Original Article

Contamination of Live Virus during Tissue Homogenizing by Ultrasonic Processor and Tissue Disperser*

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Abstract

Objective To quantitatively evaluate the contamination area and risk of a live pathogen during tissue homogenization by either ultrasonic processor or tissue disperser.

Methods A recombinant Herpes Simplex Virus (rHSV) containing GFP gene was used as the index virus, and fresh liver tissue from healthy mice was used as simulated specimen. After 10% liver homogenate was mixed with rHSV (100 TCID50/0.1 mL) in a 5 mL tube, the stability of rHSV in liver homogenate and influences of an ultrasonic processor and a tissue disperser on viral infectivity were determined by GFP expressions in cell cultures. The contaminating areas of live viruses during homogenization were evaluated by a cell culture-based sedimentary. The contamination radii were counted by measurement of the distance between the operator and the farthest GFP positive well.

Results The infectivity of rHSV in 10% liver homogenate maintained almost unchanged after it was incubated at room temperature for 30 min. Treatment with an ultrasonic processor clearly dropped down the virus infectivity, while a disperser not. Obvious spills and slashes of live viruses were observed in processes of homogenization with those two apparatuses. The contamination radii are positively related with sample volume, output energy of operator and handling time.

Conclusion Homogenizing infectious samples with an ultrasonic processor and a tissue disperser at commonly used conditions caused obvious spills and splashes of live viruses, which possesses high risk to induce Laboratory acquired infections (LAIs).

Key words: Splash; LAIs; Homogenize

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BACKGROUND

Laboratory acquired infections (LAIs) were firstly reported around the start of the twentieth century. By 1978, four studies by Pike and Sulkin had collectively identified 4 079 LAIs between 1930 and 1978, resulting in 168 deaths[1]. In the following 20 years, 1 267 LAIs with 22 deaths had been described worldwide[2]. Although the reported numbers decreased in the past decade, LAIs still occur every year and the total numbers are up to 83 cases from 2000 to 2009

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involving many agents, e.g, Brucella abortus, Cowpox virus, Anthrax bacillus, SARS virus, tularensis bacillus, Meningococcus, West Nile virus, Ebola virus, vaccinia virus, Yersinia pestis[3-5]. Certainly, those cases do not represent all LAIs, since some laboratories usually do not report overt cases or conduct surveillance programs to identify sub-clinical or asymptomatic infections.

Tissue homogenization is one of the important processes for detecting potential pathogens. Meanwhile, it is also one of the main resources to produce infectious droplets and aerosols which may cause LAIs. Although the formations of droplets and aerosols during tissue homogenization have been widely recognized, it still lacks of definite determination of the contaminating areas of live pathogens during the process. We therefore used a recombinant herpes simplex virus (rHSV) expressing GFP as the model pathogen and the mice liver tissue as the simulated sample in this study to understand the risk of contamination from laboratory homogenate operations in more detail. The contaminating scores of viral droplets and aerosols produced by an ultrasonic processor and a tissue disperser were evaluated with cell culture. Our results showed that the contamination radii were positively correlative with sample volume, output energy of apparatuses, and handling time.

MATERIALS AND METHODS

Recombinant Virus

A rHSV expressing GFP was used as the index virus and propagated in HeLa cells as described previously[6]. Briefly, cells were absorbed with viruses in FCS-free DMEM at 37 °C for 30 min, and then maintained in the medium with 2% FCS for 48 h. Virus infectivity was evaluated by observation of fluorescence of the infected cells under a fluorescent microscopy (Olypus, Japan). The cell infective titer of this virus was determined by TCID50 (50% tissue culture infective doses) by endpoint dilution, and calculated with the method of Reed and Muench.

Homogenization of Liver Tissues with an Ultrasonic Processor or a Tissue Homogenizer

The fresh liver tissue from healthy mice (Balb/c SPF) was cut into pieces. 10% (w/v) liver homogenates were prepared in FCS-free DMEM by homogenization with an ultrasonic processor (Sonics VC750 USA) or a tissue disperser (IKA T8 Germany). The working parameters of the ultrasonic processor were 10 sec with an interval of 20 sec at energy of 200, 400 W for 1, 3, and 5 min, respectively, while those of the tissue homogenizer were 9 000, 16 000, and 25 000 rpm for 0.5 and 1 min, respectively. After centrifuged at 20 000 g for 90 min, the supernatants were collected and aliquoted.

Determination of the Stability of the rHSV Infectivity in Liver Homogenates

Different amounts of rHSV (10-fold diluted) were diluted by 10% liver homogenates and maintained at room temperature for 30 min. Aliquot of the incubation was inoculated into HeLa cells and the virus infectivity was quantified as TCID50 by endpoint dilution as described above.

Determination of the Stability of rHSV during Homogenizing Liver Tissues with an Ultrasonic Processor or a Tissue Disperser

5 mL 100 TCID50 viruses were added into liver pieces and homogenized in 10 mL tubes according to the working parameters described above. After processes, the homogenates were 10-fold serially diluted with DMEM and inoculated into HeLa cells. The virus infectivity was quantified as described above.

Determination of the Contamination of rHSV during Tissue Homogenization with an Ultrasonic Processor or a Tissue Disperser

0.3 or 0.4 g liver pieces were allocated into a 5 mL tube to make the final volume of 3 or 4 mL with 3 or 4×10^3 TCID50 rHSV. To see the contamination of viral aerosols or droplets produced during tissue homogenization with an ultrasonic processor or a tissue disperser, a special device was established (Figure 1). Briefly, four pieces of 96-well plates with HeLa monolayer cells were placed on the desk. The processes of tissue homogenization were performed based on the working parameters described above. The 96-well plates were kept open for 5 min after tissue homogenization and then removed to the incubator for virus culture. The well expressing GFP was taken as the positive well and the distance (cm) from the farthest positive well to the tested tube was measured as the contaminating radius.

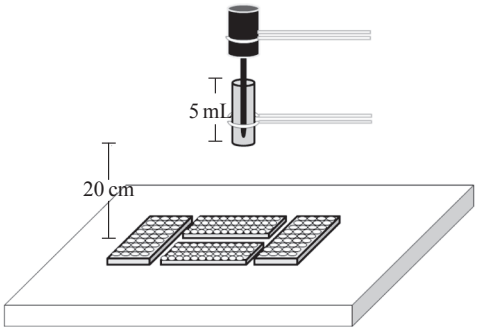

Figure 1. Schematic of tissue homogenization with an ultrasonic processor or a tissue disperser.

Statistical Analysis

Multiple linear regression analyses were conducted by using SPSS statistical program 13.0. An alpha level of 0.05 was used to determine statistical significance throughout the study.

RESULTS

Influences of Tissue Homogenizations on virus infectivity

Since the two instruments may generate considerable heat during processing, the virus infectivity will be markedly reduced with no cooling apparatus. After ultrasonic treatment, virus virulence decreased significantly with the prolonging of the handling time and increase of energy, in which virus TCID50 decreased to 10-3.6, 10-3.1, and 10-2.8/0.1 mL in 1, 3, and 5 min at the energy of 200 W, and 10-3.2, 10-2.6, and 10-2.1/0.1 mL at the energy of 400 W. Similar phenomena were observed in the processes with a tissue disperser. TCID50 of rHSV reduced to 10-4.32 and 10-4.16 after homogenizing for 30 sec or 1 min at the speed of 9 000 rpm, 10-4.16 and 10-4.11 at the speed of 16 000 rpm, and 10-3.61 and 10-3.26 at the speed of 25 000 rpm, respectively. These results indicate that the processes of tissue homogenizations with an ultrasonic processor and a tissue disperser clearly reduce virus infectivity in case of no cooling. However, under the experimental conditions, the virus infectivity is still measurable.

Evaluations of the Contaminating Areas during Tissue Homogenization

To evaluate the spill areas during homogenization at different conditions, rHSV expressing GFP was mixed with the liver tissue sample in a 5-mL tube, with a total volume of 3 or 4 mL. Based on the routine methodologies, multiple homogenizing parameters of an ultrasonic processor and a tissue disperser were designed and comparatively tested. The spilled live viruses were collected with monolayer cells in 96-well plates and the contamination radii were measured and the average radii were calculated. In preparations of the ultrasonic processor, the scores of positive wells (live virus) were clearly associated with the handling time, sample volume and ultrasonic energy (Table 1). Linear regression analyses with all variables on the log scale showed that handle time, ultrasonic energy and sample amount significantly affected the splash radius (P<0.05) and they had linearity with the spill or splash radius (F=96.933, P=0.000), adjusted R2=0.729.

Table 1. Contamination Area of Homogenizing from Ultrasonic Processor

Power (W)	Volume (mL)	Time (min)	Radius (cm)			Average Radius (cm)
			1 st#	2 nd	3 rd	
200	3	5	5	3.5	3.5	4.00
200	4	5	5.3	4.5	5.5	5.10
400	3	5	3.5	4.5	2.5	3.50
400	4	5	6.5	6.5	6.8	6.65
200	3	3	3	3.5	4	3.50
200	4	3	5.5	5.5	5.5	5.50
400	3	3	3	3.5	2.5	3.00
400	4	3	6.2	6.5	5.5	6.07
200	3	1	0	0	0	0.00
200	4	1	3.4	4.5	5.5	4.47
400	3	1	2.5	2.3	4.5	3.10
400	4	1	4.5	5	5.5	5.00

Note. #Represents homogenized time.

In preparing the tissue disperser, the ranges of positive wells were markedly related with sample volume, handling time and the revolving speed (Table 2). Statistical assays revealed that sample volume and the revolving speed significantly affected the splash radius (P<0.05). Revolving speed and sample volume had linearity with the splash radius (F=30.67, P=0.000), adjusted R2=0.629.

Table 2. Contamination Area of Homogenizing from Tissue Disperser

Rpm	Volume (mL)	Time	Radius (cm)			Average Radius
			1st	2nd	3rd#	
9 000	3	30 s	0	0	0	0
9 000	3	1 min	4.6	0	4.2	2.93
9 000	4	30 s	4.2	4.2	0	2.8
9 000	4	1 min	3.5	0	4.2	2.57
16 000	3	30 s	0	3.7	3.5	2.4
16 000	3	1 min	1.7	4.2	4.2	3.37
16 000	4	30 s	6.2	5.5	3.5	5.07
16 000	4	1 min	4	4.5	4.5	4.33
25 000	3	30 s	5.5	6.5	5.5	5.83
25 000	3	1 min	6.2	6.6	6.5	6.43
25 000	4	30 s	6.5	6.8	7.2	6.83
25 000	4	1 min	7.2	7.2	6.8	7.07

Note. #Represents homogenized time.

DISCUSSION

With improvement of containment equipments, engineering controls and great emphases on biosafety training, the incidence of LAIs is apparently reduced in the past decade[2]. However, LAIs seem to be still notified repeatedly every year, though no severe consequence has been identified since the accidence of SARS in 2004. Among the described infectious pathways, unexpected inhalation or intake is the major infectious way. Theoretically, any inappropriate handling or carelessness during the processes producing the aerosols or droplets will potentially cause acquired infection for laboratory staff, and even disaster in public.

Homogenization is the common process to release pathogens, or pathogen-associated elements, e,g, nucleotide acids or proteins, from various infectious organ-, tissue- and cell-samples. Spill, splash or aerosols may be produced simultaneously in process homogenizers[5]. In this study we have used a cell culture based method to evaluate the biorisks during tissue homogenization. Our data provide the reliable contamination areas of live viruses during homogenization with an ultrasonic processor and a tissue disperser at different conditions. It is apparent that at the commonly applied processing conditions, spill, and splash of the live viruses through droplets and aerosols are frequent and unavoidable. The contaminating radii of live viruses during processes are closely related with sample volume, energy of operation and handling time. Sample volume is the most significant factor resulting in virus contamination under our experimental conditions. Energy of operation generally influences the contaminating zone positively. However, it seems that increase of the operation energy, especially the ultrasonic one, induces remarkable contamination in the preparations of shorter handling time. Extension of handling time generally increases the contaminating zone, but not so significantly in the preparations between 3 and 5 min with an ultrasonic processor.

Although the process of homogenization of an ultrasonic processor causes more significant drop-down on virus infectivity than that of a tissue disperser, the contaminating areas of live viruses by the two methodologies are comparable under our experimental parameters. The exact reason remains unclear, possibly implying a more vigorous production of aerosols and droplets with a tissue disperser at the experimental conditions. Nevertheless, our data indicate that a tissue disperser may be more suitable for the tests of virus culture, whereas an ultrasonic processor can be used for the experiments

related with detections of viral nucleotides and antigens. Careful choice of the technique for tissue homogenization based on the purposes may help to balance convenience of objective realization and avoidance of potential biorisks.

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