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Microstructure of atmospheric particles revealed by TXM and a new mode of influenza virus transmission

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ABSTRACT

For control of influenza, firstly it is important to find the real virus transmission media. Atmospheric aerosol particles are presumably one of the media. In this study, three typical atmospheric inhaled particles in Shanghai were studied by the synchrotron based transmission X-ray microscopes (TXM). Three dimensional microstructure of the particles reveals that there are many pores contained in, particularly the coal combustion fly particles which may be possible virus carrier. The particles can transport over long distance and cause long-range infections due to its light weight. We suggest a mode which is droplet combining with aerosol mode. By this mode the transmission of global and pandemic influenzas and infection between inland avian far from population and poultry or human living in cities along coast may be explained.

1. Introduction

Epidemiological studies have found that a consistent increase in cardiac and respiratory morbidity is strongly correlated with the exposure to aerosols or particulate matter (PM), i.e. the inhaled particles PM10 (aerosol particles in aerodynamic diameter of less than 10 μm) fraction and especially fine particles PM2.5. The inhaled particles, with an aerodynamic diameter <10 μm (PM10), can reach the lower respiratory tract through main tracheal and bronchioles. The fine particles PM2.5 (aerosol particles in aerodynamic diameter of less than 2.5 μm), occupied a large fraction in PM10, can well reach the alveolus and penetrate the bloodstream [1,2]. Coarse particles in size larger than PM2.5 even PM10 tend to get caught in the nose and throat [3,4]. Somers et al. did a follow-up experiment with mice and found increased induction of DNA changes in the offspring of mice housed in a polluted location at the harbor compared with control animals housed in an unpolluted location [5]. They also found that mutation rate could be reduced by ~50% by cleansing the air with a high-efficiency-particulate-air (HEPA) filter [6]. It was reported that aerosol particles could induce heritable mutations [7]. Moreover, the aerosol particles could be a media as virus transmission, in addition to the routes of large droplets and direct contact with secretions or fomites [8]. In 2003, during the period of the outbreak of severe acute respiratory syndrome (SARS) researchers found in Sino-Japanese Friendship Hospital, Beijing that SARS virus could spread ~30 m via PM, while it is within 5 m via medias of spittle, secretions and closed contact [9].

The fine PMs, from various pollution sources (vehicles, industries, and soil), can transport over a long distance because of their long settling time [10], and this causes concerns over potential long-range infections by virus-carrying particles. It is considered that the health risk of PM predominantly associates with size and composition of the fine particles, but their microstructure can be an important factor for the adverse health effects, too. Comparing two fine particles of the same diameter, one being solid spherical and another being of complicated rough surface with pores, the latter would have greater impact to human health due to its larger area to interact with lung tissue, stronger adsorption abilities of toxic chemicals (such as polycyclic aromatic hydrocarbons), and stronger binding to pathogenic microorganisms. Therefore, it is worthwhile to study PM microstructure.

Generally, PM microstructure can be examined by scanning electron microscope (SEM) [11] and transmission electron microscopy (TEM) [12]. SEM provides surface topography of a sample and TEM provides inner information of a sample, and both provide just two dimensional information of sample microstructure. Based on a synchrotron radiation facility, however, the transmission

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X-ray microscope (TXM) enables one to "see" a sample at a series of measurement angles and obtain three dimensional structure of the sample after image-reconstruction. The measured depth of TXM can be up to a few hundreds μm, while that of TEM is only a few μm.

In this paper, microstructures of the inhaled particles emitted from coal combustion, metallurgic dust and vehicle exhaust in Shanghai are studied for the first time by synchrotron-based TXM. The structures which cannot be detected by SEM and TEM are revealed, and the role of the PM play is discussed in terms of their health impact to human body.

2. Experimental methods

It was found that the main source of aerosol particles in Shanghai are coal combustion, metallurgic dust and vehicle exhaust [13]. Samples of coal combustion were collected at Shi Dong Kou power plant, the largest coal-fired power plant in Shanghai. Samples of car exhaust were collected from a Santana 3000 car on the engine test bench using model CYQ-26 air sampler at Shanghai Institute of Internal-combustion Engine. Samples of metallurgic dust were collected from a converter for steelmaking at Bao Steel, the largest steel industry in Shanghai. The particles were delivered depressively to a 0.3 μm thick Mylar film adhered to an aluminum frame with a hole of diameter 10 mm for TXM experiments.

The TXM experiments were performed at beam-line 32-ID at the Advanced Photon Source, Argonne National Lab, IL, USA. A schematic of the experimental setup is provided in Fig.1. The electron storage ring was run at 7 GeV 102 mA. Monochromatic X-rays were produced using a double-bounced Si(1 1 1) monochromator and double-reflection vertical harmonic rejection mirrors. A set of elliptically shaped glass capillary condenser (73 mm in length and with an inner diameter ranging from 0.90 to 0.84 mm) was used to focus the incident X-rays on a sample. The sample is rotated through 180 degrees with sample projections produced at specified angle intervals and exposure times. X-rays transmitted through the sample are magnified by a Fresnel zone plate objective lens and captured by a high resolution charge couple device (CCD). In this full-field X-ray transmission measurements, the condenser with the inner diameter of 0.9 mm was chosen to maximize the vertical acceptance of the undulator beam at 65 m from the source. An Au Fresnel zone plate with a 45 nm outmost-zone width and 900 nm thickness was used as an objective lens to produce a magnified image of the sample on an optically coupled high resolution charge-coupled (CCD) device system. The estimated flux of monochromatic X-ray passed from a Si(1 1 1) double crystal monochromator and focused by the condenser was 2×10^{11}/s at 8 keV. The high brightness and the optimized condensers design to yield an excellent imaging throughput of 50 ms/frame with $\sim 1 \times 10^4$ charge coupled device counts per pixel. Spatial resolution for this TXM setup is around 30 nm. For three-dimensional images by X-ray nanotomography, a total of 141 sequential tomographic images (2D) were automatically collected from −90 to +90 degree with a 50 ms per image measurement time at X-ray energy of 8.0 keV. The image reconstruction was performed using the IDL code. All reconstructed images were treated by using the Amira 5.2 code to build three dimensional images. Details of this TXM system are provided by Shen et al. and Grew et al. [14,15].

3. Results and discussion

3.1. Microstructure of atmospheric particles

3.1.1. Coal combustion particles

The particles from coal combustion, metallurgic dust or vehicle exhaust, are mostly of spherical shape, as shown in Fig. 2, an SEM image of the particles collected from coal combustion. SEM

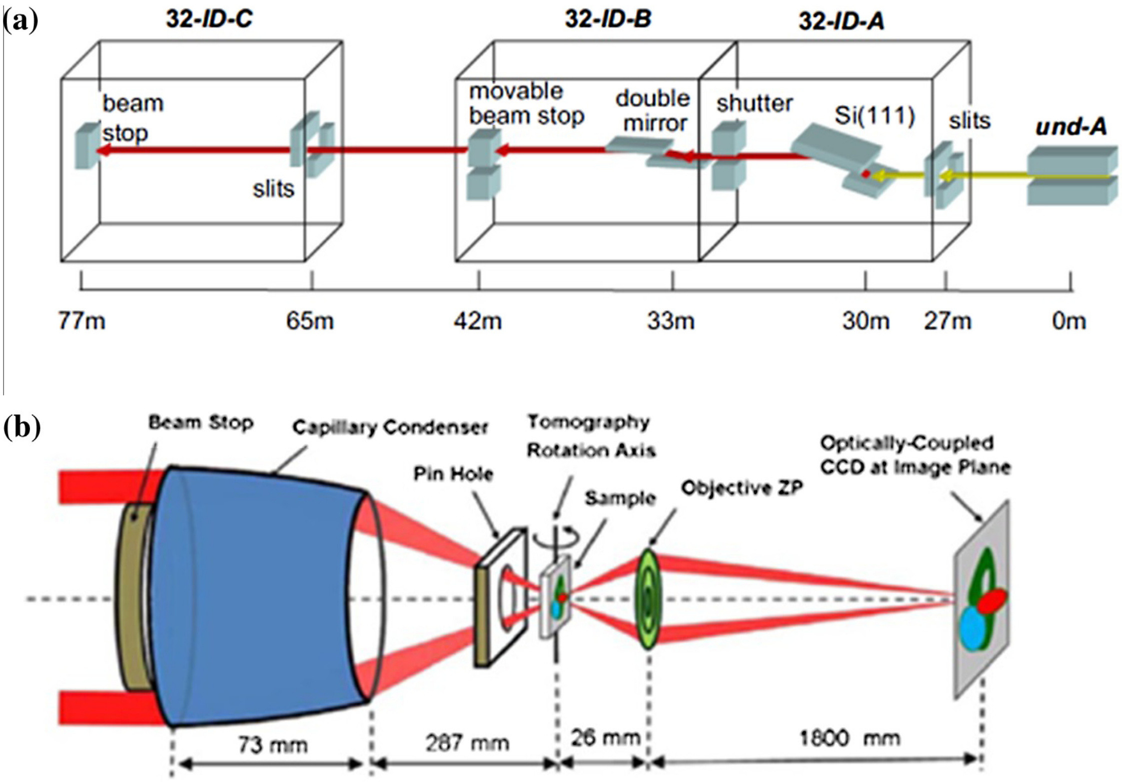

Fig. 1. A schematic of the TXM experimental setup. (a) Schematic optics layout of 32-ID (not to scale) [14], (b) schematic of the full-field TXM [15].

imaging of the particles from various emission sources reveals differences in their size distribution, particle aggregation and surface roughness, but it cannot find details of inner structure of an atmospheric particle, while TXM provides not only the surface information but also the inner structure. Fig. 3a shows the feature of a particle from coal combustion detected at a certain angle. The particle is spherical, in diameter of 9 μm, containing many pores inside and adhering several small particles aggregated on its surface. Fig. 3b is the reconstructed 3 D image with the 141 projections, and it did provide more detailed configuration of this particle. After looking at the 3D graphic at different angles we could be sure that its surface had two separate small particles with a size of around 2 μm and the inner pore locations, as indicated by the gray and white arrows, respectively. We note that an opening size at about 1.5 μm (the red arrow) in the particle surface may well be a place for accommodating virus or bacteria. Fig. 3b also shows that mass density of the particle is quite inhomogeneous, caused probably by pore overlapping.

3.1.2. Metallurgic dust particles

Fig. 4 shows a typical 3D tomography reconstruction of the particle of 6 μm diameter from metallurgic dust. It is quite different from the coal combustion particle in Fig. 3. The particle is solid, without pore inside. Its spherical surface is adhered with smaller particles than the coal combustion particle. Some of the smaller particles are aggregated. In addition, both the larger and smaller particles have rough surface.

3.1.3. Particles from vehicle exhaust

It was reported that as SEM images showed, particles emitted from vehicle exhausts are of solid sphere, with a size distribution from nm to μm, and many particles are aggregated together [16]. However, real feature of the particles from vehicle exhausts can be revealed by TXM. Fig. 5a is a patchwork of TXM images. One finds that many particles are of quite similar shapes, i.e. sphere with inner structures. The particle of ~2.5 μm diameter, indicated by the red arrow, was selected to perform the measurements for 3D tomography reconstruction. In Fig. 5b, from the measurement at −83°, the particle looks like a football, while in Fig. 5c, a 3D tomography reconstruction of the particle shows its plate-like shape, rather than the spherical shape observed generally by SEM. The TXM measurements reveal a complicated structure of the football-shaped particle. It contains smaller particles of nm size and pores in surface, and has a skeleton inner structure. Because of the strong absorption contrast, the smaller particles may well be formed by metals, which may mainly consist of Fe element, worn off from the cylinders. In addition, there are openings in the particle surface (Fig. 6).

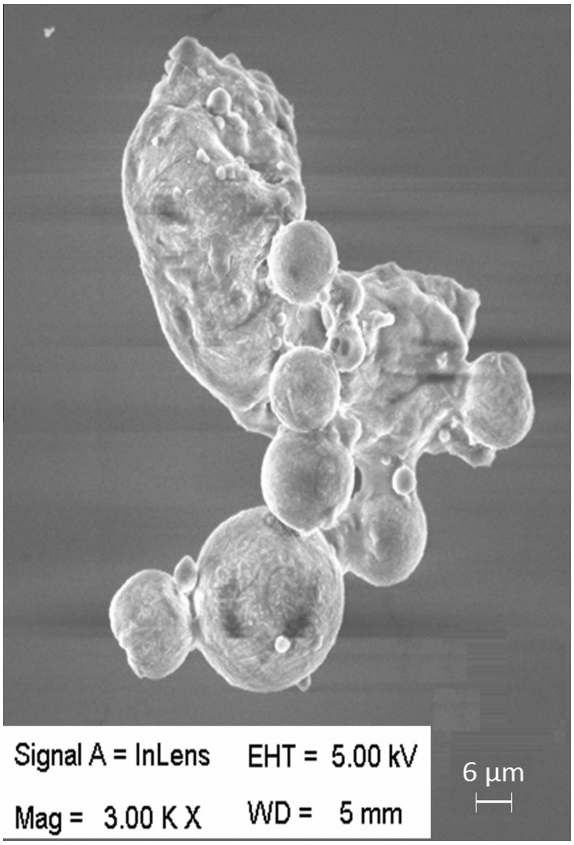

Fig. 2. SEM image of coal-combustion particles.

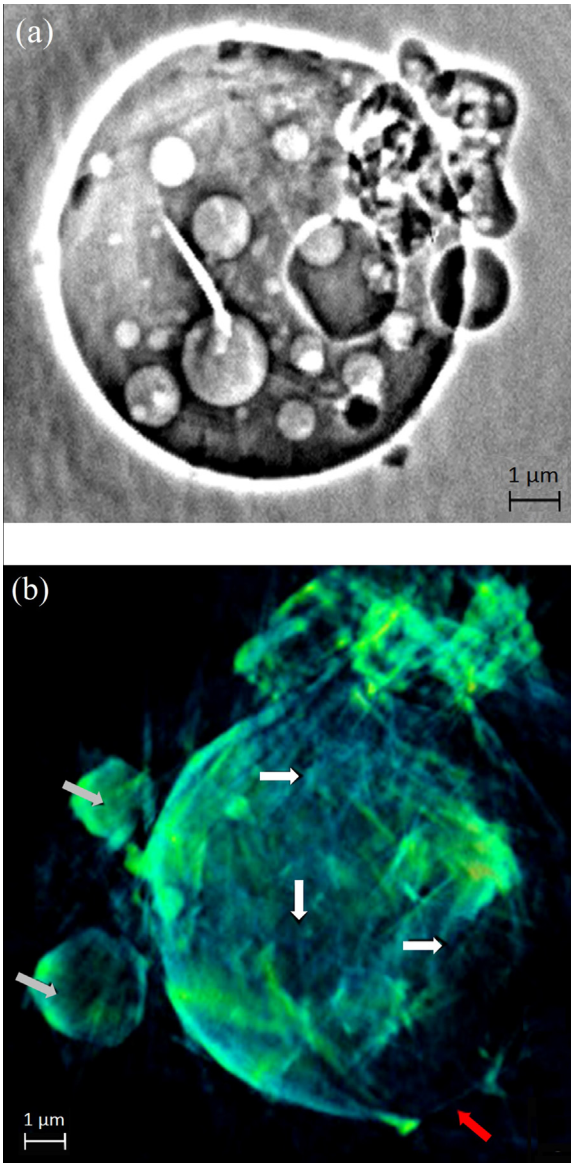

Fig. 3. TXM micrographs of a coal-combustion particles sized at 9 μm. (a) Feature of the particle detected at an angle, (b) 3D tomography reconstruction with 141 images for the particle. The gray arrows indicate separate smaller particles in the surface, the white arrows indicate the inner pores, and the red arrow indicates an open area in the surface. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

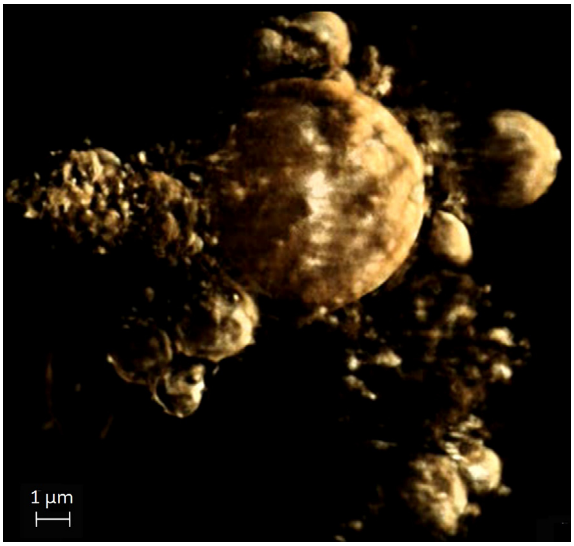

Fig. 4. 3D tomography reconstruction for a particle sized at 6 μm from metallurgic dust.

3.2. Aerosol particle is a possible media of virus transmission

Several authors have stated that large-droplet transmission is the predominant mode by which influenza virus infection is acquired [17]. The droplets containing influenza viruses are generated by coughing or sneezing from an infected person. Therefore, aerosols are infectious by the droplets, too. Despite the large-droplets transmission is predominant, but aerosol transmission is not negligible. Influenza viruses can be transmitted through aerosols, and this has been supported by published evidence [18–20]. Many virus are of spherical shape. H5N1 is of a sphere of 80–120 nm in diameter. The virus with many pins in its surface, H5N1 adsorbs cell of respiratory tract, penetrates membrane of cell and enters the nucleus. It can be imagined that the virus would adsorb on aerosol particles, especially pore-containing particles. We may figure out a pictures as shown in Fig. 5, a virus adsorbs a coal combustion particle at the position with a pore. With the time the virus may move inside or remain on the surface. Bean et al. [21] reported that the virus could survive for 24–48 h on hard nonporous surfaces such as stainless steel and plastics, and the virus inocula with complete drying on the surfaces survived only in 1.5 h. A suspended particle adsorbs moisture on the surface and pores, forming a good accommodation environment for viruses, and this seems better than an aqueous droplet, which evaporates rapidly and shrinks in size. William et al. [22] measured the amount and size of aerosol particles containing influenza virus that were produced by coughing, and they found that thirty-five percent of the influenza RNA was contained in particles >4 μm in aerodynamic diameter, while 23% was in particles 1–4 μm and 42% in particles <1 μm. These results show that coughing by influenza patients emits aerosol particles containing influenza virus and that much of the viral RNA is contained within particles in the respirable size range. The question of aerosol transmission of influenza virus has received attention by some healthcare facilities. For example, during the 2009 H1N1 pandemic, a United States Institute of Medicine (IOM) panel recommended that healthcare workers in close contact with influenza patients wear respirators to avoid infectious aerosols [22].

So far, it has been hard to explain and hard to find the transmission path of global and pandemic influenzas ever happened. The transmission of virus can be by poultry to human for short distance and by migratory birds and poultry for long distance. In 2005,

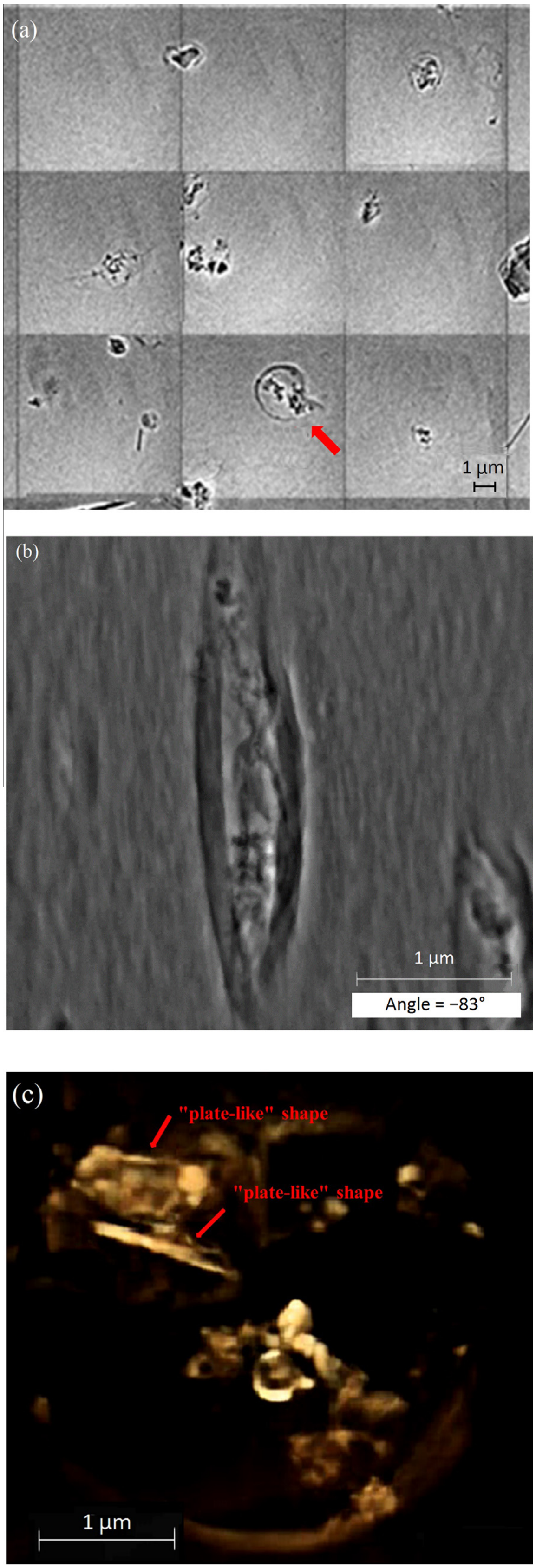

Fig. 5. Micrographs of the particles from vehicle exhausts. (a) A patchwork of TXM images of the particles on 0.3 μm thick Mylar film, a particle of 3 μm size (the red arrow) was used for tomography measurement; (b) an individual micrograph of the particle measured at −83°; (c) A 3D tomographically reconstructed image of the particle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

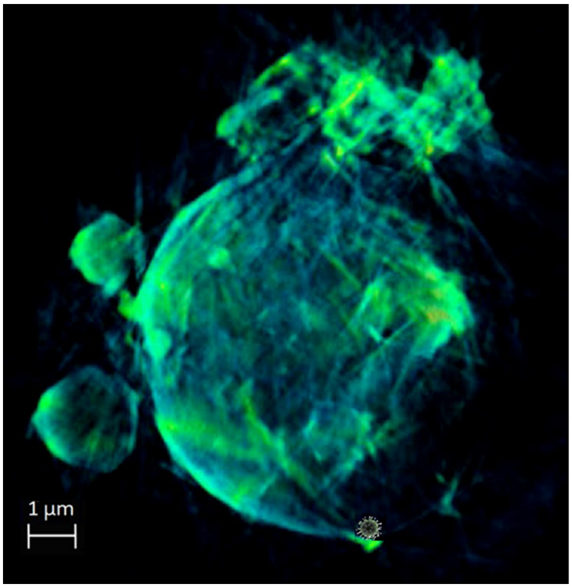

Fig. 6. A combination schema of a virus adsorbed at a surface pore on a coal combustion particle. The viruses typically have a size of 80–120 nm, in this figure the size of virus has be enlarged, otherwise it is hard to see.

2006, 2007, 2009 and 2010, Chinese microbiologists found the H5N1 virus in the birds at Qinghai Lake, which is an inland lake, hundreds kilometers away from large cities [23]. They are still busy with this study because the origin of the virus and routes of influenza transmission is not clear. We think that aerosol particles may play a role to transmit virus from poultry or human to avian, as the fine particles can transport over a long distance that they will remain airborne for a prolonged period of time because of their low settling velocity [19]. It was reported that the particles caused by earthquake occurred east of Japan on March 11, 2011 crossed the Pacific in just 3–4 days [24]. So, inland transportation of the particles should be in much shorter time, and the virus loaded in the particles survives.

After reviewing 26 studies Gralton et al. found that breathing, coughing, sneezing and talking of individuals form infections droplets of 0.05–500 μm [25]. The smaller droplets are dried rapidly in air, but when the virus-carrying droplets collide with aerosol particles, the virus may be adsorbed on the aerosol particles, and enter the pore afterwards. The virus-bearing aerosol particles can transport over a long distance, and virus may survive due to the accommodation condition of less evaporation. In this way, the viruses transmit to birds, poultry and human in places far away from original place. Therefore we suggest a combined mode of droplet and aerosol, the virus transmission between individuals who are long distances apart, even without migratory birds. The possibility of virus infection from original source to individuals far from the source depends on the virus intensity, aerosol particle concentration and structure, and wind direction and strength.

Considering the particles mentioned above, coal combustion fly particles have many pores sized from a few dozens of nanometer to one micrometer, both inside the particles or in the surface. These particles, in particular the fine particles can remain in air for a long time and transport over long distance due to their low specific gravity, leading to long-range infections if the virus is absorbed on the particles. In comparison with coal combustion fly particles the metallurgic dusts are mostly solid particles without pore, so at the same size as a coal fly particle, their transportation distance will be much shorter and the ability of carrying virus will be lower. For the vehicle exhausted particles the transportation distance and the ability of carrying virus could appear in between, based on its microstructure. The TXM results support the idea that the airborne route may be a pathway for influenza transmission. However, aerosol transmission of influenza virus is affected by the weather, the infectious dose, the amount of infectious particles aerosolized at the source, and the rate of biological decay of the infectious agent [19], further research is needed to confirm the model.

4. Conclusion

In summary, three main atmospheric particles in Shanghai were studied by TXM based on synchrotron. Our study shows that there are many pores contained in the coal combustion fly particles, which could be a possible candidate of media for transmission virus. We suggest a mode which is droplet combining with aerosol mode. By this mode the transmission of global and pandemic influenzas and infection between inland birds far from population and poultry or human living in cities along coast could be explained. Meanwhile it would be recommended that healthcare workers in close contact with the high lethality of avian influenza patients wear respirators instead of surgical masks to avoid infectious aerosols.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nimb.2015.07.050.

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