Effects of ambient $PM_{2.5}$ collected from Asian cities using cyclone technique on human airway epithelial cells

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Abstract

Recent studies have shown that air pollution is intense and hazardous in Asia compared to other parts of the world due to late and poor implementation of updated technology in automobiles and industry, as well as high population density. Respiratory diseases, including asthma, are exacerbated by air pollution. However, effects of $PM_{2.5}$ especially in Asian cities have not yet been well studied, notably on exacerbation of respiratory allergy in this continent. In this study, airway epithelial cells were exposed to crude $PM_{2.5}$ particle collected by cyclone technique from three different Asian cities, namely Sakai, Bangkok, and Taipei. We compared the cytotoxicity and inflammatory potential of $PM_{2.5}$ among the three cities by measuring IL-6 and IL-8 release. Samples from Sakai and Bangkok showed cytotoxic effects at a dose of 75 μg ml^{-1}. Moreover, $PM_{2.5}$ collected from Sakai and Bangkok induced IL-6 and IL-8 release even at low doses. IL-6 and IL-8 release was highly associated with fluoranthene derivatives, microbial factors (endotoxin and β-glucan), metals (Ti), and organic carbon (OC2, OC3) and elemental carbon (EC1) in $PM_{2.5}$. These components of $PM_{2.5}$ collected from Asian cities can contribute to cellular damage and pro-inflammatory responses in airway epithelial cells, and the effect depends on $PM_{2.5}$ sources in the locations.

Key words: Crude PM2.5, Cyclone sampler, Cytotoxicity, Pro-inflammatory response

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1. Introduction

Asian countries suffer from the worse air quality mostly because of the poor implication of regulations and time lag in introducing updated vehicle technology (Gautam *et al*., 2016). Air pollution is more prominent in the most densely populated areas of Asia (Cohen *et al*., 2017). The rapid growth of industries dramatically increased coal consumption in parts of Asia, reportedly in China, which is the major emitter of polycyclic aromatic hydrocarbons (PAH) and other particulate matter with aerodynamic dias. ≤ 2.5 μm ($PM_{2.5}$) (Wang *et al*, 2014), raising $PM_{2.5}$ and secondary organic aerosol (SOA) generation locally and distantly. A study in Taiwan (Chen *et al*., 2016) showed that $PM_{2.5}$ components, including PAH, showed differences owing to various reasons such as high temperatures, humidity and high solar radiation. Moreover, the low mixing layer and thermal inversion in the winter can reduce atmospheric dispersion, and thereby trap air pollutants that leading to higher pollutant density than usual which cause various health effects. In additional to anthropogenic pollutants, Asian dust generated from China is an important factor that affects air quality in Asian countries, such as Japan, Korea, and Taiwan, especially in the spring (Watanabe *et al*., 2011; Park *et al*., 2005; Chien *et al*., 2012). Asian dust contains large amounts of PM_{10}, a small amount of $PM_{2.5}$, and associated EC, OC and total carbon (TC), sulfate, nitrate, black carbon, and PAHs (Liang *et al*, 2013).

Previous epidemiological studies have shown that air pollution is associated with adverse cardiovascular effects in Taiwan (Liu *et al*., 2015). In Japan, respiratory

symptoms were more prevalent in individuals living near busy roads than in those whose places of residence were exposed to less traffic, suggesting that traffic-related air pollution could be a risk factor for respiratory symptoms and lung function (Nakai *et al*., 1999). However, the effects of $PM_{2.5}$ on respiratory allergy in Asian cities have not yet been studied adequately. As $PM_{2.5}$ is composed of a complex mixture of carbon nuclei associated with metals, ion, organic, inorganic, and microbial components, the determination of the contributory factors and elucidation of pathophysiological mechanisms that exacerbate respiratory diseases are also necessary (Chowdhury *et al*., 2017). It has been shown that metals and PAH are important factors that cause respiratory allergy (Bowatte *et al*., 2017; Borgie *et al*., 2015).

A large amount of $PM_{2.5}$ is needed required to evaluate respiratory health in *in vitro* assays. However, it is difficult to collect large amounts of $PM_{2.5}$ by the conventional method of collecting on a filter. In addition, it is not possible to expose the crude particle when it is attached to the filter. Thus, the conventional method requires the extraction of $PM_{2.5}$ from the filter to evaluate the effect of $PM_{2.5}$ on respiratory health, which may lead to loss of components of $PM_{2.5}$ and differences in extraction efficiency among samples. The cyclonic technique is a promising alternative for collecting sufficient PM within a limited time to obtain particles of the required size. The crude particle can be directly exposed without extraction, and therefore the risk of loss of components during the extraction process can be eliminated. Researchers are currently investigating ways to improve the performance of cyclonic technique (Avci *et al*., 2003; Zhao *et al*., 2003; Fu *et al*., 2016).

To best of our knowledge this study for the first time focused on the response of airway epithelial cells to crude $PM_{2.5}$ collected from three different Asian cities, namely Sakai in Japan, Bangkok in Thailand, and Taipei in Taiwan, using cyclone technique. We compared the proinflammatory potential of $PM_{2.5}$ among the three cities and aimed to identify the components of $PM_{2.5}$ that contribute to respiratory and allergic diseases.

2. Methods

2.1. Cell preparation

The BEAS-2B cell line, which is derived from human bronchial epithelial cells transformed by an adenovirus (12-SV40 hybrid virus), was purchased from the European Collection of Cell Cultures (Salisbury, Wiltshire, UK). To initiate cell culture, the vial containing cells was taken out from liquid nitrogen and added to serum-free LHC-9 medium (Life Technologies, Carlsbad, California). LHC-9 medium is already supplemented with retinoic acid, epinephrine, Gentamicin etc. The subculture was maintained in LHC-9 medium in an incubator in a 5% CO_2 atmosphere at 37°C. For particular experiments, the cells were seeded in 96- and 12-well collagen I-coated plates and incubated for 72 h to reach semiconfluence at the same conditions as those used for the subculture.

2.2. Collection and characterization of $PM_{2.5}$

106 $PM_{2.5}$ was collected by using a cyclone sampler placed on the 4th-floor rooftop of a
107 building in a residential area in Sakai. In Bangkok, the cyclone sampler was placed on the
108 7th-floor rooftop of a building on the roadside in a commercial district. In Taipei, the
109 sampling locations were near the main road in a commercial area, with a river nearby.
110 The collection sites are shown in Fig. 1. $PM_{2.5}$ was collected during different seasons
111 within a span of one year as follows: In Sakai, between May 6, 2016 and May 24, 2016
112 (spring), in Bangkok between August 1, 2016 and August 12, 2016 as well as August 15,
113 2016 and September 4, 2016 (rainy season), and in Taipei, between October 31, 2016 and
114 January 10, 2017 (winter). A schematic diagram and image of the cyclone sampler used
115 for the present study are shown in Fig. 2.

116 The collected PM2.5 was characterized by ion exchange chromatography for ions,
117 thermal optical reflectance for organic and elemental carbon, inductively coupled plasma
118 mass spectrometry (ICP-MS) for metals, and high-performance liquid chromatography
119 (HPLC) for PAH. Microbial materials such as endotoxin and β-glucan were measured by
120 Japan Pharmacopeia test.

121 ### *2.2.1. Ions*

122 The collected PM2.5 was measured gravimetrically and extracted with 10 mL of
123 ultrapure water. After 30 min of sonication and occasional stirring the filtered extract was
124 collected as final volume of 6mL and anion species (Cl-, NO3-, and SO42-) and cation
125 species (Na+, NH4+, K+, Mg2+, and Ca2+) were measured by Ion chromatography.

126 ### *2.2.2. Organic and elemental carbon*

Thermal/Optical Carbon Analyzer (Atmoslytic Inc.,Calabasas, CA, USA) produces four OC fractions (OC1, OC2, OC3, and OC4 at 120◦C, 250◦C, 450◦C, and 550◦C, respectively) in a He atmosphere. Elemental carbon (EC1, EC2, and EC3 fractions) was evolves at 550, 700 and 800 °C at the 2% O2/98% He atmosphere particles were installed in carbon analyzer using quartz fiber filter.

2.2.3. Metals

Particles were measured gravimetrically and went through microwave-assisted digestion using diluted nitric acid and hydrogen fluoride (3.5:1 respectively). Final volume was achieved as 50 mL and Na, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Mo, Sb, Cs, Ba, La, Ce, Hf, W, Pb and Cd were measured by ICP-MS.

2.2.4. PAH

Particle mass was measured gravimetrically and extracted by 3mL of dichloromethane. After 30 min of sonication and occasional stirring the extract was filtrated as a final volume of 5mL. The extract was pressurized by nitrogen flow to remove dichloromethane and 1mL of acetonitrile was added before Fluoranthene, Pyrene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Benzo(ghi)perylene were measured in HPLC.

2.2.5. Microbial materials

Microbial materials such as endotoxin and β-glucan were measured by Japan Pharmacopeia test. As biological components of the PM2.5 extract, we measured an

147 endotoxin and a β-glucan by kinetic-turbidimetric method using Limulus Amebocyte
148 Lysate.

149

150 2.3. Exposure and measurement

151 *2.3.1. Cell viability by WST-1*

152 Cell viability was assessed by WST-1 assay. The BEAS-2B cell suspension containing
153 7.5 x 10^4 cells ml^{-1} was seeded at a density of 70 µl/well in collagen I-coated 96-well
154 plates and cultured for three days. On day 3, the medium was discarded and cells were
155 exposed to the sample solution at an equal volume (70 µl/well). Crude $PM_{2.5}$ was
156 suspended in phosphate buffered saline (PBS) and subjected to ultrasonication at a power
157 of 40 for 3 min. BEAS-2B cells were exposed to $PM_{2.5}$ at doses of 0, 7.5, 22.5, or 75 µg
158 ml^{-1}. WST-1 reagent was added after 21 h of exposure. The doses were selected based on
159 our prior studies (Honda et al. 2017, Chowdhury et al, 2018). The amount of WST-1
160 reagent should be 1/10th of the sample volume (7 µL). After 3 h the absorbance of the
161 plate was measured at 450 nm using a microplate reader (reference wavelength 630 nm).

162 *2.3.2. Quantification of cytokines in culture supernatant by ELISA*

163 Interleukin (IL)-6 is produced at the site of inflammation and plays a key role
164 in various acute and chronic phase response via different signal-transduction pathways
165 for instance, protein kinase C, cAMP/protein kinase A, and calcium ionophore (Gabay,
166 2006; Alfaro-Moreno *et al*., 2009). Moreover, IL-6 exerts stimulatory effects on T- and
167 B-cells which favors inflammatory responses (Gabay, 2006). IL-8 is an inflammatory
168 chemokine, able to affects the function and recruitment of various inflammatory cells and

fibroblasts (Moyer *et al*, 2002). These molecules related to inflammation can affect exacerbation of asthma. IL-13 works as a central mediator of asthma though a cascade of biochemical pathway including regulation of Immunoglobulin (Ig)-E production, promoting migration of eosinophils into the lung, and upregulation of adhesion molecules which bind to eosinophils, increased flexibility of airway epithelial cells, higher mucus production, production of nitric oxide synthase by airway epithelial cells, collagen deposition in airway, proliferation of airway smooth muscle, and stimulation of airways hyper responsiveness (Corren, 2013).

After exposure to crude $PM_{2.5,}$ BEAS-2B cells were incubated for 24 h and centrifuged at 300 × *g* for 5 min before the supernatant was collected. The supernatant was stored at -80°C. The levels of interleukin (IL)-6, IL-8, and IL-13 released from BEAS-2B cells were measured by Quantikine ELISA kits (IL-6 and IL-8 from ThermoFisher Scientific Human ELISA kit). The detection limits for IL-6, IL-8, and IL-13 were < 2.00, < 6.44, and 30.57 pg ml^{-1}, respectively.

2.4. Statistical analysis

The experiments for cytotoxicity and cytokine release were performed with multiple samples (n = 3–4). The average values ± standard error of the mean were calculated for all statistical analyses. The statistical significance was examined by Dunnett's multiple comparison tests. P-value < 0.05 and P-value < 0.01 were considered significant, indicated in figures as (*) and (**), respectively. The correlation between $PM_{2.5}$ components and cytotoxicity and IL release were determined by using Pearson's

correlation coefficient with two-tailed significance by using IBM SPSS software. The correlation analysis was conducted by pooling the samples. A source appointment study was also performed based on previous literature.

3. Results

3.1. Characterization of $PM_{2.5}$

Characterization results showed the detail composition of $PM_{2.5}$ collected from the three cities Sakai, Bangkok, and Taipei. The mean mass concentration of $PM_{2.5}$ was 18.1, 16.1, and 15.6 μg m^{-3}, respectively. The components identified were OC1, OC2, OC3, EC1, EC2, EC3, EC4, inorganic ions (Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, Mg^+, Ca^{2+}), heavy metals, trace elements, and microbial elements (Fig. 3, Table 1). PAHs have been included in OC and shown separately in Table 2. The most abundant component of $PM_{2.5}$ was OC (35%) in Sakai, while that in Bangkok and Taipei was metals (22 and 26%, respectively). The OC/EC ratio in Sakai, Bangkok, and Taipei was 1.64, 1.18, and 0.99, respectively.

We designated Ca, Mg, Na, K, Al, Fe, and Ti as crustal elements and V, Cr, Ni, Zn, Cd, Pb, and Cu as anthropogenic tracers. In this study, the concentrations of crustal elements were highest in Taipei (179.08 μg mg^{-1}) and lowest in Sakai (104.1 μg mg^{-1}). However, the ratio of crustal elements to anthropogenic elements was highest in Taipei (52.17), followed by Sakai (40.87) and Bangkok (20.82).

3.2. Effects on airway epithelial cells

$PM_{2.5}$ collected from Sakai and Bangkok did not show adequate difference in cell viability, except at a concentration of 75 μg ml^{-1}. At this concentration, the samples collected from Sakai and Bangkok reduced cell activity by 11.82 and 4.36%, respectively (Fig. 4). $PM_{2.5}$ collected from all cities significantly increased IL-6 release at doses of 22.5 and 75 μg ml^{-1} ($p < 0.05$) (Fig. 5). Moreover, $PM_{2.5}$ collected in Bangkok significantly increased IL-6 levels even at a concentration of 7.5 μg ml^{-1}. In contrast, exposure to all doses of PM2.5 samples collected from Sakai and Bangkok elevated IL-8 release (Fig. 6). However, exposure to Taipei sample increased IL-8 release only at a concentration of 75 μg ml^{-1} (Fig. 6). IL-13 levels in the cells remained unaffected after exposure to the three samples at all concentrations (data not shown).

3.3. Correlation study results

Pearson's correlation between cell viability, IL-6, and IL-8 with the components of $PM_{2.5}$ is shown in Table 3. Cell viability was negatively correlated with microbial factors, such as endotoxin and β-glucan, OC2, and OC3 ($P < 0.01$, Fig. 7). IL-6 and IL-8 release showed a positive correlation with multiple components, including PAHs, inorganic and organic carbon, microbial elements, and metals. Benzo (b) fluoranthene (BbF), Benzo (k) fluoranthene (BkF), EC1, microbial elements, and Ti demonstrated the highest (Pearson correlation > 0.9) correlation with IL-6 release (Fig. 8). BbF, OC2, OC3, microbial factors, Ti, and EC1 showed the highest correlation with the IL-8 release (Fig. 9).

4. Discussion

We performed similar sets of experiments and obtained comparable results in widely discrete locations. In the present study, $PM_{2.5}$ collected at Sakai and Bangkok had cytotoxic effects only at the highest dose (75 μg ml^{-1}). In addition, this dose increased IL-6 and IL-8 release from airway epithelial cells. However, $PM_{2.5}$ collected from Taipei had comparatively lower potential to initiate respiratory inflammation via IL-6 and IL-8 release. The characterization of $PM_{2.5}$ revealed the following components: OC-1, OC2, OC3, EC-1, EC2, EC3, EC4, inorganic ions, PAH, metals, trace elements, and microbial elements. From correlation studies, it was observed that cytotoxicity is correlated with microbial elements, OC2, and OC3. In contrast, IL-6 and IL-8 release were highly correlated with PAHs, e.g. Benzene derivatives of fluoranthene, EC1, OC2, OC3, metals such as Ti, and microbial elements.

In the present study, we used crude $PM_{2.5}$ collected by cyclone method. Hence, the risk of contamination on the filter and during the process of extraction was avoided. *In vitro* experiments and characterization require adequate amounts of $PM_{2.5}$ for repeated exposure at different doses. Cyclone method is efficient for obtaining adequate $PM_{2.5}$ (Okuda *et al*., 2015). Comparing the results of this study with our previous study (Chowdhury *et al*., 2018), we concluded that crude $PM_{2.5}$ shows more profound biological effects than aqueous as well as organic extracts of $PM_{2.5}$. Aqueous extract of $PM_{2.5}$ exhibited cytotoxicity; however, both the extracts failed to show any proinflammatory response through IL-6 and IL-8 release. In the present experiment, crude $PM_{2.5}$ showed enhanced effects on cytokine release especially in higher doses, because particles have a marked effect on cellular events compared to extracts. An image of the cyclone sampler and representative diagram illustrating the process of cyclone

257 separation described by Okuda, (2015) are shown in Fig. 2. A study showed that $PM_{2.5}$
258 collected by the same cyclone model induced allergic airway inflammation (Ogino *et al*.,
259 2017).

260 Sakai is a city located in Osaka prefecture of Japan, with a population of more
261 than 800,000. It is a suburban area near Osaka city and one of the important seaports.
262 Bangkok is the capital city of Thailand and has a population of over 8.28 million, almost
263 10 times more than Sakai. Taipei, the capital of Taiwan, has a population of 2.7 million,
264 which is almost one-third of the Bangkok's population. Thus, our study includes three
265 cities with different population levels and geographical distribution. Some studies
266 (Chuersuwan *et al*, 2008, Wimolwattanapun *et al*., 2011) reported that automobile (32%)
267 and biomass burning (26%) are the two major sources of $PM_{2.5}$ in traffic sites of Bangkok,
268 and biomass burning alone is the main contributor of $PM_{2.5}$ at residential sites. Although
269 no source appointment studies have prominently shown the contributors of $PM_{2.5}$ in
270 Taipei, a study (Hsu *et al*, 2016) conducted in central Taiwan identified traffic and
271 industry emissions as the major sources of $PM_{2.5}$. Japan and Taiwan suffered from Asian
272 sand dust (ASD) in spring (Liang *et al*., 2013; Lee *et al*., 2015). A previous study
273 reported that total suspended particles (TSP), secondary aerosols formed from SO_2, NOx,
274 and hydrocarbons from diesel exhausts, and small aerosols generated from factories are
275 the sources of air pollutants in Sakai (Mizohata *et al*., 1980).

276 The theoretic reconstruction of components from the characterization data of
277 $PM_{2.5}$ and primary sources of pollution has been reported previously (Behera *et al*., 2012).
278 We used a similar method to identify the source of pollutants found in the three cities
279 considered in our study. Our results showed that the concentration of crustal elements (Ca,

280 Mg, Na, K, Al, Fe, and Ti) were higher than that of anthropogenic elements (V, Cr, Ni,
281 Zn, Cd, Pb, and Cu) by 52.17, 40.87, and 20.82 times in Taipei, Sakai, and Bangkok,
282 respectively. As the ratio of crustal elements to anthropogenic elements is the lowest in
283 Bangkok, it is assumed that anthropogenic activity is high owing to high traffic. In
284 contrast, the reason for the lowest concentration of crustal elements at Sakai (104.1 μg
285 mg^{-1}) may be because of the advanced road structure and compact city planning in Japan.
286 Taipei has the highest concentration of crustal elements as well as highest crustal to
287 anthropogenic elements ratio possibly because of the dry and windy weather in winter.
288 EC (carbon black) is generally emitted from primary combustion sources and stay in the
289 atmosphere in the particulate form. As EC undergoes a limited secondary transformation,
290 it is considered a good tracer for primary carbonaceous aerosols of combustion origin. In
291 contrast, OC can be emitted from combustion as well as evaporation of fuels and solvents
292 and often undergo secondary transformation (Turpin *et al*., 1991). As the OC/EC ratio
293 was 1.64 and 1.17 at Sakai and Bangkok, respectively, we can assume that the
294 contribution from non-combustion origin (i.e. biogenic, soil and road re-suspension, long-
295 range transport, and evaporation of fuel and solvents) is higher at Sakai and Bangkok
296 than EC, which indicates the contribution from urban sources (i.e., vehicles and industry).
297 The results of samples from Taipei showed the almost equal contribution from both non-
298 combustion and combustion origin.

299 Furthermore, Liu *et al*. recently identified the sources of different types of OC
300 and EC to determine the sources of aerosol components in Haikou, China (Liu *et al*.,
301 2017). They assigned OC1 to biomass burning, OC2, OC3, OC4, and EC1 to gasoline-
302 fueled vehicles, while EC2 was the most abundant species in the exhaust of diesel-fueled

303 vehicles. OC2 is mainly derived from coal combustion. Based on these criteria, we can
304 predict that biomass burning is negligible in Bangkok and Taipei. In addition, biomass
305 burning is not a contributor to carbon emission in Sakai. By comparing EC1 and EC2, we
306 suggest that gasoline exhaust contributes more than diesel exhaust in Sakai (EC1 32%,
307 EC2 5%) and Bangkok (EC1 41%, EC2 4%). In Taipei, the contributions from both the
308 sources were almost similar (EC1 26%, EC2 21%).

309 We observed that $PM_{2.5}$ collected at Sakai and Bangkok showed high cytotoxic
310 potential possibly due to the high density of microbial elements and organic carbons.
311 However, the amount of organic carbon that contains PAHs in $PM_{2.5}$ was the highest
312 (35%) at Sakai among the three cities, whereas that at Taipei showed the lowest
313 concentration (11%). The same pattern was observed for endotoxin and β-glucan
314 concentration i.e. 14.20 and 0.6273 EU mg^{-1} for Sakai and Taipei, respectively (Fig. 2). It
315 has been reported that $PM_{2.5}$ with higher PAHs has cytotoxic potential (Kang *et al*., 2010).
316 It is evident that endotoxin and β-glucan can induce apoptosis via inflammatory events in
317 macrophages (Murphy *et al*., 2017), however, their effect on epithelial cells showed
318 varied results (Lamkanfi *et al*., 2010). Endotoxin interferes with histone-mediated cell
319 death mechanism, especially in mammalian cells (Chen *et al*., 2014; Burris *et al*., 2015).
320 β-glucan is known to be a powerful immune stimulant (Akramiene *et al*., 2007). The
321 combination of β-glucan and endotoxin resulted in hypersensitivity and inflammatory
322 responses in airways (Fogelmark *et al*., 1994). Thus, the conjugated mechanism of β-
323 glucan and endotoxin may explain the significant release of IL-6 and IL-8 from the
324 samples collected in Sakai and Bangkok.

The concentrations of PAHs were the lowest in Taipei among the three cities (Table 2). Jung *et al*. reported that nonvolatile PAH did not correlate with asthma, however, semivolatile PAH, such as pyrene, exacerbated asthma in children (Jung *et al*., 2014). We identified fluoranthene and pyrene as semivolatile PAHs in all $PM_{2.5}$ samples, and they significantly correlated with IL-6 and IL-8 release (Table 3).

In this study, BbF and BkF correlated highly with IL-6 and IL-8 release. Previous studies have shown that BbF and BkF are the important PAHs present in diesel exhaust particles (DEP) and ambient $PM_{2.5}$ (Boland *et al*., 1999; Guo *et al*., 2017; Yang *et al*., 2013). They are included in the 16 PAH priority pollutants listed by US environmental protection agency owing to their abundance in the air, as well as toxicity. Hence, it can be suggested that BbF alone or synergistically with other $PM_{2.5}$ components causes the proinflammatory response. BkF and fluoranthene showed high correlation with the expression of cytokines associated with proinflammation. Therefore, further studies are warranted to understand the effect of fluoranthene and its derivatives alone and synergistically.

EC1 was highly correlated with cytokine release. A previous study reported that carbon nuclei might induce IL-6 expression (Totlandsdal *et al*., 2009) via involvement of NF-κB or mitogen-activated protein kinase (MAPK). Several studies have reported that IL-6 and IL-8 expression was elevated when exposed to DEP (Kim *et al*., 2016). $PM_{2.5}$ was found to increase IL-6 and IL-8 release in BEAS-2B cells probably via regulation of n405968 gene on chromosome 4 in humans (Huang *et al*., 2017).

From the present results, it can be suggested that besides the microbial factors and PAHs, organic and inorganic carbons and metals can contribute to proinflammatory

response. $PM_{2.5}$ from Taipei increased IL-6 release at doses of 22.5 and 75 μg ml^{-1}, while IL-8 release was increased at a dose of 75 μg ml^{-1}. Compared to microbial factors, ions and metals were higher in concentration in $PM_{2.5}$ collected from Taipei. The correlation result (Table 3) showed that Ti, Fe, Ce, and Mn were positively correlated ($p < 0.01$) with IL-6 and IL-8 release. Oxides of Ti (TiO_2) induced proinflammation in asthmatic mice (Jonasson *et al*., 2013). Fe alone has not been documented to be responsible for direct inflammation, however, Dunea *et al*. (2016) concluded that $PM_{2.5}$ with a high content of heavy metals, including Fe, and its long-term exposure could exacerbate existing respiratory diseases. A recent study suggested that airborne Mn might affect respiratory health, thereby causing wheezing and asthma (Rosa *et al*., 2016). Apart from Ti and Fe, Ce and Mn also correlate highly ($p < 0.01$) with IL-8 release. Vehicle emission is a source of Ce (Dale *et al*., 2017), however, it has not been documented to pose health risks in humans.

This study compared $PM_{2.5}$ collected from different city in different season. PM collection was not feasible simultaneously in same season in three different city and so this study cannot compare the ambient $PM_{2.5}$ quality in same time of the year.

5. Conclusion

Overall, $PM_{2.5}$ collected from the three Asian cities caused cytotoxicity or proinflammatory response in airway epithelial cells. The effects differed between the cities. Crude $PM_{2.5}$ collected by cyclone technique is a more efficient method for exposure studies. It is possible that IL-6 and IL-8 release was caused by fluoranthene

derivatives, microbial factors, metal ions, OC2, OC3, and EC1 present in $PM_{2.5}$. These components may contribute to the exacerbation of respiratory diseases such as asthma. We suggest further exposure studies for identifying the components correlated with carbon nuclei in airway epithelial cells to confirm our conclusion.

Disclosure: The authors declare no competing financial interest.

Tables:

Table 1. Density Organic and Elemental carbon in ng mg^{-1}

	OC1	OC2	OC3	OC4	EC1	EC2	EC3
Sakai	520	25000	100000	18000	74000	11000	2300
Bangkok	0	12000	52000	21000	65000	6200	960
Taipei	UDL	6400	30000	15000	27000	22000	2800

UDL=Under Detection Level

Table 2. PAHs detected in $PM_{2.5}$

PAH (ng mg^{-1})	Sakai	Bangkok	Taipei
Fluoranthene	1.1	0.79	0.54
Pyrene	0.62	0.98	0.39
Benzo(b)fluoranthene	2.7	2.3	0.67
Benzo(k)fluoranthene	0.70	0.82	0.17
Benzo(a)pyrene	0.47	0.88	0.14
Benzo(ghi)perylene	0.86	2.8	0.30
Total	**6.45**	**8.57**	**2.21**

Table 3. Components of $PM_{2.5}$ with Pearson's correlation (highest to lowest) and statistical significance

**= Significant difference (p-value < 0.01), *= Significant difference (p-value < 0.05) between components and cell responses through Pearson's Correlation test.

Components	Pearson correlation (viability)	P value	Components	Pearson correlation (IL-6)	P value	Components	Pearson correlation (IL-8)	P value
Endotoxin, β-glucan	-0.724*	0.027	Benzo (b) fluoranthene	0.952**	0.000	Benzo (b) fluoranthene	0.936**	0.000
OC2	-0.706*	0.034	Benzo (k) fluoranthene	0.930**	0.000	OC2	0.947**	0.000
OC3	-0.673*	0.047	EC1	0.926**	0.000	OC3	0.938**	0.000
Cl^-	0.649	0.059	Endotoxin, β-glucan	0.97**	0.000	Endotoxin, β-glucan	0.956**	0.000
Benzo (b) fluoranthene	-0.636	0.065	Ti	0.914**	0.001	Ti	0.901**	0.001
EC1	-0.566	0.112	Fe	0.893**	0.001	EC1	0.904**	0.001
Ti	-0.558	0.119	OC2	0.892**	0.001	Fluoranthene	0.872**	0.002
Benzo (k) fluoranthene	-0.551	0.124	OC3	0.892**	0.001	Benzo (k) fluoranthene	0.869**	0.002
W	0.521	0.150	Fluoranthene	0.867**	0.002	Fe	0.857**	0.003
Fluoranthene	-0.515	0.156	Rb	0.855**	0.003	Ce	0.861**	0.003
Na^+	0.512	0.159	Ce	0.861**	0.003	Mn	0.845**	0.004
Ce	-0.494	0.176	Mn	0.841**	0.005	La	0.793*	0.011
Mg^{2+}	0.493	0.177	Cs	0.838**	0.005	Hf	0.784*	0.012
Fe	-0.481	0.190	Benzo (a) pyrene	0.829**	0.006	Rb	0.779*	0.013
Mn	-0.481	0.199	Co	0.819**	0.007	Cs	0.776*	0.014
Na	0.430	0.248	K	0.809**	0.008	Co	0.748*	0.021
La	-0.414	0.268	Hf	0.813**	0.008	Ba	0.743*	0.022
Al	0.396	0.292	Pyrene	0.808**	0.008	K	0.735*	0.024
Benzo (a) pyrene	-0.381	0.311	Ba	0.802**	0.009	Ni	0.732*	0.025
Rb	-0.38	0.313	La	0.774*	0.014	Benzo (a) pyrene	0.714*	0.031
Hf	-0.367	0.331	Ni	0.764*	0.016	Pyrene	0.705*	0.034
NH_4^+	-0.365	0.334	OC4	0.755*	0.019	OC4	0.690*	0.040
Cs	-0.360	0.341	Cu	0.713*	0.031	Cu	0.620	0.075
Co	-0.322	0.398	Benzo (ghi) perylene	0.701*	0.035	NH_4^+	0.617	0.077
SO_4^{2-}	0.315	0.408	Ca	0.687*	0.041	V	0.602	0.086
Ba	-0.308	0.420	Ca^{2+}	0.681*	0.043	Mo	0.571	0.108
K	-0.304	0.426	Mo	0.682*	0.043	Benzo (ghi) perylene	0.550	0.125
Pyrene	-0.298	0.436	V	0.645	0.061	Ca	0.535	0.138
Ni	-0.296	0.439	Pb	0.600	0.088	Ca^{2+}	0.530	0.142
Se	0.295	0.441	Zn	0.575	0.105	Pb	0.460	0.213
EC2	0.272	0.478	As	0.517	0.154	As	0.414	0.268
OC4	-0.237	0.539	NH_4^+	0.508	0.163	Zn	0.409	0.274
Benzo (ghi) perylene	-0.232	0.549	K^+	0.496	0.174	EC3	0.394	0.294
Cr	0.202	0.602	Cd	0.895	0.175	Cd	0.384	0.307
NO_3^-	0.173	0.656	Sb	0.479	0.192	Sb	0.384	0.308
Ca	-0.171	0.659	NO_3^-	0.478	0.251	K^+	0.374	0.321
Cu	-0.156	0.688	EC3	0.352	0.353	NO_3^-	0.329	0.388
Ca^{2+}	-0.154	0.692	Cr	0.343	0.366	Cr	0.285	0.457
V	-0.134	0.732	Se	0.299	0.434	Cl^-	-0.251	0.515
K^+	0.119	0.760	SO_4^{2-}	0.287	0.453	Se	0.202	0.602
Sb	0.117	0.765	Cl^-	-0.179	0.645	SO_4^{2-}	0.181	0.642
Cd	0.114	0.771	W	-0.140	0.720	W	-0.152	0.695
Mo	-0.106	0.786	Na	0.132	0.735	EC2	0.130	0.739
As	0.084	0.830	EC2	0.120	0.758	Na^+	-0.051	0.897
EC3	-0.008	0.983	Al	0.088	0.821	Al	0.048	0.902
Zn	-0.006	0.987	Mg^{2+}	0.056	0.886	Na	0.045	0.909
Pb	-0.002	0.996	Na^+	0.046	0.907	Mg^{2+}	-0.032	0.936

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