clinically, should be

carefully monitored by workers, nurses, doctors and therapists.

Currently, the exact molecular mechanism on how FIR radiation induces instability of RNA from airborne microorganisms is unknown. As RNA is subjected to spontaneous degradation under with high pH, metal ions and high temperature (Russell and Harries, 1968; Kaga et al., 1992), it is possible that it might become fragile and break after the airborne microorganism is overheated after absorbing excessive FIR energy.

Taken together, this study showed that single strand RNA from total airborne particles was degraded after FIR radiation, indicating that RNAs of RNA virus and other microorganisms in them are unstable under FIR radiation. As RNA virus, such as influenza virus and COVID-19 virus, is spreading worldwide and causes large casualties, our study implies that FIR radiation might be a cheap, convenient, and efficient method that can be used immediately in clinic to treat RNA virus.

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Method

Materials and reagents. The FIR heating fan was purchased from a local supermarket. Its physical properties are listed below: defined voltage: model: DF825 (Pioneer, Inc); 220 V; weight: 1.4 kg; defined wattage: 400/800 W. The rooms used for the experiments were two rooms with similar size (width x length x height: 2.5 meter x 8.5 meter x 2.4 meter) in the first floor near a fresh food market in Beijing. Before those experiments, the air-tight windows of the two rooms were open to let the environmental air to entry. From December 1, 2013 to June 1, 2014, air particle samples were taken every two weeks. DEPC (diethyl pyrocarbonate) was purchased from Sigma, and Trizol was purchased from Thermo Fisher company.

Sample collection. Before sampling, the air-tight windows and doors were closed to prevent interference of environmental air particle. In control room, there were not any treatments. In another room, a FIR fan was used to radiate the air for different time. Then, a simplified air-particle collector (Figure 1C) was used to accumulate particles in the air. The 1000 ml collection bottle was first filled with 300 ml DEPC-treated RNase-free water with 0.001% gelatin. The inlet of this sampler was put at a fixed height of 0.5 meter above the ground. The vacuum pump has a speed on 6 L/min (6 liters air per minutes) to suck air. After 10 hours, a total of 3.6 m^3 of air was sampled. The collected samples were stored at - 80°C.

total RNA extraction. One hundred-milliliter samples were taken and subjected for ultrafiltration using Amicon Ultra-15 centrifugal filters. After the samples were concentrated to about 2 ml, the enriched samples (100 µl each tube) were extracted by Trizol (Thermo).

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Following the manufacturer's protocol, total RNA of the sample was collected. Briefly, 100 µl of the enriched sample was mixed with 1 ml Trizol, and incubated at room temperature for 5 minutes to completely degrade cell membranes. After incubation, the lysed samples were added 200 µl of chloroform, and mixed gently. Then, the sample was subjected for centrifugation (12,000 g for 15 minutes at 4°C). Upper aqueous layer was then pipetted into a new tube and 500 µl isopropanol was added. The mixture was centrifuged for 12 minutes at 12,000 g at 4°C. the supernatant was discarded and washed three times by 75% ethanol. Then, the pellet was air dried and dissolved into 20 µl DEPC-treated RNase-free water.

Total genomic DNA extraction. When 100 µl of the enriched sample was transferred into a 1.5 ml tube containing 400 µl of lysis buffer (1 mM EDTA, 1% SDS, 10 mM NaCl, 20 mM Tris–HCl, pH 8.2). Then, 100 µL Proteinase K (20 mg/mL) was added into the tube. After the whole mixture was vortexed to be homogeneous, it was incubated at 60°C for 6 hours. Equal volume of cold absolute ethanol was further added into the mixture and incubated for 1 hour on ice. The total mixture was subjected for high-speed centrifugation (14,000 rpm for 20 minutes), and the pellet was washed by 400 µl of pre-cold 75% ethanol for three times. After the pellet was air dried, it was dissolved in 200 µl autoclaved ddH_2O.

DNA agarose gel electrophoresis. After equal volume of samples were mixed with loading buffer, the mixture was loaded into wells of 0.6% TBE agarose gel. Initially, 3 V/cm was applied for 30 minutes, then 9 V/cm was applied for about 1 hour. Then the bands stained with ethidium bromide. Images were taken using a UV transilluminator (Bio-Rad).

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RNA non-denaturing agarose gel electrophoresis. Taking 20 µl RNA aliquot and heating the sample at 70°C for 2 minutes, then immediately putting it on ice. After equal volume of RNA samples were mixed with loading buffer, the mixture was loaded into wells of 1.2% TAE agarose gel. Initially, 5 V/cm was applied for 30 minutes, then 8 V/cm was applied for about 1 hour. Then the bands were stained with ethidium bromide. Images were taken using a UV transilluminator (Bio-Rad).

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Figure legends

Figure 1. Schematic representation of experimental design. (A) A diagram of FIR fan. It can swing its head and emit FIR radiation. (B) Tow rooms with same volume were used for those experiments. One is the control room without FIR radiation, the other was treated by FIR radiation with varied time. (C) A diagram for the manually prepared airborne microorganism collector. For preventing RNase contamination, DEPC treated bottle and water were used.

Figure 2. the FIR radiation induced degradation of RNA but not double strand genomic DNA in airborne microorganisms. (A) After FIR radiation, genomic DNA extracted from airborne microorganisms remained intact. (B) After FIR radiation, RNA from airborne microorganisms were degraded in terms of treatment period.

Figure 3. Two models for treatment of RNA virus, such as influenza virus and COVID-19 virus, by FIR radiation. (A) Elimination of environmental RNA virus by FIR radiation to prevent spreading and contamination of RNA virus. (B) Curing RNA virus infected patients by applying FIR radiation directly into the patients' mouths or on the patients' skin.

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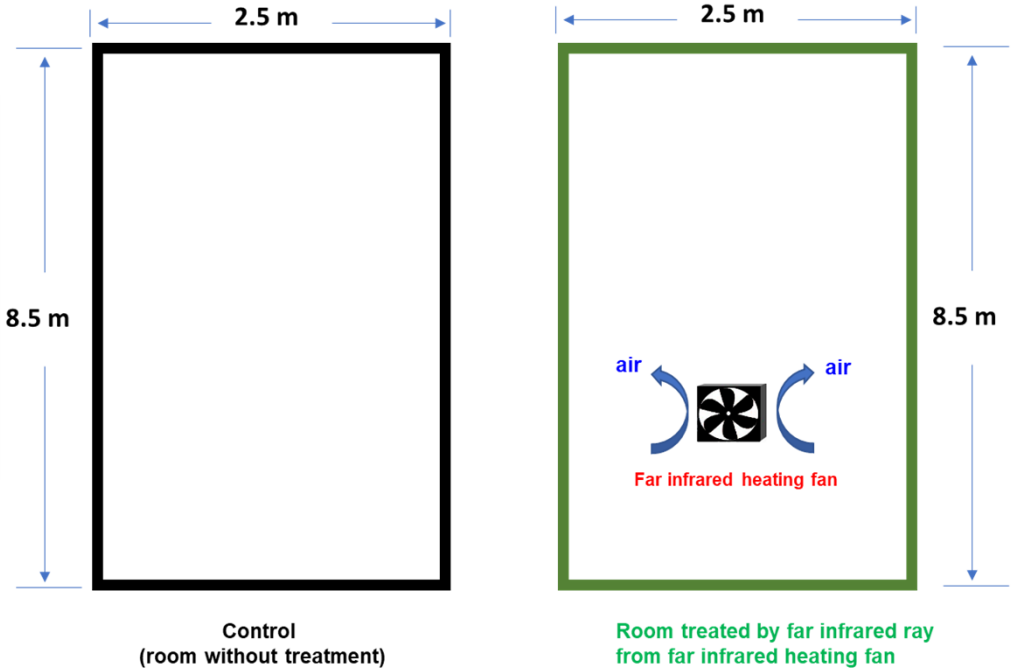
Figure 1

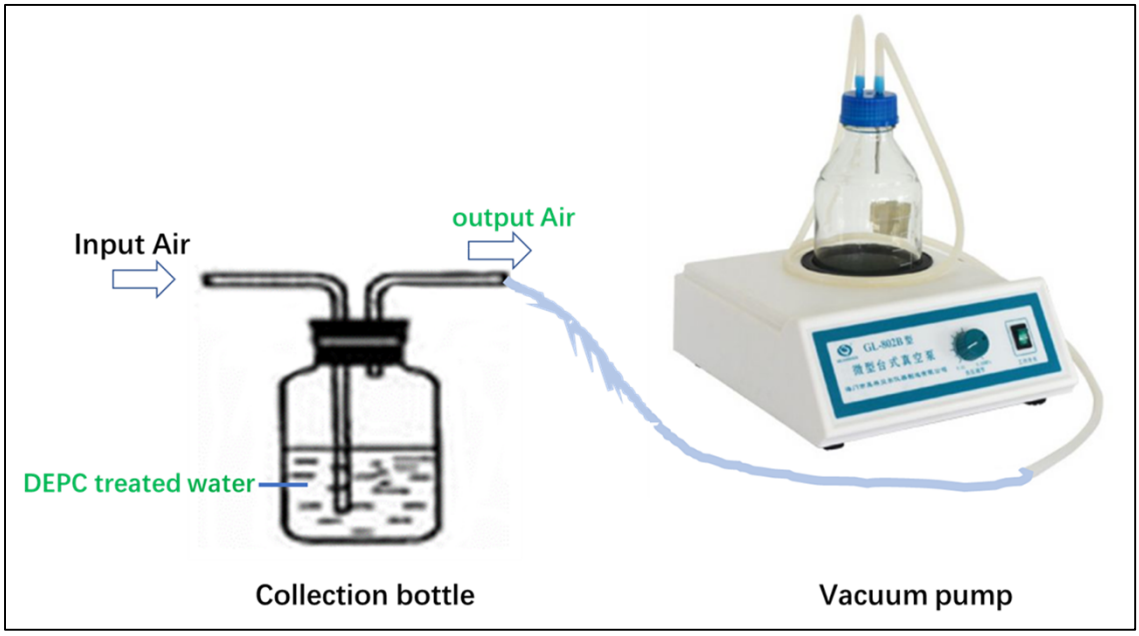
A

Far infrared heating fan

B



C



Simplified air particle Collector

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Figure 2

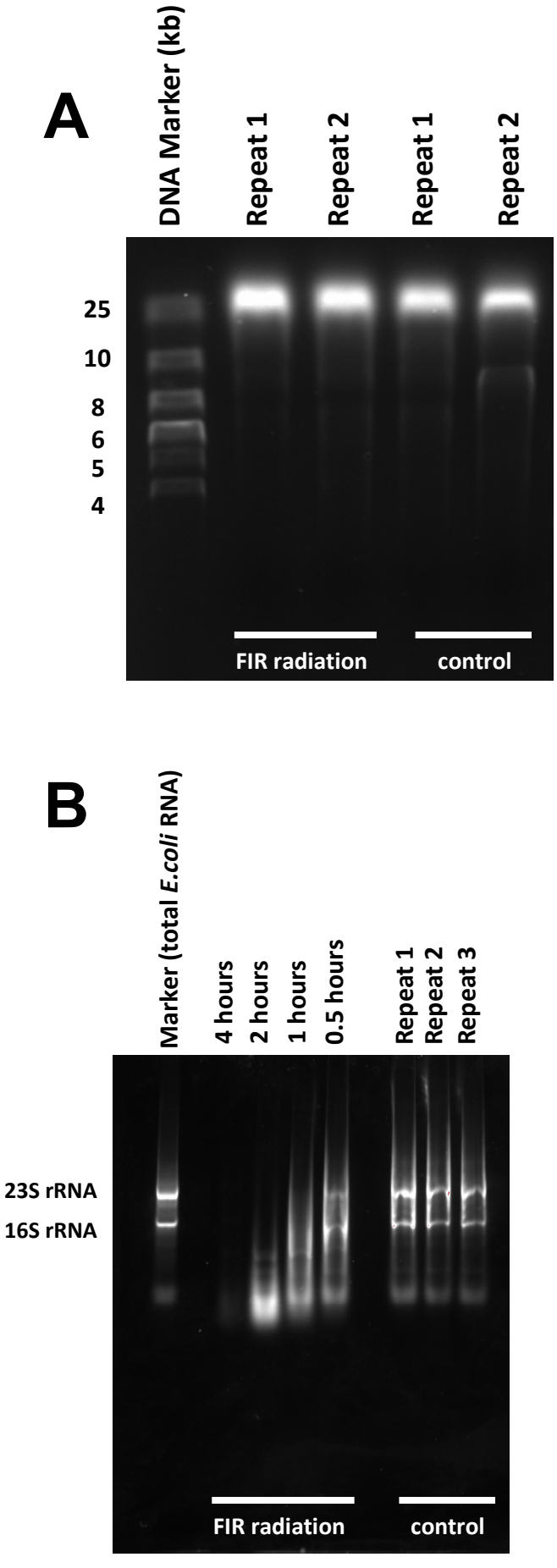

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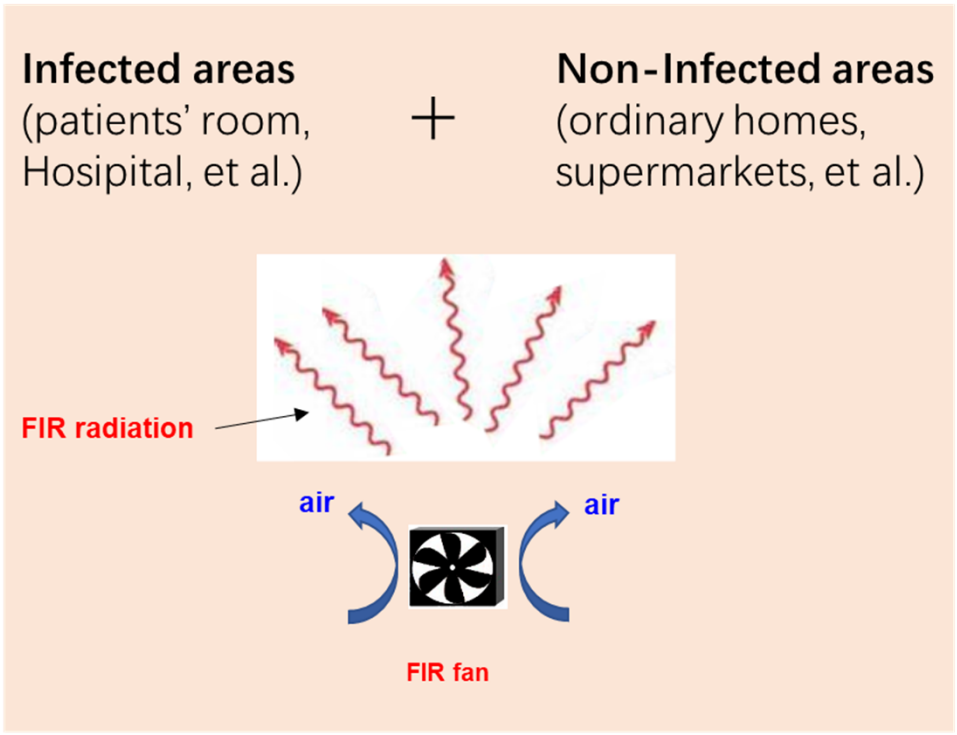
Figure 3

A



B