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The oxidative capacity of indoor source combustion derived particulate matter and resulting respiratory toxicity

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Abstract

Indoor air pollution sources with emissions of fine particles ($PM_{2.5}$), including environmental tobacco smoke (ETS) and incense smoke (IS) deteriorate indoor air quality and may cause respiratory diseases in humans. This study characterized the emission factors (EFs) of five types of tobacco and incense in Hong Kong using an environmental chamber. Human alveolar epithelial cells (A549) were exposed to $PM_{2.5}$ collected from different indoor sources to determine their cytotoxicity. The $PM_{2.5}$ EF of ETS (109.7±36.5 mg/g) was higher than IS (97.1±87.3 mg/g). The EFs of total polycyclic aromatic hydrocarbons (PAHs) and carbonyls for IS were higher than ETS, and these two combustion sources showed similar distributions of individual PAHs and carbonyls. Oxidative damage and inflammatory responses (i.e. DNA damage, 8-hydroxy-desoxyguanosine (8-OHdG), tumor necrosis factor-α (TNF-α) and interlukin-6 (IL-6)) of A549 cells was triggered by exposure to $PM_{2.5}$ generated from ETS and IS. Different indoor sources showed different responses to oxidative stress and inflammations due to the accumulation effects of mixed organic compounds. High molecular weight PAHs from incense combustion showed higher correlations with DNA damage markers, and most of the PAHs from indoor sources demonstrated significant correlations with inflammation. Exposure to anthropogenic produced combustion emissions such as ETS and IS results in significant risks (e.g. lung cancer) to the alveolar epithelium within the distal human respiratory tract, of which incense emissions posed a higher cytotoxicity.

Keywords: biomarkers, DNA, environmental tobacco smoke, incense smoke, inflammation, oxidative damage

1. Introduction

Chronic exposure to airborne particulate matter (PM), especially fine particles (i.e. $PM_{2.5}$, less than 2.5 μm aerodynamic diameter), has been linked to cardiopulmonary diseases in humans (Gualtieri et al., 2010). There is a direct link between the global burden of respiratory diseases and the environment, whereby indoor air pollution (AP) from tobacco and incense smoke, burning of fossil fuels and industrial sources are highlighted as contributing to most respiratory conditions (FIRS, 2017). Reducing the risk from lung carcinogens such as combustion derived air pollution, which is now classified as carcinogenic to humans (Loomis et al., 2014), is needed. Respiratory diseases have become a global health issue due to increasing human morbidity, mortality and health-care costs, with ambient AP being the greatest global risk factor (Mannino and Buist, 2007; Ronkko et al., 2018).

Cohen et al. (2017) estimated that deaths attributable to ambient $PM_{2.5}$ increased to 4.2 million in 2015, representing 7.6% of total global deaths. In many parts of the World, smoking and incense burning (WHO, 2010) are the main sources of indoor $PM_{2.5}$, and when these activities occur the poor ventilation in residences can lead to extremely high indoor pollution levels (Chao et al., 1998; Chuang et al., 2013a). Studies has proved that exposure to high concentrations of indoor particulates can result in lung function reduction and respiratory diseases (Long et al., 2001). Therefore, knowledge of indoor source emission levels and the resulting health effects on the human respiratory system are essential for assessing the health impacts of anthropogenic indoor air pollution.

Combustion-related indoor activities, including smoking and incense burning, can

74 emit significant amounts of fine particles within a short time period (Perrino et al.,
75 2016). These particles from combustion exist in indoor air can increase the potential
76 exposure to the human respiratory system and resulting in cardiopulmonary diseases
77 (Steinvil et al., 2008). Polycyclic aromatic hydrocarbons (PAHs) have been identified
78 as significant carcinogenic compounds in combustion-derived $PM_{2.5}$, (Han et al., 2015).
79 Particulate carbonyls, mainly emitted from indoor combustion activities, consistently
80 showed higher concentrations in indoor environments when compared to outdoor
81 environments, and have been listed as air toxins (Wang et al., 2007). These organic
82 species are found in ETS and IS and have been shown to trigger the expression of
83 cytokines and chemokines in the respiratory epithelium and induce irreversible damage
84 in the respiratory system (Ho et al., 2016; Li et al., 2003). The health end-points of
85 exposure to $PM_{2.5}$ is driven by the formation of Reactive Oxygen Species (ROS) as the
86 primary source of oxidative stress, causing cell dysfunction, inflammatory reactions
87 and concomitant lung injury (Chuang et al., 2011a). The carcinogenic organic
88 compounds can form mutagenic DNA adducts and lead to oxidative DNA lesions by
89 ROS generations (Oh et al., 2011). Therefore, DNA is one of the critical targets for ROS
90 initiated by airborne particles (Danielsen et al., 2009).

91 Environmental Tobacco Smoke (ETS) is a mixture of particulate and gaseous
92 pollutants including thousands of organic components. The combination or synergistic
93 effects of the different phase pollutants may increase the risk of pulmonary disease (Wu
94 et al., 2012). Tobacco smoking has been linked to lung diseases and lung cancer as one
95 of the common etiological risk factors (Cruz et al., 2011). Exposure to ETS within the

indoor environment has shown carcinogenic effects on humans (Slezakova et al., 2009). Bernstein et al. (2008) estimated that exposure to ETS at home could increase the risk of developing asthma by 40–200%. The burning of incense for religious ceremonies or to perfume the air in enclosed environments has created a series of public health issues Worldwide (WHO, 2010). Previous studies indicated that particles from incense combustion contain toxic pollutants that have been associated with ROS formation, DNA damage, respiratory illness and lung cancer (Chuang et al., 2013a; Ho et al., 2005). As one of the main chemical contents of $PM_{2.5}$, PAHs and carbonyl compounds can readily absorb metals, on to their surfaces, and these are responsible for the cellular processes culminating in cell death (Chuang et al., 2011b; Lui et al., 2016).

Although it is known that $PM_{2.5}$, emitted from various combustion sources, showed adverse respiratory effects on humans (Ho et al., 2016; Wang et al., 2015; Wu et al., 2012), there is still a lack of knowledge about the toxicological properties of PM from single indoor sources. In this study, chamber experiments were undertaken to obtain $PM_{2.5}$ samples from different types of tobacco and incense emissions. The objectives of this study were to characterize the organic chemical properties and cytotoxicity of $PM_{2.5}$ from different indoor sources, and identify the organic species correlated with respiratory oxidative stress, DNA damage and inflammation.

2. Methodology

2.1 Collection of indoor combustion samples

Five types of tobacco and incense were selected as typical indoor combustion

sources (Table 1). Combustion emission tests were conducted in a stainless steel environmental chamber (3.2 m × 3.2 m × 2.5 m) with an effective volume of 18.26 m^3. The system was described in a previous study (Huang et al., 2012). Before each experiment, the chamber was fully cleaned with a sponge and ozone (O_3) was introduced by an ozone-generator (Model 2001, Jelight Company Inc., Irvine, CA, USA) to remove any residual particle and gas pollutants. During the experiments the chamber was maintained at controlled conditions (air exchange rate 0.36 h^{-1}, relative humidity 50%, temperature 23 °C) that reproduced typical indoor environments. The background level for $PM_{2.5}$ was below 30 μg m^{-3}, CO and CO_2 were below 5 ppm and 1000 ppm respectively. Each type of tobacco and incense were burned in the chamber until it burned-out, and the sampling continued until the concentrations of $PM_{2.5}$, CO and CO_2 declined to background level. The combustion of tobacco was conducted by a cigarette smoking machine, which can simulate the real condition of a tobacco that puffed by a smoker actively. The details of the indoor source materials and chamber experiments are shown in Table 1, and each experiment was repeated 3 times.

A Dust-Trak air monitor (Model 8530, TSI Inc., USA) after calibration was used to measure $PM_{2.5}$ concentrations, and zero-setting was done before each sampling. A portable Q-Trak (Model 7575, TSI Inc., USA) was used to monitor CO and CO_2 concentrations in the chamber. $PM_{2.5}$ source samples were collected by a particle sampler (MEDVOL, DRI, USA) with a flowrate of 113 L min^{-1}. Two quartz and two Teflon filters (47 mm; Whatman, UK) were positioned in the filter holders separately (Figure S1). More details about the MEDVOL sampler are described in Chen et al.

(2007). The sampling inlet was positioned 1.5 m above the floor of the chamber with a flowrate of 5 L min^{-1}. Blank samples were collected for each type of filter in each set of experiment. After sampling, filters were kept in -20 °C refrigerator for further chemical and biological analysis. All filters were weighed by a microbalance (±1 μg sensitivity, Sartorius AG MC5, Germany) before and after sampling. Filters were equilibrated for 24 h at 23 ± 0.5 °C temperature and 50 ± 5% relative humidity (RH) before weighing.

2.2 Chemical analysis

Organic carbon (OC) and elemental carbon (EC) were analyzed on quartz filters using a DRI Model 2001 Thermal/Optical Carbon Analyzer (Atmoslytic Inc., Calabasas, CA, USA) by thermal/optical reflectance (TOR) method following the IMPROVE_A protocol. This method for carbon analysis has been widely used in $PM_{2.5}$ chemical analysis since 1990s (Chow et al., 1993). More detailed descriptions on the method can be found in Cao et al., (2012).

The concentrations of 19 PAHs were analyzed by in-injection port thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS; Agilent Technologies, USA). Two hole punches of the quartz filter sample (0.526 cm^2 ×2) were cut into small pieces and transferred into the injector liner of an Agilent 6890 GC/5975 MS detector (Santa Clara, CA, USA) by a TD (thermal desorption) tube. Separation of the eluting compounds was performed by an HP-5MS (30 m long × 0.25 mm I.D.× 0.25 μm film thickness, Agilent Technologies, USA) capillary column. The evaluation of TD-GC/MS

methods and more details on organic compounds analysis are presented in Ho et al., (2008).

The carbonyl compounds in the indoor source samples were analyzed by hydroxylamine hydrochloride chromatography/mass spectrometry (PFBHA-GC/MS; Agilent Technologies, USA). The quartz filters were extracted with ultrapure methanol and rotary evaporator to 5 ml, and purged with nitrogen at room temperature. The dried sample was re-dissolved by PFBHA solution and acidified to pH 2 and stood for 1 day, and then further extracted to 1 μl of hexane analyte for GC-MS analysis. This method is widely used and has been validate by Yu et al., (1993). The details of this method was described in Lui et al., (2016).

2.3 Emission factors calculation

Emission factors (EFs) of ETS and incense were calculated by dividing the mass of combustion emissions by the mass of the material burnt, and they are expressed as milligrams or micrograms of emission per gram of consumed material (mg/g or μg/g). The EFs of particulate pollutants were calculated as:

$$EF = \frac{m_{filter}}{V_{filter}} \times \frac{V_{chamber}}{m_{material}} \quad (1)$$

where EF is the EFs of pollutants for the specific indoor sources; m_{filter} is the mass of pollutant collected on the filter in μg/ng/pg; V_{filter} is the sampling volume through the filter in m^3; $V_{chamber}$ is the total volume of the environmental chamber in m^3; $m_{material}$ is the mass of the materials burnt (difference between original weight and residual ash) during the sampling period in μg.

184

185 **2.4 Bioreactivity analysis**

186 2.4.1 Cell culture and treatment

187 Human alveolar epithelial cells (A549, American Type Culture Collection,
188 Rockville, MD, USA) were cultured in RPMI (Roswell Park Memorial Institute) cell
189 culture medium (10% feotal bovine serum, 100 U ml^{-1} penicillin and 100 mg ml^{-1}
190 streptomycin) with 5% CO_2 at 37 °C and 95% humidity. The cells were exposed to 0,
191 100 and 200 μg ml^{-1} of $PM_{2.5}$ for 24 hours. The $PM_{2.5}$ solutions were extracted by
192 methanol and re-dissolved with phosphate buffer saline (PBS) after drying with
193 nitrogen. Cells were analyzed for cell viability and ROS, and the supernatants were
194 analyzed for cytokines.

195

196 2.4.2 Cell viability

197 MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay was
198 used for cell metabolic activities. A549 cells were seeded on 96-well Transwell plates
199 and exposed to different concentrations of $PM_{2.5}$ extractions to a final volume of 200 μl
200 for 24 hours. After that, the cells were treated with MTT solution (10%; Sigma Aldrich,
201 St. Louis, MO, USA) for color development at 37 °C for 4 hours. The optical density
202 (OD) of each well was measured by a microplate reader (ELx800, BioTek, VT, USA)
203 at 540 nm. Cell viability (%) was presented after adjusting for the control.

204

205 2.4.3 Reactive oxidative species

ROS was determined by the fluorogenic cell-based method using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) as the indicator, which has been commonly used for environmental toxicology (Eruslanov and Kusmartsev, 2010; Montesinos et al., 2015). After 24 hours exposure to $PM_{2.5}$, DCFH-DA was added to the A549 cells, and cultured for 30 min. Each well was washed with PBS to remove the DCFH-DA that did not combined with cells. The fluorescence intensity (IF) was determined by a Light Luminescence Plate Reader (VICTOR™ X; PerkinElmer, Waltham, USA) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

2.4.4 Plasmid scission assay (PSA)

The plasmid scission assay (PSA) is a method that uses a biological indicator to determine the capability of $PM_{2.5}$ to induce oxidative DNA damage (Lui et al., 2019). This *in vitro* method measures oxidative damage to plasmid DNA induced by free radicals generated on particle surfaces (Lui et al., 2019). Oxidative damage initially causes the supercoiled DNA to relax, and further damage results in linearization. The sum of the percentage of relaxed and linear DNA is the oxidative damage rate. Ultra-pure water (conductivity 18.2 MΩ; Millipore, China) was used as a procedure blank throughout the experiment.

The $PM_{2.5}$ samples were suspended in molecular grade water (Sigma-Aldrich, UK) at different concentrations (50, 100, 500, 1000 μg ml^{-1}). The plasmid ΦX174 RF DNA molecule (Promega, UK), which is vulnerable to ROS, was added in a final volume of

20 ng and incubated with $PM_{2.5}$.

Gels (0.6% Agarose; Bioline, UK) were prepared using Tris/Borate/EDTA (TBE) buffer solution (Thermo Scientific, UK) diluted 10 times with agarose and the solution was heated by microwave (EMS-820; Electron Microscopy Services, USA) to clarity and transparency. The solidified gel was placed in an electrophoresis cell (DYCP-34A type; NANBEI, China) containing 10 times diluted TBE buffer.

Bromophenol blue stain (14 μL; Sigma-Aldrich, UK) was added to the DNA-PM2.5 samples and placed on a rocking platform (Bio-Rad, UK) for 4 hours. Post-mixing, 20μL of the DNA-PM2.5 mixtures were aliquoted into each gel well. Three parallel samples were made for each sample. Ethidium bromide (EB; 20 μL; Sigma-Aldrich, China) was added to both sides of the electrophoresis tank (NANBEI, China). After the EB was fully dissolved in the buffer, the laboratory electrophoresis power supply (DYY-6C; NANBEI, China) was turned on and operated at 30 Volts for 16 hours.

Post-electrophoresis, the optical densities of three different DNA morphologies (i.e. super-coiled, relaxed and linear) in the gel were captured using a gel documentation system (ChemiDoc, Bio-Rad, UK) and the GeneTools (Version 4.3.10; Syngene, USA) image analysis software program was utilized to calculate the toxic dose of $PM_{2.5}$ causing 50% DNA damage (TD_{50}) via a non-linear regression exponential rise to maximum model. In the final calculation, the DNA damage of ultra-pure water was subtracted from the DNA damage caused by particles. Additional information about the PSA procedure can be found in Chuang et al., (2011b).

2.4.5 Determination of cytokines

Enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., MN, USA) was used to determine 8-hydroxy-desoxyguanosine (8-OHdG), tumor necrosis factor-α (TNF-α) and interlukin-6 (IL-6) levels according to the manufacturer's instructions (Chuang et al., 2018).

2.5 Statistical analysis

Pearson's correlation coefficient analysis was used to identify the correlations between chemical compounds and bioreactivity caused by $PM_{2.5}$ exposure, including cell viability, oxidative-inflammation cytokines and DNA damage. All the data were analyzed by IBM SPSS statistics 22.0 (IBM ®, New York, NY). The significance level was $p < 0.05$.

3. Results and discussion

3.1 Emission factors of indoor sources

3.1.1 $PM_{2.5}$ and carbonaceous compounds

The EFs of $PM_{2.5}$ and chemical components for different indoor sources are shown in Table 2. The average $PM_{2.5}$ EF for ETS was slightly higher (109.7±36.5 mg/g) than incense with $PM_{2.5}$ EF of 97.1±87.3 mg/g. The $PM_{2.5}$ EFs of different tobacco brands ranged from 55.6 to 156.8 mg/g, while the differences among five incense brands were much larger (16.8 to 253.7 mg/g) (Figure 1). The larger differences among incenses were related to the different components in fragrant plant materials for incense sticks production (Jetter et al., 2002). Chuang et al., (2012) measured two types of incenses

in a church with $PM_{2.5}$ EFs of 417.2±72.9 and 290.1±94.3 mg/g. The carbonaceous fractions (OC and EC) showed the greatest contributions in $PM_{2.5}$ EFs to the indoor sources. The average EF of OC for ETS was as high as 61.2±19.5 mg/g, which contributed 55.8% to the total $PM_{2.5}$ EFs. The EF of OC for incense was lower than ETS (45.0±39.5 mg/g), and with a lower contribution of 46.3% to $PM_{2.5}$ EFs. Comparing with tobacco, incense was burned more completely, which has led to more transformations of organic components to gaseous pollutants (i.e., CO_2 and CO) (Wang et al., 2007). The contributions of OC to $PM_{2.5}$ emissions among different types of tobacco (s.d. 1.2%) and incense (s.d. 4.9%) were similar, though their EFs had larger variations; indicative of the specific chemical characteristics for each of the indoor sources.

3.1.2 PAHs

The EFs of 19 detected PAHs from each indoor source are listed in Table 2. The EFs of total PAHs (∑PAHs) for incense ranged from 5.93±2.71 to 24.93±0.86 μg/g (Table 2, Figure 1) with an average EFs of 14.98±6.17 μg/g, which is 19.3% higher than ETS. The average EFs of ∑PAHs for ETS was 12.08±4.22 μg/g, ranging from 5.77±2.19 to 18.72±8.36 μg/g. The relatively high PAHs EFs of incense was attributed to the higher contributions of ∑PAHs to $PM_{2.5}$ (0.015%), especially the PAHs EFs of I-5 showed 0.050% contributions to $PM_{2.5}$. For ETS, the average contribution of ∑PAHs to $PM_{2.5}$ were 0.011%, ranging from 0.010% to 0.013%. Non-volatile particulate PAHs that are harmful to the human respiratory system were emitted during the combustion

for both incense and tobacco; which would increase the risks of lung cancer (Tse et al., 2011). The EFs of individual PAHs ranged from 0.07±0.02 to 1.92±0.63 μg/g and 0.08±0.07 to 1.78±0.73 μg/g for ETS and incense. Different types of tobacco and incense shared common PAHs EFs profiles. The most abundant PAHs emitted from tobacco and incense burning was chrysene, contributed 15.9% and 11.9% to ∑PAHs, followed by fluoranthene (13.6% and 10.6%), pyrene (13.7% and 9.8%), benzo[a]anthracene (8.6% and 10.0%) and benzo[a]pyrene (6.7% and 7.3%), respectively. ETS and incense showed similar PAHs constitution profiles in indoor environments because they both emitted pollutants from combustion. Similar results have been reported by a smoking study (Slezakova et al., 2009) and a chamber study for incense burning (Lui et al., 2016). The contributions of different ring number PAHs to ∑PAHs for ETS and incense are shown in Figure S2. Tobacco and incense burning emitted PAHs that mainly consisted of 4-ring PAHs (53.3% and 43.1%) and 5-ring PAHs (21.7% and 28.4%). Slezakova et al., (2009) concluded that PAHs with 5 rings and 6 rings were more abundant at sampling sites influenced by tobacco smoke, indicating the significant influences of combustion sources on PAHs emissions. Previous studies have proved that PAH structures containing 4 or more rings were more carcinogenic and correlated with human pulmonary diseases (Lin et al., 2013; Niu et al., 2017). Therefore, indoor pollution from tobacco and incense burning would significantly impact on carcinogenic effects in human respiratory systems.

3.1.3 Carbonyls

The average EFs of carbonyls for indoor sources are listed in Table 2, 5 high-molecular-weight mono-carbonyl (C > 6) and di-carbonyl compounds in particulate phase were detected. The average EFs of total carbonyls (∑carbonyls) for incense was the highest (30.02±29.0 μg/g), with a wide range of 2.58±0.33 to 83.88±3.31 μg/g (Figure 1) due to the variations of incense materials. ETS also showed relatively high ∑carbonyls EFs of 24.33±8.1 μg/g, and the EFs levels for different brands did not vary significantly (from 11.22±1.19 to 33.03±8.75 μg/g). The contributions of individual carbonyls (Figure S3) for ETS and incense showed similar patterns, of which methylglyoxal (33.7% and 40.4%) accounted for the largest proportion followed by heptaldehyde (25.7% and 21.3%). Octaldehyde and glyoxal also showed relatively higher contributions for EST and incense. This result agreed with a previous study on incense burning carbonyl characteristics that glyoxal and methylglyoxal were the most abundant components (Lui et al., 2016). Pang and Lewis (2011) reported that some carbonyls (such as such as benzaldehyde, 2,5-dimethylbenzaldehyde, 1-penten-3-one, glyoxal and methylglyoxal) were found only in the particle phase from cigarette smoke due to their low vapor pressures or physicochemical characteristics, and particulate glyoxal and methylglyoxal were always at high levels.

3.2 Cytotoxicity of indoor sources

3.2.1 Cell viability

The bioreactivity of A549 cells after exposure to different indoor sources at two $PM_{2.5}$ concentrations are shown in Figure 2. With increasing $PM_{2.5}$ exposure

concentration, the cell viability showed a decreasing trend. The average cell viability of incense was 59.3% and 55.4% at $PM_{2.5}$ doses of 100 μg/ml and 200 μg/ml, respectively. For ETS, the average cell viability at 100 μg/ml and 200 μg/ml $PM_{2.5}$ exposure were 68.8% and 63.5%. The cytotoxicity induced by incense was higher than ETS. Previous studies has proved that $PM_{2.5}$, especially PM emitted from combustion sources, showed significant and dose-dependent reduction in cell viability (Ho et al., 2016; Sun et al., 2018). Chuang et al. (2013b) suggested that incense burning $PM_{2.5}$ would activate an oxidative stress response leading to a apoptotic phenotype and respiratory cell dysfunction.

3.2.2 Oxidative capacity

Oxidative stress has been recognized as one of the main mechanisms for PM-mediated cytotoxicity; initiated ROS would target cellular compounds (i.e., proteins, lipids and nucleic acids) and induce cellular damage (Marchetti et al., 2019). The fluorescence intensity of different indoor sources indicating the ROS generation levels are shown in Figure 2. ETS showed the highest oxidative potential with average fluorescence intensities of 3363.1 and 4826.7 at 100 μg/ml and 200 μg/ml, respectively. The oxidative reactions induced by incense were 22.9% and 29.1% lower than tobacco at 100 μg/ml and 200 μg/ml $PM_{2.5}$ exposure. ROS including free radicals and non-radicals can increase oxidative stress on DNA, protein and lipid (Oh et al., 2011). 8-OHdG is a well characterized biomarker of ROS induced DNA damage, and showed a similar variation among the different indoor sources with ROS (Figure 2). The average

8-OHdG levels triggered by 200 μg/ml $PM_{2.5}$ from ETS and incense were 509.2 and 418.9 pg/ml, respectively. The correlations of ROS and 8-OHdG production at 200 μg/ml $PM_{2.5}$ exposure are shown in Figure S4, with a Pearson's correlation of R=0.82. $PM_{2.5}$ generated from ETS showed higher oxidative potential to A549 cells than incense, and this may be attributed to the differences in the contributions of individual toxic PAHs and carbonyls. Previous epidemiological and toxicological studies has proved that PM generated from combustion processes would lead to an increase in oxidative potential and to be a risk factor for cardiopulmonary morbidity and mortality (Chuang et al., 2013a). The PM induced oxidative stress could be generated either directly by oxidant's organic and metal components, or indirectly by further cellular responses (Rabha et al., 2018). PAHs and carbonyls in $PM_{2.5}$ generated from indoor sources would induce ROS production at different levels due to the variations in chemical composition. Tobacco and incense emissions had a greater contribution from high-molecular-weight (HMW) PAHs, which has been correlated with oxidative stress in human lung cells (Leung et al., 2014). A lung toxicity study by Marchetti et al., (2019) found that the higher PAH content in wood and charcoal PMs enhanced the expression of metabolizing and oxidative stress-related enzymes.

The percentage of oxidative DNA damage determined by PSA was shown by the three states of plasmid DNA: supercoiled (no damage), relaxed (minor damage), and linear (severe damage) as shown in Figure 3. The corresponding logarithmic regression lines of different indoor sources for TD_{50} calculations are shown in Figure S5. The indoor sources all caused oxidative DNA damage in a dose-dependent manner. At 100

383 μg/ml $PM_{2.5}$ exposure dose, the DNA damage induced by ETS (29.2%) was much
384 higher than incense (15.0%), while increasing $PM_{2.5}$ concentration resulted in
385 decreasing variations of DNA damage among the three sources. The average TD_{50}
386 concentration (Figure 2) for incense (1557.9 μg/ml) was significantly lower than ETS
387 (2233.4 μg/ml), indicating the higher oxidative potential for incense at high exposure
388 $PM_{2.5}$ levels. Two main biochemical pathways can lead to the observed effects on
389 cellular DNA damage. Either non-cellular characteristics of the particles including size,
390 surface reactivity and chemical components etc. and/or the cellular properties including
391 ROS generation ability, initiation of inflammation and alteration of signaling pathways
392 (Reche et al., 2012). Oh et al., (2011) has confirmed the important role of $PM_{2.5}$ organic
393 extracts inducing significant increases of oxidative DNA damage including oxidized
394 purines and pyrimidines. With the release of ROS, single- and double strand DNA
395 breaks were generated by a sequence of radical reactions within the DNA backbone,
396 and even single-strand breaks could impede the ensuing transcription, replication and
397 repair processes (Bertram and Hass, 2008). Previous studies have proved that PAHs and
398 PAH-derived compounds not only trigger bulky DNA adducts and oxidative DNA
399 damage, but also could modulate DNA repair mechanisms, cell cycle progression, and
400 cell fate (Ronkko et al., 2018). The high proportion of PAHs generated during
401 combustion by tobacco and incense would induce higher levels of DNA damage even
402 at low exposure levels. Navasumrit et al. (2008) found that inhalation of incense smoke
403 could significantly increase 8-OHdG levels and DNA strand breaks in temple workers.
404 Comparing our simulation results with previous studies in real indoor environments,

the high oxidative DNA damage capacity of tobacco agreed with a previous study on indoor air in a smoker's living room (Shao et al., 2007), which also led to the higher risks of environmental tobacco exposure. Previous studies found that over 50% adults and children were exposed to passive smoking and about half of the households in Hong Kong burn incense, which adversely affect respiratory health (McGhee et al., 2002; Xie et al., 2014; Zhang et al., 2019). Chuang et al., (2012) has proved that although people stay in church for short time, exposure to the high levels of PM emitted by candles and incense would induce irreversible health effects. A study conducted in various indoor environments with personal measurements concluded that residential indoors and personal OC and PAHs should be of great concern for human respiratory health (Chen et al., 2020).

3.2.3 Inflammatory response

Inflammation has been recognized as one of the important factors for developing respiratory diseases by oxidative stress (Dilger et al., 2016). The inflammatory reactions presented by TNF-α and IL-6 induced by $PM_{2.5}$ from different indoor sources are shown in Figure 2. With the elevating of $PM_{2.5}$ doses, all the sources showed higher inflammatory responses on TNF-α and IL-6 at 200 μg/ml $PM_{2.5}$ exposure. Similar trends for the indoor sources were also shown for the average values of TNF-α and IL-6; incense induced higher inflammatory markers levels than ETS. For example, the TNF-α and IL-6 concentrations in A549 cells at 200μg/ml incense extracts exposure were 25.2 and 49.1 pg/ml, respectively; whereas TNF-α and IL-6 triggered by ETS was 12.9% (21.9 pg/ml) and 15.4% (41.5 pg/ml) lower than incense. Tobacco and incense

emissions both showed high potential for inflammatory responses, while the differences in chemical composition may cause the variations seen in inflammation. The higher inflammatory responses of incense correlated with the results of cell viability, which finally results in greater cell damage to the respiratory system (Schmidt et al., 2017).

Oxidative stress triggered by particle deposition in the human respiratory system would further activate the transcription of pro-inflammatory mediators like IL-6 and TNF-α, which would lead to airway inflammation and diseases (He et al., 2018). Although TNF-α and IL-6 showed similar trends in the indoor sourced $PM_{2.5}$ samples, the effects of $PM_{2.5}$ from different brands of incense and tobacco on bioreactivities were still varied. C-2 and C-3 showed higher TNF-α levels among the five tobaccos, while C-4 and C-1 were higher than others for IL-6. Among the five incenses I-5 and I-1 induced higher TNF-α and IL-6 concentrations, while the other three incenses showed different responses. C-1 and C-2 showed similar levels in TNF-α, but the IL-6 level of C-1 was much higher than C-2. The different effects of indoor sourced $PM_{2.5}$ on TNF-α and IL-6 was caused by the different pathways in regulating the secretion of cytokines (Longhin et al., 2018). Previous studies has proved that cytokines in cells are released either from secretory granules or via constitutive secretory pathways that instead have more dynamic vesicular carriers (Stow et al., 2009).

3.3 Correlations of chemical components and bioreactivity

In order to identify any associations between $PM_{2.5}$ chemical compounds and oxidative-inflammatory responses from different indoor sources, Pearson's correlation coefficients (R) were calculated and these are shown in Table 3. Moderate ($0.3 < R <$

449 0.7) and strong (R > 0.7) correlations were found in some specific chemical compounds
450 and this is highlighted in Table 3. OC showed moderate correlation with DCFH level
451 for total indoor sources and ETS, while strong correlations with TNF-α and IL-6 for
452 incense were found. Moderate negative correlations with cell viability were only
453 observed for the PAHs of total indoor sources. DCFH was poorly correlated with PAHs,
454 only BghiP from ETS and DaeP from incense showed significant correlations. For
455 incense sources, moderate to strong correlations were found between HMW PAHs and
456 8-OHdG, among which, 5-ring and 6-ring PAHs showed higher correlations. For total
457 indoor sources and ETS, only DaeP, IcdP and COR showed significant correlations with
458 8-OHdG. Most of the individual PAHs showed significant correlations with TNF-α and
459 IL-6. For the total indoor sources the LMW PAHs were more correlated with the
460 inflammation markers. Similar conclusions were also found for incense, PHE, ANT and
461 FLT showed moderate to strong correlations with inflammatory responses. Only a few
462 carbonyls were observed with significant correlations with biomarkers: Gly and Mgly
463 were correlated with TNF-α for total indoor sources, C8 was correlated with TNF-α and
464 C-6 was correlated with IL-6 for ETS. These results are consistent with the conclusions
465 of Ronkko et al., (2018) that BaP and BkF showed moderate to strong correlations with
466 genotoxic responses.

467 OC and PAHs in $PM_{2.5}$ from indoor sources were highly correlated with the
468 oxidative and inflammatory responses of A549 cells. Previous studies have indicated
469 the important role of PAHs in $PM_{2.5}$ in inducing oxidative damage and inflammation in
470 human lung cells (Ovrevik et al., 2010). It was also reported that organic compounds,

471 especially PAHs in $PM_{2.5}$ were positively correlated with genotoxic mechanisms of
472 inducing DNA-adduct and oxidative DNA damage (Gualtieri et al., 2010). The
473 significant correlations between PAHs and bioreactivity were mainly attributed to
474 indoor source emissions, especially combustion sources like ETS and incense,
475 containing soot-based particles with chemical-rich surfaces that were able to cause
476 oxidative and inflammatory markers formation. *In vitro* studies has demonstrated that
477 high mutagenic and DNA adduct-forming potential is associated with the neutral and
478 slightly polar fractions including PAHs and their derivatives (Besis et al., 2017).
479 Dergham et al. (2015) also proved the positive correlations of 8-OHdG and PAHs. In
480 this study, the oxidative-DNA damage was more correlated with HMW PAHs, which
481 were always in the particle phase; while the LMW PAHs were more correlated with
482 inflammatory responses, which were volatile and partially in the gas-phase. Different
483 individual PAHs from different indoor sources showed variations on cytotoxicity due
484 to the influences of the mixture components. The toxic properties of particles could be
485 either inhibited or enhanced when the chemical compounds are combined (Marchetti et
486 al., 2019). In addition to organic compounds, the inorganic compositions in PM are also
487 potential factors for PM-induced cytotoxicity. A previous study on PM toxicological
488 effects also demonstrated that some organic and inorganic chemical compounds were
489 preferentially associated with early oxidative responses, whereas others in the later
490 oxidative and/or inflammatory cytokine secretion (Dergham et al., 2015). Akhtar et al.,
491 (2010) found that the biological responses were more responsive to metals as compared
492 with secondary inorganic ions and organic compounds. The synergistic cytotoxic

effects of water soluble and insoluble components were observed with long exposure time, and they were predominantly associated with ROS and cell membrane disruption, respectively (Zou et al., 2016). The interactions between chemical compounds and the oxidative and inflammatory responses of A549 cells were ambiguous, and therefore still need further detailed studies.

4. Conclusion

This is the first study to compare the cytotoxicity effects of major indoor sources, ETS and incense, and combined the results with $PM_{2.5}$ emission characteristics. The results indicated the great potential of combustion emissions $PM_{2.5}$ on inducing human respiratory diseases. The EFs of $PM_{2.5}$ chemical components from different sources varied, ETS and incense showed similar chemical contributions on PAHs and carbonyls. Oxidative-DNA damage and inflammatory reactions were found when exposed to $PM_{2.5}$, while different indoor sources showed different responses to oxidative stress and inflammations due to the accumulation effects of mixed organic compounds. More studies on exposure and bioreactivity levels of $PM_{2.5}$ emissions are needed to investigate the oxidative and inflammatory pathways in human respiratory systems. These studies on the different mechanisms of indoor emission exposure are required to support policies decreasing exposure levels and mitigation of chronic respiratory diseases in indoor environments. Good ventilation and a reduction in combustion emissions are the basic mitigating requirements to achieve safer indoor air.

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Figure Captions

Table 1 Combustion characteristics of different indoor sources in the chamber

Table 2 Emission factors of different indoor combustion sources

Table 3 Correlations of oxidative and inflammatory cytokines with chemical species of indoor sources emissions at 200 μg/ml $PM_{2.5}$ exposure

Figure 1 The emission factors of $PM_{2.5}$, OC, total PAHs and total carbonyls for different types of indoor sources

Figure 2 Bioreactivities of A549 cells exposed to 100 μg/ml and 200 μg/ml $PM_{2.5}$ from different indoor sources (a-e) and median lethal dose (LD50) of indoor sources samples (f).

Figure 3 Examples of gel image showing oxidative damage on supercoiled DNA induced by (a) tobacco; (b) incense; (c) cooking

Figure 4 Corresponding risk factors of indoor sources at 200 μg/ml $PM_{2.5}$ exposure dose

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745 Table 1 Combustion characteristics of different indoor sources in the chamber

Indoor source	Abbre-viation	Initial weight (g)	Combustion Weight (g)	Sampling duration (min)	$PM_{2.5}$ background level (μg m^{-3})	CO_2 background level (ppm)	CO background level (ppm)
Tobacco							
Black Marlboro	T-1	1.68	0.65	60	29.0	689.0	2.1
Double Happiness	T-2	1.82	0.65	45	30.0	728.0	2.3
Red Marlboro	T-3	1.87	0.72	35	28.0	681.0	2.7
Lotus King	T-4	1.75	0.58	35	29.0	709.3	3.3
CAPRI	T-5	1.17	0.54	45	30.0	620.7	3.0
Incense							
Golden Unpacked	I-1	0.94	0.61	40	27.0	889.5	2.0
Black incense	I-2	2.17	1.70	120	18.0	612.0	3.0
No smoke incense	I-3	0.79	0.57	60	29.0	577.0	3.0
Fumakilla	I-4	0.28	0.26	35	28.0	534.3	2.0
Zebra	I-5	1.79	1.48	60	30.0	642.0	3.0

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750 Table 2 Emission factors of different indoor combustion sources

	Abbrevi-ations	Tobacco	T-1	T-2	T-3	T-4	T-5	Incense	I-1	I-2	I-3	I-4	I-5
$PM_{2.5}$ (mg/g)		109.7±36.5	55.6±12	81.1±7.5	122.6±25.8	156.8±30.5	132.3±83.2	97.1±87.3	126.1±19.6	16.8±12.2	64.2±2.9	253.7±42.6	24.8±5.7
Organic carbon (mg/g)	OC	61.2±19.5	31.3±7.1	46.4±4.5	69.5±14.6	84.3±15.8	74.6±47.5	45±39.5	59.7±9.5	6.8±5.0	29.5±1.7	115.1±21.3	13.8±3.2
Elemental carbon (mg/g)	EC	2.0±0.6	1.0±0.1	1.6±0.2	2.0±0.3	2.9±0.6	2.4±1.2	1.9±2.0	2.7±0.7	0.1±0.1	0.8±0.1	5.5±1.3	0.4±0.1
PAHs (μg/g)													
Acenapthene	AC	0.40±0.13	0.19±0.03	0.31±0.08	0.50±0.21	0.44±0.25	0.57±0.43	0.52±0.55	1.4±1.77	0.02±0.01	0.18±0.04	0.92±0.40	0.06±0.04
Fluorene	FLO	0.49±0.26	0.36±0.13	0.3±0.12	0.29±0.17	1.00±0.70	0.50±0.31	1.10±1.16	1.47±0.33	0.08±0.05	0.46±0.08	3.21±1.28	0.28±0.13
Phenanthrene	PHE	0.84±0.37	0.34±0.04	0.53±0.16	0.86±0.14	1.34±0.4	1.13±0.08	1.01±0.78	0.8±0	0.14±0.07	0.68±0.39	2.48±0.51	0.97±0.68
Anthracene	ANT	0.32±0.12	0.21±0.15	0.34±0.27	0.53±0.36	0.29±0.12	0.22±0.11	0.24±0.14	0.37±0.21	0.03±0.01	0.16±0.06	0.42±0.12	0.22±0.05
Fluoranthene	FLT	1.65±0.62	0.73±0.44	1.41±0.17	1.43±0.23	2.47±1.2	2.20±1.12	1.58±0.73	2.62±0.28	0.47±0.16	1.46±0.47	1.29±0.39	2.08±0.25
Pyrene	PYR	1.65±0.63	0.70±0.41	1.44±0.14	1.46±0.24	2.55±1.28	2.11±0.92	1.46±0.73	2.74±0.32	0.55±0.19	1.58±0.46	1.38±0.45	1.05±0.46
Benzo[a]anthracene	BaA	1.04±0.31	0.55±0.18	0.98±0.08	1.11±0.35	1.53±0.76	1.01±0.65	1.49±0.69	2.72±0.37	0.62±0.3	1.53±0.36	1.12±0.32	1.47±0.15
Chrysene	CHR	1.92±0.63	0.91±0.39	1.87±0.15	2.00±0.52	2.88±1.5	1.92±1.3	1.78±0.73	3.01±0.42	0.75±0.36	1.71±0.43	1.86±0.59	1.57±0.14
Benzo[b]fluoranthene	BbF	0.46±0.17	0.23±0.08	0.43±0.03	0.47±0.13	0.77±0.39	0.40±0.27	0.91±0.31	1.35±0.21	0.49±0.21	1.17±0.28	0.7±0.24	0.84±0.06
Benzo[k]fluoranthene	BkF	0.46±0.13	0.24±0.10	0.41±0.03	0.49±0.10	0.61±0.38	0.56±0.42	0.76±0.38	1.42±0.18	0.39±0.14	0.93±0.21	0.42±0.09	0.64±0.10
Benzo[a]fluoranthene	BaF	0.30±0.09	0.16±0.06	0.28±0.04	0.32±0.10	0.45±0.21	0.28±0.19	0.47±0.21	0.83±0.1	0.24±0.11	0.56±0.13	0.35±0.09	0.37±0.04
Benzo[e]pyrene	BeP	0.42±0.12	0.21±0.08	0.42±0.02	0.44±0.11	0.6±0.31	0.41±0.28	0.78±0.28	1.23±0.16	0.45±0.2	0.93±0.25	0.62±0.16	0.67±0.07
Benzo[a]pyrene	BaP	0.81±0.23	0.4±0.16	0.75±0.05	0.94±0.22	1.08±0.53	0.86±0.61	1.09±0.5	1.82±0.27	0.48±0.22	1.47±0.36	0.66±0.13	1.01±0.10
Perylene	PER	0.35±0.42	0.09±0.05	0.16±0.02	0.17±0.05	1.19±1.38	0.13±0.09	0.23±0.09	0.38±0.06	0.12±0.04	0.24±0.06	0.26±0.04	0.16±0.02
Indeno[1,2,3-cd]pyrene	IcdP	0.4±0.17	0.17±0.05	0.35±0.03	0.33±0.06	0.66±0.32	0.52±0.36	0.67±0.39	1.35±0.13	0.31±0.13	0.84±0.12	0.3±0.05	0.56±0.06
Benzo[ghi]perylene	BghiP	0.34±0.21	0.11±0.05	0.22±0.03	0.19±0.09	0.5±0.21	0.66±0.45	0.45±0.22	0.81±0.11	0.25±0.1	0.57±0.05	0.22±0.05	0.39±0.04
Dibenzo[a,h]anthracene	DahA	0.10±0.04	0.06±0.02	0.11±0.03	0.16±0.03	0.12±0.06	0.05±0.01	0.16±0.07	0.27±0.05	0.06±0.02	0.16±0.02	0.19±0.06	0.10±0.02
Coronene	COR	0.07±0.03	0.04±0.01	0.07±0.03	0.05±0.01	0.12±0.07	0.07±0.05	0.11±0.06	0.18±0.01	0.04±0.02	0.11±0.01	0.18±0.09	0.05±0

Dibenzo(a,e)pyrene	DaeP	0.07±0.02	0.06±0.01	0.09±0.03	0.04±0.02	0.10±0.05	0.06±0.03	0.08±0.07	0.14±0	0.02±0.01	0.01±0.01	0.2±0.02	0.04±0
Total PAHs		12.08±4.22	5.77±2.19	10.46±1.04	11.78±2.4	18.72±8.36	13.66±7.68	14.98±6.17	24.93±0.86	5.93±2.71	14.77±3.51	16.76±4.65	12.51±2.02
Carbonlys (μg/g)													
Hexaldehyde	C6	2.32±1.05	1.19±0.04	-	1.44±0.31	3.75±1.17	2.88±1.35	2.13±1.79	3.43±1.47	0.16±0.02	1.70±0.28	4.89±0.15	0.49±0.06
Heptaldehyde	C7	6.25±2.05	3.06±0.05	-	6.62±0.43	8.79±2.33	6.55±2.79	6.39±6.86	6.2±1.72	0.60±0.09	4.17±1.58	19.53±0.93	1.47±0.07
Octaldehyde	C8	2.44±0.88	1.8±0.73	-	3.94±0.06	2.09±0.48	1.92±0.74	3.24±3.01	5.59±1.48	0.31±0.01	1.89±0.95	7.91±0.48	0.48±0.15
Nonaldehyde	C9	2.51±0.84	1.35±0.15	-	2.14±1.33	3.56±0.73	2.97±1.44	2.54±2.17	3.53±0.92	0.29±0.01	1.83±0.46	6.27±0.85	0.77±0.09
Decaldehyde	C10	0.81±0.36	0.33±0	-	0.91±0	-	1.20±0	0.55±0.51	1.4±0.06	0.06±0.02	0.28±0	-	0.47±0.12
Glyoxal	Gly	2.38±0.97	0.99±0.06	-	3.62±0.49	2.79±0.83	2.1±0.74	3.29±3.21	3.15±0.78	0.24±0.07	2.86±0.15	9.3±1.47	0.88±0.31
Methylglyoxal	Mgly	8.21±3.45	2.73±0.56	-	9.92±0.44	12.04±3.96	8.13±2.46	12.12±12.45	10.64±3.05	0.93±0.1	9.36±2.26	35.98±2.37	3.71±0.54
Total Carbonlys		24.33±8.1	11.22±1.19		28.14±1.63	33.03±8.75	24.95±9.34	30.02±29	33.47±9.36	2.58±0.33	21.89±4.83	83.88±3.31	8.27±1.04

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758 Table 3 Correlations of oxidative and inflammatory cytokines with chemical species of indoor
759 sources emissions at 200 μg/ml $PM_{2.5}$ exposure

	Total					Tobacco				Incense			
	Cell viability	DCFH	8-OHdG	TNF-α	IL-6	DCFH	8-OHdG	TNF-α	IL-6	DCFH	8-OHdG	TNF-α	IL-6
OC	0.391*	**0.367***	0.068	-0.104	-0.105	-0.463	-0.051	**0.522***	-0.535*	0.029	-0.244	**0.887****	**0.964****
EC	0.238	-0.399*	-0.526*	-0.347*	-0.335*	0.255	0.219	-0.061	0.280	0.002	-0.500	0.138	0.326
AC	-0.147	0.052	-0.017	0.114	-0.008	-0.364	-0.158	0.350	-0.371	0.313	0.082	0.056	0.002
FLO	-0.188	-0.105	-0.022	**0.486****	**0.581****	0.304	0.074	-0.142	**0.550***	0.074	-0.314	0.217	0.345
PHE	-0.169	-0.106	0.002	**0.564****	**0.586****	0.098	-0.102	-0.395	0.048	-0.023	-0.002	**0.636***	**0.670****
ANT	-0.183	0.112	0.220	**0.700****	**0.462****	-0.311	-0.038	0.474	-0.310	0.226	0.171	**0.822****	**0.787****
FLT	**-0.341***	-0.052	0.152	**0.748****	**0.665****	0.368	0.430	-0.139	0.163	0.239	0.329	**0.809****	**0.724****
PYR	**-0.471****	0.031	0.314	**0.570****	**0.425****	0.378	0.451	-0.155	0.134	0.375	**0.644****	0.419	.0199
BaA	**-0.435****	-0.133	0.150	**0.747****	**0.575****	0.116	0.433	0.435	0.015	0.345	**0.550***	**0.679****	0.512
CHR	**-0.403***	-0.004	0.288	**0.704****	**0.520****	0.176	0.506	0.366	-0.016	0.348	**0.589***	**0.604***	0.430
BbF	**-0.455***	-0.364*	-0.022	**0.576****	**0.425***	0.250	0.500	0.328	0.139	0.272	**0.618***	0.419	0.228
BkF	**-0.478****	-0.325	0.006	**0.562****	**0.386***	-0.008	0.266	0.392	-0.066	0.357	**0.677****	0.390	0.172
BaF	**-0.481****	-0.324	0.021	**0.571****	**0.395***	0.104	0.409	0.445	0.026	0.351	**0.685****	0.393	0.173
BeP	**-0.431****	-0.193	0.139	**0.594****	**0.401***	0.150	0.507	0.446	0.001	0.315	**0.674****	0.348	0.143
BaP	**-0.477****	-0.167	0.143	**0.710****	**0.513****	-0.037	0.374	**0.534***	-0.204	0.260	**0.538***	**0.579***	0.395
PER	-0.220	0.107	0.304	0.237	0.297	0.346	0.216	-0.222	0.309	0.322	**0.666****	0.166	-0.032
IcdP	**-0.519****	-0.178	0.138	**0.653****	**0.491****	0.493	**0.575***	-0.072	0.299	0.385	**0.667****	0.502	0.274
BghiP	**-0.484****	-0.301	0.024	**0.487****	**0.390***	**0.554***	0.297	-0.655*	0.391	0.348	**0.696****	0.358	0.136
DahA	**-0.471****	-0.164	0.173	**0.637****	**0.372***	-0.366	0.141	**0.783****	-0.468	0.360	**0.684****	0.332	0.113
COR	**-0.460****	-0.165	0.182	**0.511****	**0.352***	0.399	**0.531***	0.118	0.309	0.313	**0.660****	0.127	-0.089
DaeP	-0.207	0.125	**0.400***	0.339	0.317	0.270	0.450	0.326	0.342	**0.635***	**0.737***	0.015	-0.294
C6	0.153	-0.270	-0.447*	-0.224	-0.229	0.587	0.379	-0.553	**0.614***	-0.037	-0.179	-0.091	-0.118
C7	0.221	-0.419*	-0.391*	-0.061	-0.189	-0.100	-0.069	0.077	-0.116	-0.460	-0.293	-0.378	-0.215
C8	-0.217	-0.155	-0.021	0.224	-0.044	-0.562	-0.451	**0.620***	-0.358	0.334	0.223	-0.378	-0.452
C9	0.299	-0.418*	-0.617*	-0.257	-0.402*	0.278	0.127	-0.255	0.374	0.071	0.298	0.044	-0.005
C10	0.095	-0.334	-0.351	-0.187	0.049	0.103	-0.376	-0.630	-0.343	0.302	-0.440	0.212	-0.042
Gly	-0.342	-0.679*	-0.450*	**0.461***	0.094	-0.566	-0.394	0.439	-0.656*	-0.890*	-0.756**	-0.177	0.020
Mgly	-0.153	-0.711*	-0.548*	**0.371***	0.150	-0.095	-0.023	-0.046	-0.307	-0.718*	-0.580*	0.070	0.287

760 *p<0.05, **p<0.01

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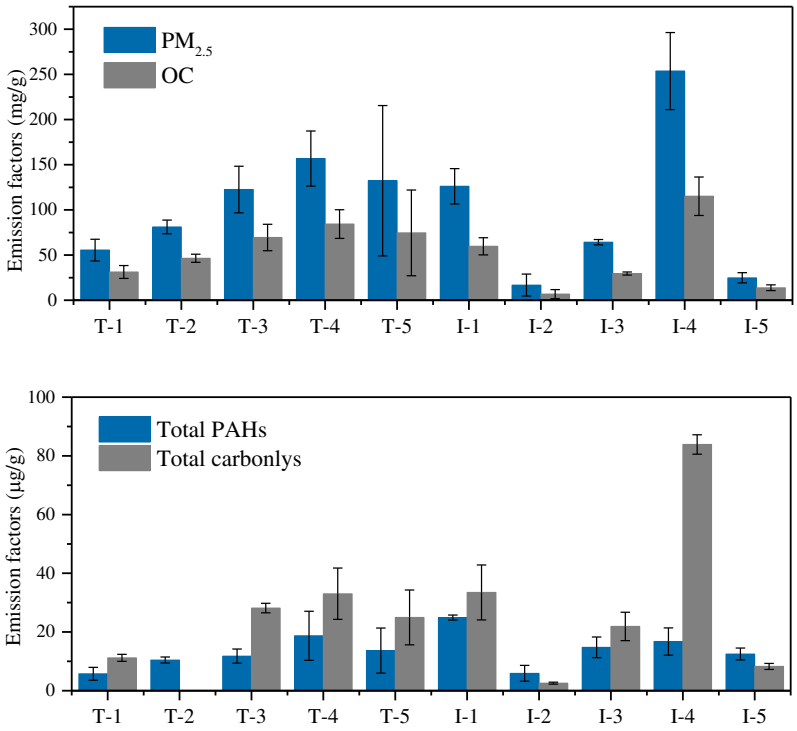

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763 Figure 1 The emission factors of $PM_{2.5}$, OC, total PAHs and total carbonyls for different types of
764 indoor sources

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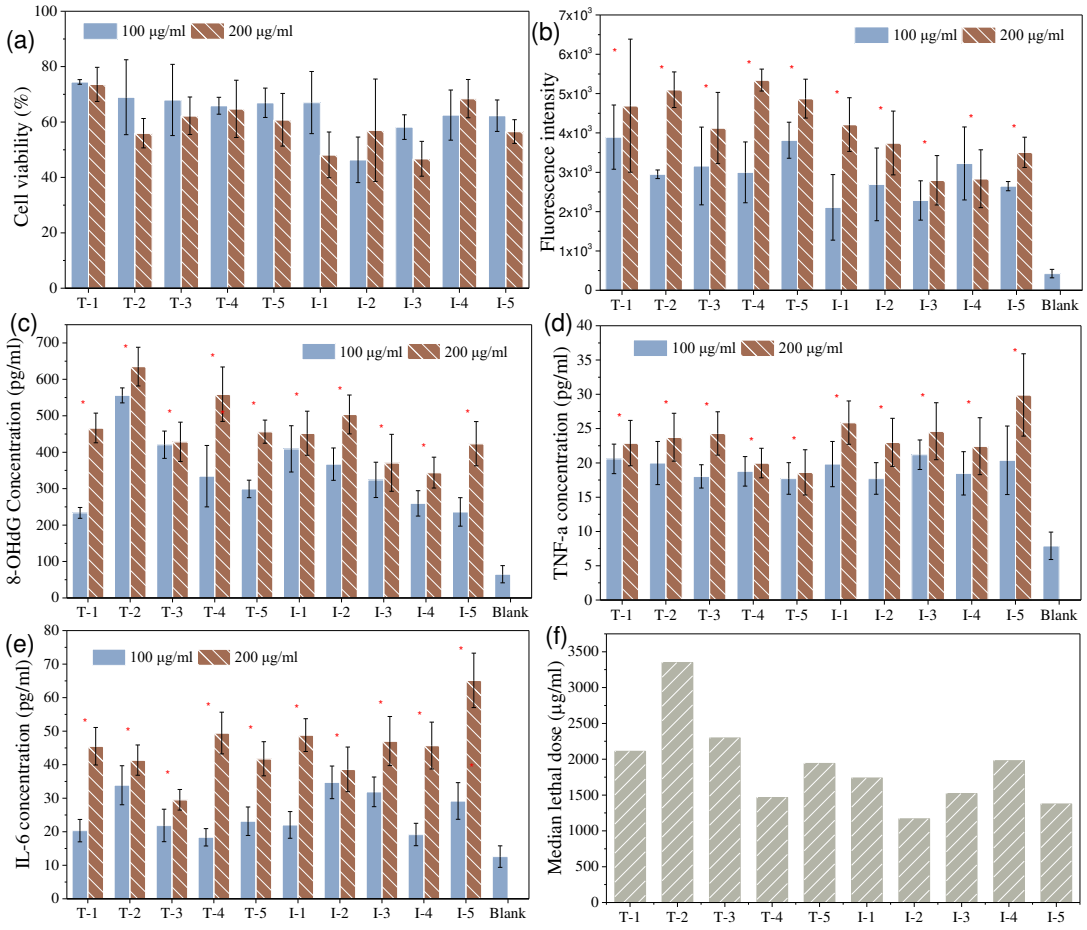

Figure 2 Bioreactivities of A549 cells exposed to 100 μg/ml and 200 μg/ml $PM_{2.5}$ from different indoor sources (a-e) and median lethal dose (LD50) of indoor sources samples (f). T-1~T-5 and I-1 ~I-5 represented ETS and incense from different brands. * Significant difference in comparison of BG at the same concentration ($p < 0.05$)

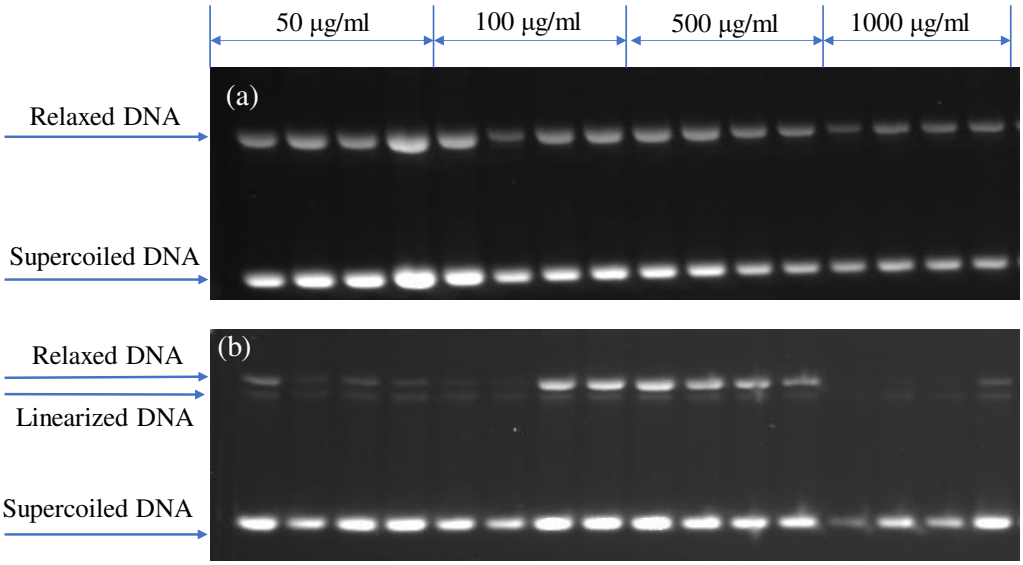

Figure 3 Examples of gel image showing oxidative damage on supercoiled DNA induced by (a) ETS and (b) incense