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Original Article

Approach of design for air mass balance in an aseptic processing area for cell-based products

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ABSTRACT

Introduction: The manufacture of cell-based products requires assuring sterility through all processes, with aseptic processing in a cleanroom. The environment consists of a critical processing zone (CPZ) that can ensure a level of cleanliness that allows cell culture containers to be opened, and a support zone (SZ) adjacent to it and accessed by an operator. In this study, an environment for cell manufacturing was proposed by designing an air mass balance in an aseptic processing area (APA).
Methods: We considered the distribution of particle concentration related to the airflow of clean air passing through a high efficiency particulate air (HEPA) filter and the location of the particle emission sources and set up a model dividing the SZ into two zones vertically: the upper and lower zones in a cleanroom, considering three cases practically. Both the air inlet and outlet were located outside the cleanroom and were connected to the CPZ directly by air ducts (Case 1). The inlets of the CPZ were located in the lower or upper zones of the SZ inside the cleanroom, and the outlets were located in the upper zone (Case 2 or Case 3, respectively). We analyzed how the cleanliness of the APA was affected by different locations of the inlet and outlet of the CPZ by varying the particle emission rate or air change rate.
Results: In Case 1, changes in the particle emission rate or air change rate within the SZ did not affect the particle concentration in the CPZ. In Case 2, an increase in the particle emission rate led to an increase in the particle concentration of the CPZ. In Case 3, the particle concentration of the CPZ was not affected by the particle emission rate. Cases 2 and 3 showed differences in particle concentrations between the CPZ and SZ, indicating that the location of the air inlet of the CPZ had an impact on the cleanliness of both zones. The partial circulation of air between the SZ and CPZ exhibited an additional air cleaning effect, leading to a reduction in the particle concentration in the SZ in Cases 2 and 3.
Conclusions: These results suggest that the appropriate location of the air inlet and outlet can construct the cleanliness of the APA, which reduces the risk of microbial contamination. In addition, we consider that this approach can realize an APA design policy, which eliminates the need for air ducts between the outside of the cleanroom and the equipment for the CPZ, reduces the requirements for gowning, thereby reducing the required air change rate.

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1. Introduction

In recent years, regenerative therapy and cell therapy using cell-based products have progressed [1,2]. Cell-based products are administered directly to patients, so safety including sterility assurance is required as well as efficacy. The manufacture of cell-based products, cell manufacturing, involves cell processing, in which cells collected and isolated from patients or donors are placed in containers, and expansion cultures are conducted repeatedly until

Abbreviations: APA, aseptic processing area; BSC, biological safety cabinet; CFD, computational fluid dynamics; CPZ, critical processing zone; HEPA, high efficiency particulate air; SZ, support zone.

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Nomenclature

α_i	particle concentration in Zone i [(particle)·m^{-3}]
Fij	airflow rate from Zone i to Zone j [m^{3}h^{-1}]
t	time [h]
p	particle penetration ratio of HEPA filter [–]
β	average particle emission rate [(particle)·h^{-1}]
v_1	linear velocity of clean air in Zone 1 [ms^{-1}]
A_1	bottom area of Zone 1 [m^2]
A	floor area of Zone 2 [m^2]
H	height of Zone 2 [m]
Vi	volume of Zone i [m^3]
R_{AC}	air change rate of Zone 2 [h^{-1}]

the number of cells required for the products is reached [3]. As cell-based products are products of living cells, terminal sterilization cannot be applied in the manufacturing process to ensure the sterility of the products [2,3], which requires aseptic processing through all the processes [3]. The purpose of aseptic processing is to prevent microbial contamination from the outside into the products [4]. Several studies have demonstrated a correlation between the number of airborne microorganisms and airborne particles [5–7]. The sterility of aseptic processing area (APA) is achieved by controlling airborne microorganisms, and the design of the structural equipment is assessed using airborne particles [7].

Cell processing such as opening of cell culture containers by an operator can be carried out in open system with a chamber [8]. A zone in a chamber enclosed by physical or air barriers can be adopted for cell processing to prevent microbial contamination from the external environment [8]. The inside of the chamber is an aseptic environment with the lowest controlled number of particles and microorganisms in the environment involved in cell processing and it is equivalent to Grade A (ISO Class 5) [9,10] and said critical processing zone (CPZ) [10]. The surrounding of CPZ is the support zone (SZ) in which an operator works [10]. The SZ requires cleanliness control, such that the cleanliness of the CPZ is not affected by the work of the operator [8]. The SZ composed in a cleanroom where CPZ is installed is an environment in which the airborne particle concentration is controlled [8,11,12]. Particle sources in a cleanroom consist of an operator, materials and equipment and the main particle emission source is the operator [13–15]. In addition, a biological safety cabinet and an isolator system are well known to be used for CPZ, and the locational patterns of these ducts for intake and exhaust of air are variety [16]. Therefore, engineers who design an APA rely on their experience for cleanliness in the APA, requiring a calculation method to evaluate.

Whyte et al. [11] reported that the cleanroom was supplied with clean air and the particle concentration was controlled by ventilation. In a cleanroom, locating of the supply and exhaust air and particle emission sources can change the ventilation efficiency and particle concentration distribution [17,18]. Several methods have been developed to calculate the particle concentration in cleanrooms using variables such as the air change rate, particle emission rate, and particle penetration rate through air filters [11,19]. However, there have been few calculation methods considering air mass balance in APA with CPZ and SZ.

The purpose in this study is to propose an approach to designing an APA for aseptic processing with the air cleanliness. We developed a kinetic model for particle concentration in a space consisting of three zones in APA; CPZ, upper and lower spaces of SZ, describing the impact of airflow rate on the cleanliness. In addition, we set three cases with different locations of air intake and exhaust ports of CPZ, discussing the impact of the ventilation and the particle emission by operator in SZ under considering the management based on the structural design in cell manufacturing.

2. Methods

2.1. Cases of facility structure for APA

We considered the distribution of particle concentration related to the airflow of clean air passing through a high efficiency particulate air (HEPA) filter and the location of the particle emission sources and set up a model dividing the SZ into two zones vertically: the upper and lower zones in a cleanroom, considering three cases practically. We analyzed how the cleanliness of the APA was affected by different locations of the inlet and outlet of the CPZ by varying the particle emission rate or air change rate.

We assumed the facility structure model of the APA as shown in Fig. 1 as follows. The APA for cell processing involving container opening consists of a CPZ (Zone 1) capable of ensuring cleanliness under unidirectional flow to allow for container opening, and an adjacent zone called an SZ (Zone 2), which does not affect the cleanliness of the CPZ. Fresh air outside of the APA (Zone 3) is introduced into Zone 2. And the facility structure model consists of the SZ divided vertically into two zones (lower space; Zone 2(A) and upper space; Zone 2(B)) and the CPZ, and three cases (Cases 1–3) with different locations of the air inlet and outlet of the equipment for the CPZ.

The equipment for Zone 1 comprises an air inlet duct, which supplies air to Zone 1 from outside the equipment (Zone 4), and an exhaust duct, which exhausts from Zone 1 through the air outlet of the equipment (Zone 5). In Zone 2(A), the particle emission occurs by the operator and the particles cannot travel against the downflow of air, preventing the influx of particles into the upper zone (Zone 2(B)).

The structural patterns for Cases 1–3 are set to be different locations of inlets and outlets from equipment consisting of Zones 1, 4, and 5 as follows. In Case 1, the air inlet and outlet of the equipment are outside of the APA (Zone 3). In Case 2, the air inlet is inside of Zone 2(A) and outlet is inside of Zone 2(B). In Case 3, the air inlet and outlet are inside of Zone 2(B). In addition, HEPA filters are installed in each case as shown in Fig. 1. Examples of equipment configurations expected for each case are as follows: a Class II B2 biological safety cabinet for Case 1, a Class II A2 biological safety cabinet for Case 2, and an isolator system for Cases 1–3. Furthermore, the influence of operator intervention on aseptic processing within the biological safety cabinet is not considered in calculating particle concentration within the CPZ.

2.2. Kinetics for the particle concentration based on air mass balance in each zone

The air mass balance equations were constructed to determine the change in the particle concentration, α_i [(particle)·m^{-3}] in each zone at time t [h].

The cleanroom, which internally constitutes Zone 2, was set as a structural facility surrounded by walls to separate it from external influences, forming a space with a floor area of A [m^2] and a height of H [m]. The ventilation airflow rates for Zone 2, represented by $F_{32(B)}$, $F_{2(B)2(A)}$, and $F_{2(A)3}$ [m^{3}h^{-1}], were set to be equal to $R_{AC}AH$ [m^{3}h^{-1}] when the air change rate of the cleanroom was R_{AC} [h^{-1}] (Supplement Material Fig. S1). The particle penetration ratio of the HEPA filter is denoted by p and is set to be constant. There are emission sources of particles from operator, raw materials and equipment. The sources from raw materials and equipment are assumed to be negligible [13,14], and the average particle emission

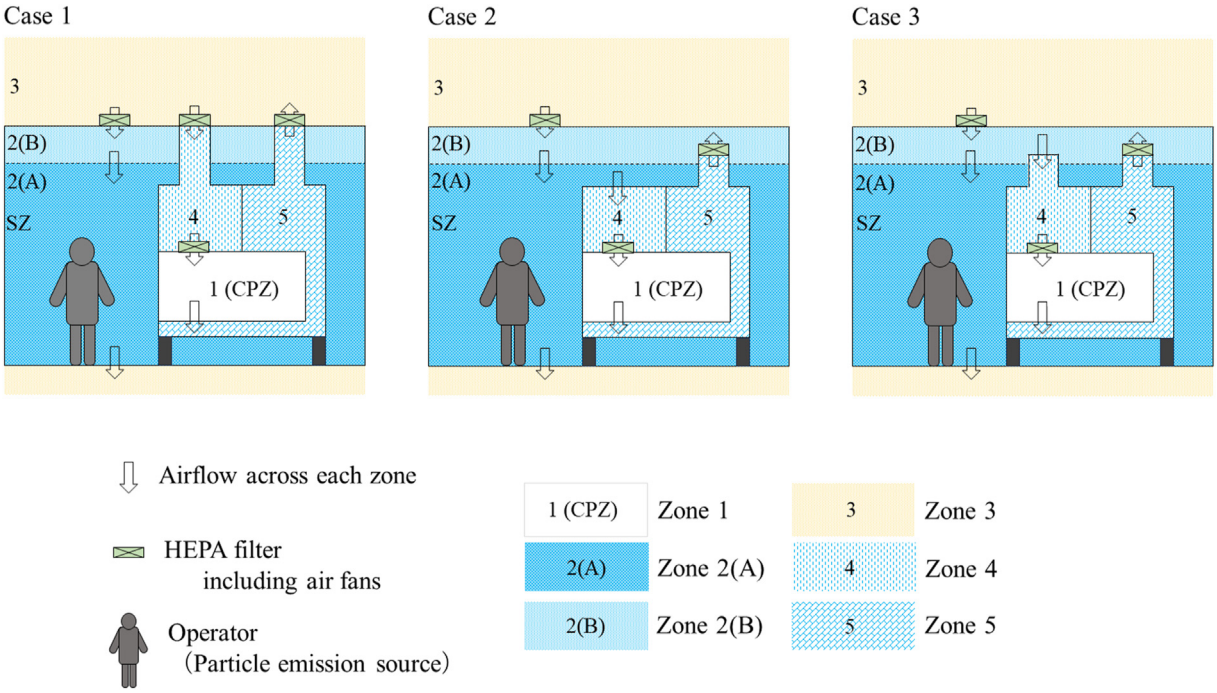

Fig. 1. Side view of the facility structure model of the aseptic processing area (APA) and three cases with the different locations of air inlets and outlets of the equipment for the critical processing zone (CPZ). Zone 1 is the CPZ inside the equipment. Zone 2 is the support zone (SZ) in the cleanroom where the equipment is installed. Zone 2(A) and Zone 2(B) are zones divided vertically as virtual divisions of Zone 2. Zone 3 is the zone outside Zone 2. Zone 4 is the supply air duct zone from outside the equipment to Zone 1. Zone 5 is the exhaust duct zone from Zone 1 to the outside of the equipment. Zones 1, 4, and 5 together constitute the equipment. The dashed line indicates the boundary line dividing Zone 2 vertically into Zone 2(A) and Zone 2(B) as virtual divisions. Case 1 is a setup in which the air supply and exhaust of the equipment do not affect Zone 2 by supplying air from Zone 3 to Zone 4 and exhausting from Zone 5 to Zone 3 directly with air ducts. Case 2 is a setup in which air supply is from Zone 2(A) to Zone 4 and exhaust is from Zone 5 to Zone 2(B). Case 3 is a setup in which the air supply is from Zone 2(B) to Zone 4, and the exhaust is from Zone 5 to Zone 2(B). The equipment expected for each case includes a biological safety cabinet (class II B2) for Case 1, a biological safety cabinet (class II A2) for Case 2, and an isolator system for Cases 1–3.

rate, β [(particle)·h^{-1}] from the operator is set to be constant. The emitted particles or those brought in from different zones were set to quickly become uniform and steady within their reach.

The linear velocity of clean air in Zone 1 was set to v_1 [ms^{-1}], and the bottom area of Zone 1 was set to A_1[m^2]. The airflow rates representing the equipment's intake and exhaust, as well as the airflow within the equipment, were set to F_{34}, $F_{2(A)4}$, $F_{2(B)4}$, F_{41}, F_{15}, F_{53}, and $F_{52(B)}$ and were set to be equal to v_1A_1 [m^{3}s^{-1}]. When there is an intake and exhaust of equipment within Zone 2, the airflow v_1A_1 [m^{3}s^{-1}] of the equipment is added to or subtracted from the airflow for ventilation in Zone 2.

The change in the number of particles per time in Zone 1 can be expressed by Eq. (1). The particle concentration in Zone 1, α_1 [(particle)·m^{-3}] in steady-state can be expressed by Eq. (2).

$$\frac{dV_1\alpha_1}{dt} = F_{41}\alpha_4 p - F_{15}\alpha_1 \tag{1}$$

$$\alpha_1 = \frac{F_{41}\alpha_4 p}{F_{15}} \tag{2}$$

The change in the number of particles per time in Zone 2(A) can be expressed by Eq. (3). The particle concentration in Zone 2(A), $\alpha_{2(A)}$ [(particle)·m^{-3}] in steady-state can be expressed by Eq. (4).

$$\frac{dV_{2(A)}\alpha_{2(A)}}{dt} = F_{2(B)2(A)}\alpha_{2(B)} - F_{2(A)4}\alpha_{2(A)} - F_{2(A)3}\alpha_{2(A)} + \beta \tag{3}$$

$$\alpha_{2(A)} = \frac{F_{2(B)2(A)}\alpha_{2(B)} + \beta}{F_{2(A)4} + F_{2(A)3}} \tag{4}$$

The change in the number of particles per time in Zone 2(B) can be expressed by Eq. (5). The particle concentration in Zone 2(B), $\alpha_{2(B)}$ [(particle)·m^{-3}] in steady-state can be expressed by Eq. (6).

$$\frac{dV_{2(B)}\alpha_{2(B)}}{dt} = F_{32(B)}\alpha_3 p + F_{52(B)}\alpha_5 p - F_{2(B)4}\alpha_{2(B)} - F_{2(B)2(A)}\alpha_{2(B)} \tag{5}$$

$$\alpha_{2(B)} = \frac{F_{32(B)}\alpha_3 p + F_{52(B)}\alpha_5 p}{F_{2(B)4} + F_{2(B)2(A)}} \tag{6}$$

The change in the number of particles per time in Zone 4 can be expressed by Eq. (7). The particle concentration in Zone 4, α_4 [(particle)·m^{-3}] in steady-state can be expressed by Eq. (8).

$$\frac{dV_4\alpha_4}{dt} = F_{2(B)4}\alpha_{2(B)} + F_{2(A)4}\alpha_{2(A)} + F_{34}\alpha_3 p - F_{41}\alpha_4 \tag{7}$$

$$\alpha_4 = \frac{F_{2(B)4}\alpha_{2(B)} + F_{2(A)4}\alpha_{2(A)} + F_{34}\alpha_3 p}{F_{41}} \tag{8}$$

The change in the number of particles per time in Zone 5 can be expressed by Eq. (9). The particle concentration in Zone 5, α_5 [(particle)·m^{-3}] in steady-state can be expressed by Eq. (10).

$$\frac{dV_5\alpha_5}{dt} = F_{15}\alpha_1 - F_{52(B)}\alpha_5 - F_{53}\alpha_5 \tag{9}$$

$$\alpha_5 = \frac{F_{15}\alpha_1}{F_{52(B)}+F_{53}} \tag{10}$$

Eqs. (2), (4) and (6) are revised in Cases 1, 2 and 3 as follows.

In Case 1, the steady-state particle concentrations, α_1, $\alpha_{2(A)}$, and $\alpha_{2(B)}$ [(particle)·m^{-3}] can be derived to Eqs. (11)–(13), respectively. The α_1 is derived from Eq. (2).

$$\alpha_1 = \alpha_4 p = \alpha_3 p^2 \tag{11}$$

As there is no intake or exhaust airflow of the equipment within Zone 2, the $\alpha_{2(A)}$ and $\alpha_{2(B)}$ are derived from Eqs. (4) and (6), respectively.

$$\alpha_{2(A)} = \frac{F_{2(B)2(A)}\alpha_{2(B)} + \beta}{F_{2(A)3}} = \alpha_{2(B)} + \frac{\beta}{F_{2(A)3}} \tag{12}$$

$$\alpha_{2(B)} = \frac{F_{32(B)}\alpha_3 p}{F_{2(B)2(A)}} = \alpha_3 p \tag{13}$$

In Case 2, the steady-state particle concentrations, α_1, $\alpha_{2(A)}$, and $\alpha_{2(B)}$ [(particle)·m^{-3}] can be derived to Eqs. (14)–(16), respectively. The α_1 is derived from Eq. (2).

$$\alpha_1 = \alpha_4 p = \alpha_{2(A)} p \tag{14}$$

Because $F_{2(B)2(A)}$ becomes the sum of $F_{2(A)4}$ and $F_{2(A)3}$, the $\alpha_{2(A)}$ is derived from Eq. (4).

$$\alpha_{2(A)} = \alpha_{2(B)} + \frac{\beta}{F_{2(A)4} + F_{2(A)3}} \tag{15}$$

As there was no air supply from Zone 2(B) to the equipment, the $\alpha_{2(B)}$ is derived from Eq. (6).

$$\alpha_{2(B)} = \frac{F_{32(B)}\alpha_3 p + F_{52(B)}\alpha_5 p}{F_{2(B)2(A)}} \tag{16}$$

In Case 3, the steady-state particle concentrations, α_1, $\alpha_{2(A)}$, [(particle)·m^{-3}] can be derived to Eqs. (17) and (12), respectively. The α_1 is derived from Eq. (2).

$$\alpha_1 = \alpha_4 p = \alpha_{2(B)} p \tag{17}$$

As there is no intake or exhaust from the equipment in Zone 2(A), the $\alpha_{2(A)}$ is derived from Eq. (4) to Eq. (12). In Zone 2(B), air is supplied from Zones 3 and 5, and air is discharged to Zones 2(A) and 4. Therefore, $\alpha_{2(B)}$ can be expressed using Eq. (6). To investigate the influence of particle emission rate in Zone 2(A) on the cleanliness of Zones 1, 2(A), and 2(B), the particle concentration in each zone, α_1, $\alpha_{2(A)}$, and $\alpha_{2(B)}$ was plotted against the average particle emission rate, β in each case. And to investigate the influence of the air change rate in Zone 2 on the cleanliness of Zones 1, 2(A), and 2(B), the particle concentration in each zone, α_1, $\alpha_{2(A)}$, and $\alpha_{2(B)}$ was plotted against the different air change rate, R_{AC} in each case. Here, β was set to be 1.7 × 10^5 (particle)·h^{-1} when an operator stays still with wearing a coverall type garment and 1.8 × 10^7 (particle)·h^{-1} when an operator is in a knee-bending position with wearing a coverall-type garment as low and high particle emission rates, respectively, based on the report by Romano et al. [20].

The values of the parameters used to calculate the particle concentration in each zone are listed in Table 1.

Table 1
Parameter values used for calculating the particle concentration in each zone The particle concentration of the air in Zone 3 was determined using the value from Ref. [21]. The particle penetration rate of the HEPA filter was adopted based on the one specified by H14 EN1822 for particle sizes of 0.2–0.5 μm [22].

Parameter	Symbol	Value	Unit
Area of Zone 2	A	12	m^2
Height of Zone 2	H	3.0	m
Linear velocity in Zone 1	v_1	0.45	ms^{-1}
Bottom area of Zone 1	A_1	0.80	m^2
Particle concentration in Zone 3	α_3	3.5 × 10^7	(particle)·m^{-3}
Particle penetration ratio of HEPA filter	p	5.0 × 10^{-5}	–

3. Result

3.1. The impact of particle emission rate on particle concentration in zones 1, 2(A), and 2(B) under constant air change rate conditions

Fig. 2 shows the result of α_1, $\alpha_{2(A)}$, and $\alpha_{2(B)}$ against the average particle emission rate, β in each case. Regarding Zone 1, α_1 kept constant at 8.8 × 10^{-2} and 4.0 × 10^{-2} in Cases 1 and 3, respectively, and α_1 in Case 1 was 2.2 times higher than that in Case 3. In Case 2, α_1 was 4.0 × 10^{-2} at β = 1.0 × 10^4, which was same value as that in Case 3. With increasing β, α_1 in Case 2 increased and when β was 2.3 × 10^6, the α_1 showed the same value as that in Case 1. Furthermore, as β increased, α_1 in Case 2 increased logarithmically.

Regarding Zone 2(A), $\alpha_{2(A)}$ was 1.8 × 10^3 at β = 1.0 × 10^4 in Case 1 and increased by 5.0 % when β increased from 1.0 × 10^4 to 1.0 × 10^5, and further increased by 45 % when β increased from 1.0 × 10^5 to 1.0 × 10^6. Beyond β of 1.0 × 10^7, $\alpha_{2(A)}$ increased logarithmically. In Case 2, $\alpha_{2(A)}$ was 8.0 × 10^2 at β = 1.0 × 10^4, which was 0.45 times lower than that in Case 1. With increasing β, $\alpha_{2(A)}$ in Case 2 showed the same increasing rate as that in Case 1. In Case 3, $\alpha_{2(A)}$ was almost same as that in Case 2 at β = 1.0 × 10^4. However, as β approached 1.0 × 10^7, $\alpha_{2(A)}$ in Case 3 converged to that in Case 1.

Regarding Zone 2(B), $\alpha_{2(B)}$ kept constant at 1.8 × 10^3 in Case 1 and 8.0 × 10^2 in Cases 2 and 3, and $\alpha_{2(B)}$ in Case 1 was 2.2 times higher than that in Cases 2, 3.

3.2. Impact of air change rate in Zone 2 on the particle concentration in zones 1, 2(A), and 2(B) under constant particle emission rate conditions

Fig. 3 shows the result of α_1, $\alpha_{2(A)}$, and $\alpha_{2(B)}$ against the different air change rate, R_{AC} in each case. Regarding Zone 1, under condition of low particle emission rate, in Case 1, α_1 kept constant at 8.8 × 10^{-2} regardless of R_{AC}. In Case 2, α_1 was 4.6 × 10^{-2} at R_{AC} = 30 and α_1 was 1.7 × 10^{-2} at R_{AC} = 5, showing that lowering the R_{AC} reduced α_1. In Case 3, α_1 was 4.0 × 10^{-2} at R_{AC} = 30 and α_1 was 1.1 × 10^{-2} at R_{AC} = 5, showing that lowering R_{AC} reduced α_1. In both Cases 2 and 3, reducing R_{AC} resulted in a lower particle concentration in Zone 1, consistently showing lower results compared to those of Case 1. Under condition of high particle emission rate, in both Cases 1 and 3, α_1 showed same profile as those under condition of low particle emission rate, respectively. In Case 2, α_1 was 4.5 × 10^{-1} at R_{AC} = 30 and α_1 was 6.3 × 10^{-1} at R_{AC} = 5, with α_1 increasing with decreasing R_{AC}. In addition, α_1 in Case 2 was always higher compared to that in Case 1.

Regarding Zone 2(A), under condition of low particle emission rate, at R_{AC} = 30, $\alpha_{2(A)}$ was 1.9 × 10^3 and 9.5 × 10^2 in Cases 1 and 3, respectively. At R_{AC} = 5, $\alpha_{2(A)}$ was 2.7 × 10^3 and 1.2 × 10^3 in Cases 1 and 3, respectively, showing an increase in $\alpha_{2(A)}$ with a decrease in R_{AC}. In Case 2, $\alpha_{2(A)}$ was 9.2 × 10^2 at R_{AC} = 30, and $\alpha_{2(A)}$ was 3.3 × 10^2 at R_{AC} = 5. In contrast to Cases 1 and 3, a decrease in R_{AC}

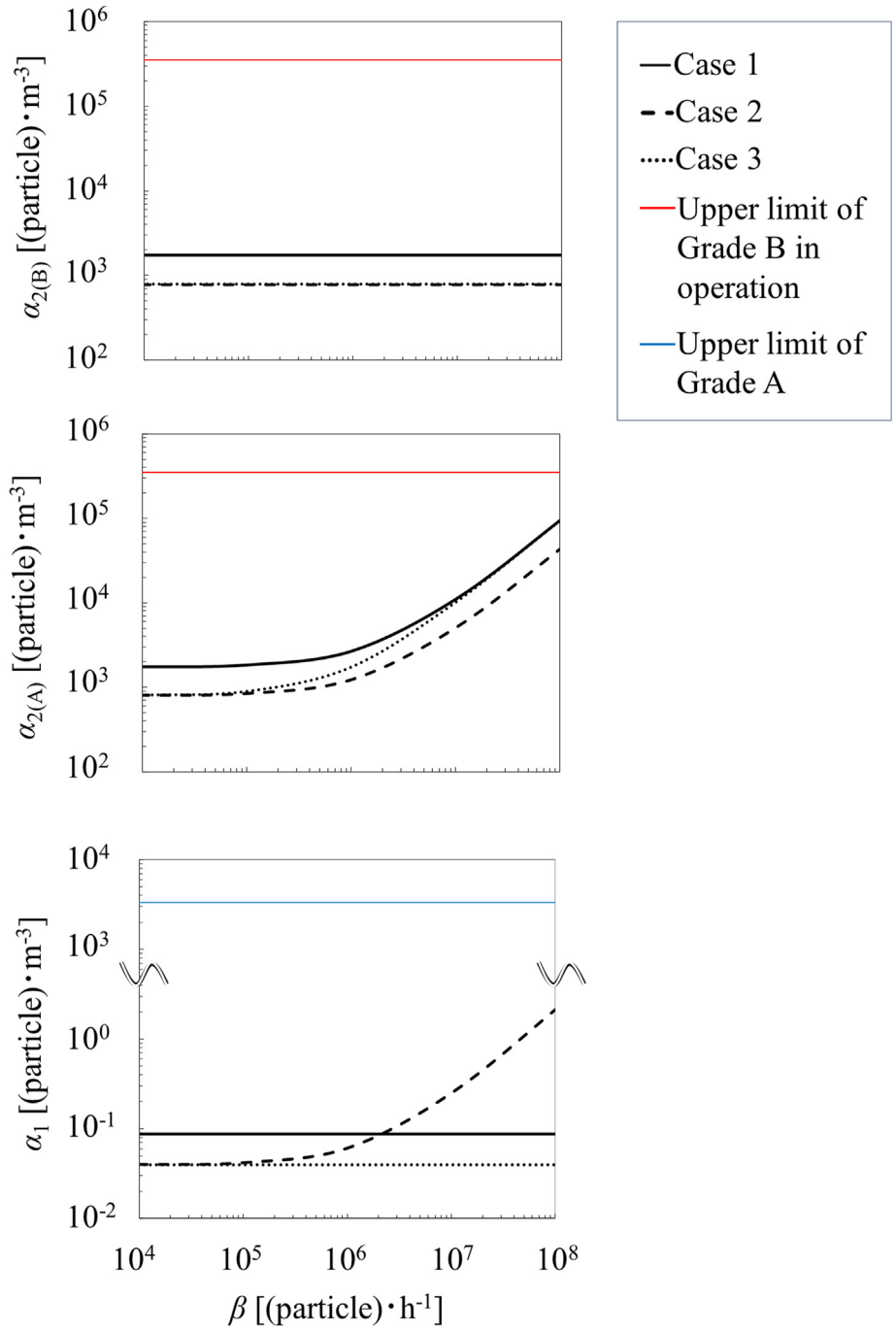

Fig. 2. Particle concentration in Zone 1, Zone 2(A), and Zone 2(B) for each case with respect to the particle emission rate.

led to a decrease in $\alpha_{2(A)}$. In Cases 2 and 3, $\alpha_{2(A)}$ was consistently lower compared to that in Case 1. Under condition of high particle emission rate, both Cases 1 and 3 showed almost same profile for $\alpha_{2(A)}$. At $R_{AC} = 30$, $\alpha_{2(A)}$ in Cases 1 and 3 were 1.9×10^4 and 1.8×10^4, respectively, at $R_{AC} = 5$, $\alpha_{2(A)}$ in both cases was approximately 1.0×10^5. In both cases, a decrease in R_{AC} led to an increase in $\alpha_{2(A)}$. In Case 2, $\alpha_{2(A)}$ was 8.9×10^3 at $R_{AC} = 30$, and $\alpha_{2(A)}$ was 1.3×10^4 at $R_{AC} = 5$, showing that a decrease in R_{AC} led to an increase in $\alpha_{2(A)}$. Case 2 showed the lowest $\alpha_{2(A)}$ among the three cases, and the change in $\alpha_{2(A)}$ in response to variations in R_{AC} was the smallest. Furthermore, under low particle emission rate condition, Case 2 showed a decrease in $\alpha_{2(A)}$ with a decrease in R_{AC}, whereas under high particle emission rate condition, a decrease in R_{AC} resulted in an increase in $\alpha_{2(A)}$.

Regarding Zone 2(B), $\alpha_{2(B)}$ showed similar behavior between low and high particle emission rate conditions in each case. In Case 1, $\alpha_{2(B)}$ kept constant at 1.8×10^3 regardless of R_{AC}. In Cases 2 and 3, $\alpha_{2(B)}$ showed almost same profiles, with $\alpha_{2(B)}$ in both cases being 8.0×10^2 at $R_{AC} = 30$ and decreasing to 2.1×10^2 at $R_{AC} = 5$, showing a decrease in $\alpha_{2(B)}$ with decreasing R_{AC}.

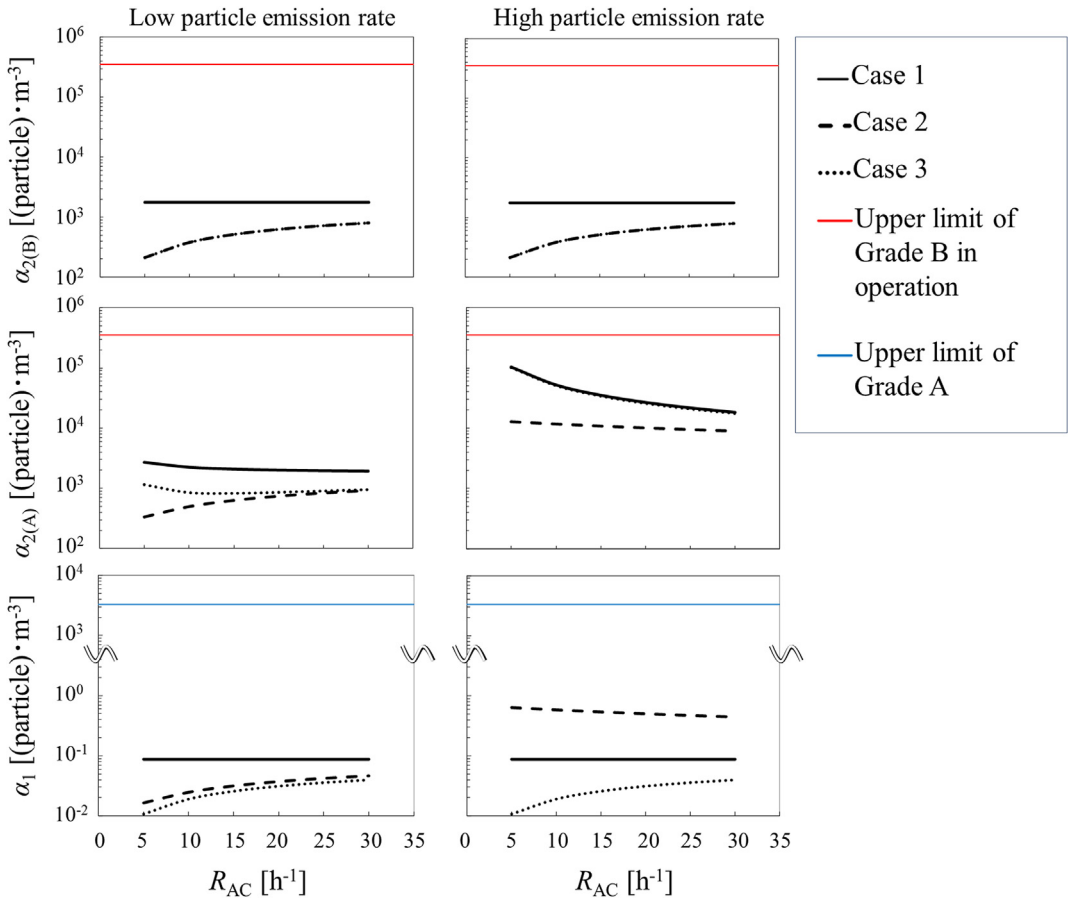

Fig. 3. Particle concentration of Zone 1, Zone 2(A), and Zone 2(B) against air change rate in both low and high particle emission rate conditions.

4. Discussion

In aseptic manufacturing, the design of particle control in an APA is important because it controls microbial contamination. Especially the location of air inlet and outlet in CPZ and SZ of the APA is one of the critical discussion points to maintain the aseptic environment by using equipment that can provide the CPZ, such as a safety cabinet or isolator system [16,23]. In addition, our proposal of vertically spatial division in SZ needs further consideration from the viewpoint of practical control using the equipment. In our structural models for APAs, Case 1 is widely used in pharmaceutical companies with large manufacturing scales, where it is free from contamination risk. On the other hand, in cell manufacturing where the manufacturing scale is relatively small, the structure is designed on a risk basis, and the airflow from the CPZ through the HEPA filter is exhausted into the SZ as in Cases 2 and 3. Mainly, a safety cabinet which draws in air from Zone 2(A) can be assumed in Case 2, and an isolator system which draws in air from Zone 2(B) in Case 3. However, unlike relatively large APAs in aseptic pharmaceutical manufacturing, there is little knowledge on design concepts for relatively small APAs in cell processing facilities. Therefore, we discuss microbial contamination risk, its impact on the location of air intake and exhaust in three cases shown in Fig. 1, and design approach of APAs with the practical management using the equipment.

4.1. Microbial contamination risk in APA by independent air intake and exhaust of CPZ

In Case 1, the constant value of α_1 regardless of β in Fig. 2, and the constant value of α_1 regardless of R_{AC} in Fig. 3, suggest that the concentration of microorganisms in Zone 1 is constant irrespective of the particle emission rate and air volume for ventilation in Zone 2, if the correlation between the number of microorganisms and that of particles is constant. When we calculated the concentration of microorganisms based on the correlation, 1.0 microorganism per 1.0×10^3 particles equal to and larger than 0.5 μm, given by Fedotov [5], which was relatively higher than others in the ratio of microorganisms to particles [6,7], the concentration of microorganisms in Case 1 can be estimated to be approximately 8.8×10^{-5} (bio-particle)·m^{-3} from the result that α_1 in Case 1 was 8.8×10^{-2}. Microbial contamination in an open container depends on the effective open area of the container perpendicular to the airflow and cumulative open time [24]. If we calculate the microbial contamination in an open container, assuming a 10 cm dish as the effective area and using an airflow rate $F_{41} = 1.3 \times 10^3$ m^{3}h^{-1} for Zone 1, it is estimated that 8.9×10^2 h of continuous airflow would introduce 1.0 bio-particle. In this study, we regard this probability calculated from α_1 in Case 1 as a criterion.

The increase of $\alpha_{2(A)}$ with the rise of β in Case 1 in Fig. 2, and the decrease of $\alpha_{2(A)}$ with the increase of R_{AC} in Case 1 in Fig. 3, are consistent with the data Whyte et al. [11] showed that particle concentration in cleanrooms increases with an increase in particle emission rate and can be reduced with an increase in ventilation airflow rate. This correspondence suggests that our air mass balance equation can be used to investigate the cleanliness of the SZ. They showed through equations that the particle concentration within the cleanroom is controlled by the ventilation airflow. This suggests that $\alpha_{2(A)}$ and $\alpha_{2(B)}$ can be calculated by dividing the sum of introduced particles and generated particles by the total airflow, which includes both the cleanroom's ventilation airflow and the

equipment's intake and exhaust airflow. From Fig. 2, the result of $\alpha_{2(B)}$ in Case 1 indicates that the cleanliness of Zone 2(B) is a constant environment filled with air containing particles immediately after passing through the HEPA filter from Zone 3. Because the local ventilation effect is high near the air supply outlets located at a distance from the operator, which is the particle emission source, such as at the ceiling of the cleanroom. Since Novoselac et al. [17] showed that the distance from the particle emission source causes a distribution in the concentration of particles in the cleanroom, Zone 2(B) is considered to represent the zone with the lowest particle concentration when the cleanroom is divided into zones by the particle concentration distribution. We verified that the particle concentration of the two zones divided vertically can be indicated appropriately with $\alpha_{2(A)}$ and $\alpha_{2(B)}$.

4.2. Contribution of air intake and exhaust in SZ to designing appropriate APA

The result of α_1 in Case 2 shown in Fig. 2, which increased with the increase in β unlike the constant value observed in Case 1, suggests that when the air inlet of the equipment is located in Zone 2(A), the particle emission rate in the SZ increase, causing α_1 to exceed the criterion. This increases the possibility of more particles coming into contact with the products during the opening of containers within the equipment. Furthermore, the correlation between particle and microorganism concentrations could increase the probability of microbial contamination. It is suggested that when the air inlet of the equipment is located in Zone 2(A), the particle emission rate should be controlled to achieve the particle concentration criterion in Zone 1. From Fig. 2, it was found that by suppressing β to below 2.3×10^6, $\alpha_{2(A)}$ could be reduced, and α_1 could also meet the criterion. Therefore, by restraining the particle emission rate from the operator, even when the equipment intakes air from zones affected by particle emission, it is considered that the APA, including both the CPZ and SZ, can be constructed with cleanliness equal to or cleaner than that of Case 1. From Fig. 2, the result of $\alpha_{2(A)}$ in Case 2 showing lower values compared to that in Case 1 with respect to β, was analyzed to be due to the addition of air with particles removed by the HEPA filter in the equipment to the air for ventilation in the SZ. The air intake and exhaust of the equipment effectively reduced particle concentration in the cleanroom. The mechanism by which $\alpha_{2(B)}$ in Case 2 was lower than that in Case 1 can be attributed to as follows: the air exhausted from the equipment to Zone 2(B) ($F_{52(B)}$) was combined with the air for ventilation in the SZ ($F_{32(B)}$), thereby increasing the total airflow. Additionally, $F_{52(B)}$ exhausted from the equipment and passed through the HEPA filter resulted in a lower particle concentration than the $F_{32(B)}$. This suggests that, by adding the air exhausted from the equipment to Zone 2(B), which is the zone with the lowest particle concentration in the SZ, an environment with a further reduced particle concentration can be established. However, despite the reduction in particle concentration in Zone 2(A) and Zone 2(B) due to the equipment's air intake and exhaust, $\alpha_{2(A)}$ and $\alpha_{2(B)}$ still exhibit differences of more than 1.0×10^3 (particle)$\cdot m^{-3}$ when compared to α_1 as an criterion. This shows that there is still a significant difference in particle concentration between the CPZ and SZ. This suggests that there is a high concern for particles to be introduced into Zone 1 when the substances move directly from the adjacent Zone 2(A) to Zone 1. From the above, it is suggested that when the equipment intakes air from zones affected by particle emission, suppressing the particle emission rate from the operator enables the construction of an APA with reduced particle concentration in both the CPZ and SZ compared to Case 1. However, if the particle emission rate increases, it may lead to an increase in particle concentration in the SZ, which causes an increase in the particle concentration in the CPZ, thereby deteriorating the cleanliness, including the particles and microorganisms of the CPZ.

The result shows that α_1 in Case 3 is lower than that in Case 1 and constant regardless of β in Fig. 2, suggesting that even when the equipment intakes air from the SZ, locating the equipment's inlet in Zone 2(B) allows for the construction of CPZ that meets the α_1 criterion independently of the particle emission rate. This result, unlike Case 2, which depends on the particle emission rate, is attributed to the changing location of the air inlet of the equipment from Zone 2(A), which is affected by particle emission, to Zone 2(B), where there is no influence of particle emission. This suggests that considering the distribution of the particle concentration within the SZ when locating the air inlet of the equipment is crucial for establishing the CPZ. The independence from the particle emission rate suggests that it does not rely on the types of cleanroom garments worn and the activity of an operator, which can affect the particle emission rate, including factors such as the material types of the garments [20]. The profile of $\alpha_{2(B)}$ in Case 3 shows almost same as that in Case 2 in Figs. 2 and 3, indicating that locating the air outlet of the equipment Zone 2(B) reduces the particle concentration in Zone 2(B), irrespective of whether the air inlet of the equipment is located in Zone 2(A) or Zone 2(B) thanks to air passing through HEPA filters in the equipment. In Fig. 2, the decreasing trend of $\alpha_{2(A)}$ in Case 3 approaches $\alpha_{2(A)}$ in Case 2 when β is under 1.0×10^6 is due to the dominance of $\alpha_{2(B)}$ in Eq. (12), while the increasing trend of $\alpha_{2(A)}$ in Case 3 approaches $\alpha_{2(A)}$ in Case 1 when β is over 1.0×10^6 is analyzed to be due to the dominance of β in Eq. (12). This suggests that there are differences in the effectiveness of reducing the particle concentration in Zone 2(A) in Case 3 based on the equipment's air intake and exhaust depending on the range of the particle emission rate, that is, depending on the degree of suppression of particle emission by the operator gowning. In Case 3, since both equipment's intake and exhaust occur in Zone 2(B), the air flow exhausted from the equipment cannot be added to the ventilation airflow ($F_{32(B)}$) in Zone 2(A), but when β is low and $\alpha_{2(B)}$ dominates in Eq. (12), it was shown that the particle reduction effect of the air intake and exhaust of equipment in Zone 2(B) extended to Zone 2(A). The formation of Zone 2(B) is influenced by various factors reported to affect airflow patterns within cleanrooms, such as the location of the air inlet and outlet of the cleanroom, objects obstructing airflow, pressure and temperature differences, and activities such as door opening, closing, and operator movement [25]. Based on these factors, an understanding and management of airflow are required. Even when the equipment intakes air from within the SZ, it is suggested that locating the air inlet of the equipment by considering the airflow and particle distribution can allow the construction of a CPZ that meets the criterion regardless of the influence of the particle emission rate. Specifically, a CPZ meeting the criterion can be achieved irrespective of the degree of particle emission suppression by operator gowning.

From the results of α_1, $\alpha_{2(A)}$, and $\alpha_{2(B)}$ in Fig. 2, Cases 2 and 3 showed that the particle concentration between the CPZ and the SZ mutually influenced each other as the equipment intakes and exhausts air within the SZ. Changes in the particle concentration within the SZ due to the intake and exhaust of the equipment were reflected in the particle concentration within the CPZ. It is suggested that the APA with low particle concentrations of both CPZ and SZ can be constructed by appropriately locating the air inlet of the equipment and controlling the particle emission rate. From the results of the three cases, it was found that the CPZ that meets the criterion can be established regardless of whether the equipment intakes air from Zone 3, Zone 2(A), or Zone 2(B) by suppressing the particle emission rate with the appropriate gowning of an operator. Furthermore, it was found that by locating the equipment's air inlet

in Zone 2(B), as in Case 3, a CPZ that meets the criterion can be established without the need for ducts connecting the outside of the cleanroom and the equipment containing the CPZ, regardless of the degree of suppression of particle emission by the operator gowning.

Furthermore, the additional impact on cleanliness caused by the exhaust air from the equipment is significant. In Cases 2 and 3, the particle concentration in Zone 2 (both Zones 2(A) and 2(B)) decreased compared to that in Case 1 shown in Figs. 2 and 3 because the air in Zone 2 was drawn into the equipment, passed through the HEPA filter inside the equipment, and was then exhausted back into Zone 2. This resulted in an additional ventilation effect in Zone 2 because the airflow generated in Zone 2 by the equipment contributed to an increase in the ventilation airflow in the cleanroom. This can be considered as the "additional air cleaning effect" described by Whyte et al. [11] or the equipment "acting as a particle sink" described by Loomans et al. [18]. These effects refer to both the removal of particles by the HEPA filter inside the equipment and the apparent improvement in ventilation owing to the increase in airflow within the cleanroom. The particle concentration of Zone 2(B) in Cases 2 and 3 were lower than that in Case 1. The particle concentration of Zone 2(A) in Case 2 under low particle emission rate conditions was lower than that in Case 1. These particle concentrations tended to decrease with decreasing air change rate owing to the airflow in Zone 2 having a higher proportion of $F_{52(B)}$, which has a lower particle concentration than $F_{32(B)}$. Compared to the particle concentration of $F_{32(B)}$ used for cleanroom ventilation, which is 1.8×10^3 (particle)$\cdot m^{-3}$, the air exhausted from the equipment, $F_{52(B)}$, has a lower particle concentration after passing through the equipment's HEPA filter. Specifically, even when air is drawn from Zone 2(A) with air change rate of 5 h^{-1} under conditions of high particle emission, the particle concentration exhausted from $F_{52(B)}$ is 3.2×10^{-5} (particle)$\cdot m^{-3}$. This was 10^8 orders of magnitude lower than the particle concentration of $F_{32(B)}$, which resulted in significantly cleaner air. Therefore, replacing the ventilation air from $F_{32(B)}$ to $F_{52(B)}$ in the cleanroom decreased the particle concentration in Zone 2 under steady-state conditions. The lower trend of the particle concentration of Zone 2(A) in Case 3 compared to that in Case 1 under low particle emission conditions can be attributed to the movement of air with reduced particle concentration owing to the influence of $F_{52(B)}$ in Zone 2(B) to Zone 2(A). In this model, when the air change rate of the cleanroom was 30 h^{-1}, the ventilation airflow was 1.1×10^3 m^3h^{-1}, and when it was 5 h^{-1}, the ventilation airflow was 1.8×10^2 m^3h^{-1}. In both cases, an additional airflow of 1.3×10^3 m^3h^{-1} is provided by the equipment. Because the airflow of the equipment remained constant, the proportion of the equipment airflow increased as the air change rate of the cleanroom decreased. The airflow of the equipment relative to the volume of Zone 2 corresponds to the air change rate of Zone 2: In this model, the equipment's airflow of 1.3×10^3 m^3h^{-1} corresponds to an apparent air change rate of 36 h^{-1} in Zone 2, which is equivalent to or greater than the air change rate in pharmaceutical cleanrooms reported [18]. When the airflow from the equipment is adequately added relative to the volume of the cleanroom, the ventilation airflow of the cleanroom can be reduced. When the particle emission rate was low or high, the behavior of the particle concentration in Zone 2(A) differed between Cases 2 and 3. In Case 2, the air exhausted from Zone 2(B) by the equipment was subsequently drawn into the equipment after reaching Zone 2(A), resulting in the addition of airflow from the equipment across the entirety of Zone 2. In Case 3, both the intake and exhaust of the equipment occurred in Zone 2(B); thus, no additional airflow from the equipment was added in Zone 2(A). Furthermore, when the equipment takes in air from Zone 2(A), the number of particles removed by the HEPA filter inside the equipment is greater than when air is drawn from Zone 2(B). Even under high particle emission rate conditions, in contrast to Cases 1 and 3, Case 2 showed suppressed increases in the particle concentration in Zone 2(A) relative to the decrease in the air change rate, indicating the presence of an "additional air cleaning effect." However, concerns have been raised regarding increasing the possibility of more particles coming into contact with the products during cell processing involving container opening, as the particle concentration of Zone 1, affected by the particle emission rate, exceeded the criterion. From the above, it is found that the difference in the location of the equipment air inlet leads to a difference in the effectiveness of "additional air cleaning effect", and air change rate can be reduced from the level which is recommended by guidance for manufacture of sterile medicinal products in PIC/S [26] and FDA [27].

4.3. Approach to structural design of APA with management

In designing and operating an APA for cell processing involving container opening, the factors to consider include airflow management for cleanliness control, operator gowning to suppress the particle emission rate, and the air inlet location of the equipment comprising the CPZ. Below, we discuss these factors in the design phase. When the ventilation air volume is designed based solely on the cleanroom airflow, the "additional air cleaning effect" of the equipment is not considered in cleanroom ventilation, potentially leading to excessive ventilation [18,28,29]. In addition, an increase in ventilation airflow leads to higher fan energy costs; therefore, from an energy cost perspective, it is desirable to maintain cleanliness with appropriate ventilation airflow. Our analyzing from Eqs. (15) and (16) shows that the "additional air cleaning effect" of the equipment is enhanced with decreasing the volume of the SZ, that is to say $F_{32(B)}$ is lower, or increasing total airflow of the equipment depending on the number of the equipment or airflow volume per an equipment, in other words, $F_{2(A)4}$ and $F_{52(B)}$ are higher. When designing the ventilation airflow in the cleanroom where equipment is installed and the equipment performs air intake and exhaust, it is possible to calculate the required ventilation airflow by considering the "additional air cleaning effect" when the equipment is in operation. In particular, we consider that when no operators are present in the SZ, such as during non-operating hours, the ventilation airflow can be significantly reduced. This reduction is possible because the particle emission rate is lower in the absence of operators.

In the present study calculation result describe the cleanliness in APA without any fluctuation of environmental and operational properties. In actual operation, a decrease in the air change rate means a reduction in the air supply for ventilation from outside the cleanroom, which can alter the airflow patterns within the cleanroom [25]. For both Cases 2 and 3, it is crucial to consider the changes in the particle concentration distribution along with alterations in the airflow patterns when reducing the air change rate. Airflow in cleanrooms is reported to vary depending on various factors such as temperature, internal structures, and air volume. Therefore, the design and management of airflow are important when forming an intact space, such as in Zone 2(B). In addition to actual measurements, predictions and simulations of airflows have been reported using computational fluid dynamics (CFD) [30]. Therefore, airflow simulations using CFD can be used to form an intact space.

When the air inlet of the equipment is located in Zone 2(A), as in Case 2, an environment where the particle concentration is lower than that in Case 1 in both the CPZ and its SZ can be constructed by suppressing the particle emission rate within the cleanroom, even under conditions in which the air change rate of the cleanroom is

reduced. Methods for suppressing the particle emission rate from an operator include wearing cleanroom garments that cover the entire body and minimize skin exposure, as reported [20]. To sufficiently suppress the particle emission rate, careful selection of cleanroom garment materials and consideration of operations such as washing, drying, sterilization, and clothing materials worn under the garments are required. Operator's experience, expertise, education, and training are also crucial factors [31]. The environment of the SZ, where the particle emission rate is suppressed and high cleanliness is maintained, corresponds to Grade B according to the cleanliness management standards for pharmaceutical manufacturing [9]. Grade B is considered necessary when the equipment draws air from the SZ that is affected by particle emission sources. An example of equipment installed in a Grade B environment is a biological safety cabinet (BSC) [12,16]. The use of BSC that introduce operators' hands and materials directly into the CPZ inside the BSC from the SZ via air barriers may pose potential risks because of the introduction of the hands and surfaces of materials to which particles and microorganisms can adhere [16,32].

In actual manufacturing environments, the hands and arms of operators inserted into the BSC can act as contamination sources, increasing the risk of microbial contamination due to the potential for microorganisms to fall into the workspace [33]. This interaction is considered the primary risk for microbial contamination during processing in a BSC within a Grade B environment [15]. Consequently, the calculated results, which did not account for intervention-related contamination risk, likely underestimate contamination potential when the equipment is a biological safety cabinet, as in Cases 1 and 2. Therefore, compared to the calculations presented in this study, the actual probability of contamination is expected to be higher. To minimize the risk of contamination by particles and microorganisms, the particle concentration within the SZ, where operators are present, should be kept as low as possible. This objective can be achieved by suppressing the particle emission rate through the use of appropriate gowning and control of operator activities.

When an “intact space” like Zone 2(B) is formed where the particle concentration is low and unaffected by the particle emission sources, it can be utilized as the location of air inlet for the equipment. In such cases, the necessity for gowning can be reduced or deemed unnecessary. A relatively low cleanliness control environment with lower necessity of gowning corresponds to Grades C or D [9]. Compared with Grade B, in Grades C or D, the costs of heating, ventilation, and air conditioning systems are significantly lower, and gowning, which has a low effect on suppressing the particle emission rate, can be adopted [12]. However, the adoption of gowning that is less effective in suppressing the particle emission rate means increase in the number of particles of all sizes emitted by operators [20]. Particles of relatively larger sizes, ranging from $\geq$5.0 μm to $\geq$30 μm, may serve as carriers for microorganisms [13], leading to an increase in particle deposition rate and accumulation on surfaces [34]. In the SZ, where gowning, which has a low effect on suppressing the particle emission rate, can be adopted, the number of particles and microorganisms adhering to both the surfaces of the operators and materials is expected to increase. Before starting operation in the CPZ, if there are a few particles adhering to the surfaces of the materials, the cleanliness of the CPZ can be ensured by simple dust removal or, if necessary, disinfection methods such as microbial removal. However, if many particles adhere to the surfaces of the materials, the use of an advanced decontamination system such as a decontamination pass box is required [23]. When introducing materials into the CPZ from the SZ managed under Grade C or D conditions, where relatively higher particle concentrations are expected compared with Grade B, the use of a decontamination pass box allows for operations that do not affect the cleanliness of the CPZ [8]. By locating the air inlet and outlet of the equipment in an “intact space” and avoiding intervention through operation within a sealed environment mediated by gloves installed on the equipment, it is possible to construct the CPZ that suppresses the number of particles coming into contact with the products even when the equipment is installed in the SZ where relatively high particle concentrations are expected. Isolator systems are a specific example of such enclosed equipment [8,12,23,29]. It has been suggested that, by using an isolator system that passes materials through a decontamination pass box when introducing them from the SZ into the CPZ inside the isolator system, it is possible to conduct an operation that does not affect the cleanliness of the CPZ [8,12,23].

A design approach that considers the location of the air inlet of the equipment can enable the construction of an APA that can reduce fan energy for ventilation and does not require an air duct connected to the equipment from outside the cleanroom. The method based on the air mass balance in the SZ divided vertically into two zones, and the CPZ enabled a design approach for constructing the APA, including the actual operation of cell processing, such as introducing materials and operator interventions from the SZ into the CPZ.

5. Conclusion

In the model dividing the SZ into two vertical zones, it was shown that the location of the air inlet of the equipment in the cleanroom affected the particle concentration in both the CPZ inside the equipment and its SZ. That is, the location of the air inlet of the equipment within the cleanroom affects the cleanliness of the APA. When the equipment draws air from the zones affected by the particle emission sources, the CPZ in the equipment is constructed, which satisfies the particle concentration criterion by suppressing the particle emission rate in the SZ. The partial circulation of air within the cleanroom, where the air is drawn into the equipment, passes through its HEPA filter, and is then exhausted back into the cleanroom, shows the “air cleaning effect” that enhances the apparent ventilation within the cleanroom. This effect can reduce the air change rate within the cleanroom. When an intact zone unaffected by the particle emission sources is formed upstream of the airflow, it is suggested that the CPZ in the equipment can be constructed, which meets the particle concentration criterion despite the condition under high particle emission rate by locating the air inlet of the equipment in this zone. By dividing the SZ into two vertical zones based on the airflow and location of the particle emission sources, we proposed a design approach for constructing an APA using open and closed system equipment comprising CPZs, such as BSCs and isolator systems. We expect that an approach to designing an environment divided regionally can realize cell manufacturing in an APA, which eliminates the need for an air duct connected to the equipment from outside the cleanroom, reduces the air change rate, and allows for a more simplified gowning of operators.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

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