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Adsorption of CMIT/MIT on the Model Pulmonary Surfactant Monolayers

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Abstract: Polyhexamethylene guanidine (PHMG) is a guanidine-based chemical that has long been used as an antimicrobial agent. However, recently raised concerns regarding the pulmonary toxicity of PHMG in humans and aquatic organisms have led to research in this area. Along with PHMG, there are concerns about the safety of non-guanidine 5-chloro-2-methylisothiazol-3(2H)-one/2-methylisothiazol-3(2H)-one (CMIT/MIT) in human lungs; however, the safety of such chemicals can be affected by many factors, and it is difficult to rationalize their toxicity. In this study, we investigated the adsorption characteristics of CMIT/MIT on a model pulmonary surfactant (lung surfactant, LS) using a Langmuir trough attached to a fluorescence microscope. Analysis of the π-A isotherms and lipid raft morphology revealed that CMIT/MIT exhibited minimal adsorption onto the LS monolayer deposited at the air/water interface. Meanwhile, PHMG showed clear signs of adsorption to LS, as manifested by the acceleration of the L_o phase growth with increasing surface pressure. Consequently, in the presence of CMIT/MIT, the interfacial properties of the model LS monolayer exhibited significantly fewer changes than PHMG.

Key words: Langmuir trough, CMIT/MIT, PHMG, lung surfactant, fluorescence microscope, adsorption

1 Introduction

Guanidine-based antimicrobials have been widely employed for the disinfection, sterilization, and preservation of various products, such as food and cosmetics[1-4]. The efficacy of polyhexamethylene guanidine (PHMG) is primarily attributed to the robust interaction between the positively charged guanidine group at physiological pH and the negatively charged phosphatidylglycerol (PG) lipid bilayer in the bacterial cell membranes[5-7]. In contrast, PHMG has negligible effects on zwitterionic lipid membranes, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), the primary components of fish and mammalian cell membranes[8, 9]. For these reasons, guanidine-based antimicrobials are believed not to harm host cells and selectively target only microorganisms intended for destruction.

However, a few traumatic incidents have recently killed or induced permanent disabilities in many people. In South Korea, PHMG was used as a humidifier disinfectant additive from 1998 to 2011, which resulted in more than 1,500 deaths[10, 11]. In Russia, PHMG was used in illegally manufactured vodka, affecting more than 12,500 patients[12]. These incidents highlight significant concerns regarding the potential toxicity of PHMG to humans. Recent studies have revealed that exposure of zebrafish to PHMG at certain concentrations leads to the generation of reactive oxygen species and the induction of pulmonary toxicity, including inflammation and fibrosis[13-15]. Furthermore, Lim *et al.* revealed that PHMG can strongly interact with and bind to PC groups via direct force measurements with a surface force apparatus (SFA) and adsorption studies using a Langmuir trough[16]. These studies have raised concerns regarding the potential adverse effects of guanidine-based chemicals on human and aquatic health.

In conjunction with PHMG, non-guanidine antimicrobials such as 5-chloro-2-methylisothiazol-3(2H)-one/2-methylisothiazol-3(2H)-one (CMIT/MIT), have also received increasing attention for their toxicity. CMIT/MIT has also been widely used as a biocide in cosmetics, humidifier dis-

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infectants, and personal care products, and recently, concerns regarding lung toxicity of the CMIT/MIT have become an issue, especially in South Korea[17–19] along with PHMG. PHMG has been proven to cause lung fibrosis, but the pulmonary toxicity of CMIT/MIT is still controversial, so research is actively underway to verify the toxicity of CMIT/MIT. To assess the toxicity of CMIT/MIT, Song *et al.* quantified the inflammatory cell count in bronchoalveolar lavage fluid (BALF) after CMIT/MIT instillation in mice. They found that intratracheally instilled CMIT/MIT (2720 ng/g tissue after 5 min) increased the inflammatory cell counts in the BALF[20]. In addition, given the wide range of products containing CMIT/MIT, studies have reported variations based on different exposure routes (nasal, inhalation, and ingestion), concentrations, and forms of exposure[21–23]. In contrast, Kim *et al.* reported that appropriate amounts of CMIT/MIT ingredients in cosmetic products applied to the skin are not toxic to humans[24]. As one can see from these studies, the toxicity of CMIT/MIT may depend on the exposure routes, doses, and many other factors. Thus, drawing a firm conclusion regarding safety is difficult and dangerous. However, fundamental studies on the adsorption characteristics of CMIT/MIT have yet to be conducted.

Herein, rather than focusing on the actual toxicity of CMIT/MIT, we investigated the adsorption characteristics of CMIT/MIT to model pulmonary surfactants (referred to as lung surfactants (LS)) using a Langmuir trough attached to a fluorescence microscope. Adsorption is the initial step for toxins to pass through the LS, and when other molecules are adsorbed to the LS, the mechanical characteristics and/or phase behavior of the LS film are known to change. Figure 1 represents the target system that CMIT/MIT adsorption to pulmonary surfactant in a human body. By investigating the π-*A* isotherm changes (using a Langmuir trough) and imaging the lipid raft morphology changes (using a fluorescence microscope), we observed relatively small changes in the interfacial characteristics with increasing concentration of CMIT/MIT from 0 to 0.1 wt% (which is close to the concentration of PHMG and CMIT/MIT in commercial products), possibly indicating little adsorption of CMIT/MIT to the model LS monolayer.

2 Materials and Methods

2.1 Materials

The LS consisted of dipalmitoylphosphatidylcholine (DPPC; Avanti Polar Lipids), palmitoyl oleoylphosphatidylglycerol (POPG; Avanti Polar Lipids), and palmitic acid (PA; Avanti Polar Lipids) at a 7:2:1 wt% ratio[25, 26]. PHMG (25% in water) was purchased from BOCSCI, Inc. Methyl chloroisothiazolinone (CMIT, Sigma Aldrich, 99%) was diluted to a final concentration of 25 vol% in deionized water (Milli-Q). LS was tagged with fluorescent (Texas Red)-labeled DHPE (N-(Texas Red sulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, Biotium) for lipid domain observation.

2.2 π-*A* isotherm measurement by using Langmuir trough

The Langmuir trough (KSV NIMA) was used for π-*A* isotherm measurement of the LS monolayer, and the surface pressure was measured using a Pt Wilhelmy plate. LS was dissolved in chloroform at a final 1 mg/mL concentration. The 20 μL of LS solution was spread by gently tipping onto the air/water interface in a controlled (20 ± 1℃) Langmuir trough. After applying, the system was equilibrated for 15 min to allow the solvent to evaporate. After monolayer preparation, the toxic chemicals were injected into the sub-phase with the targeted final concentrations of 0.001, 0.005, 0.01, 0.05, and 0.1 wt%, followed by 45 min equilibration. The air/water interface was compressed to a 10 mm/min target rate at a fixed barrier pressure of 50 mN/m.

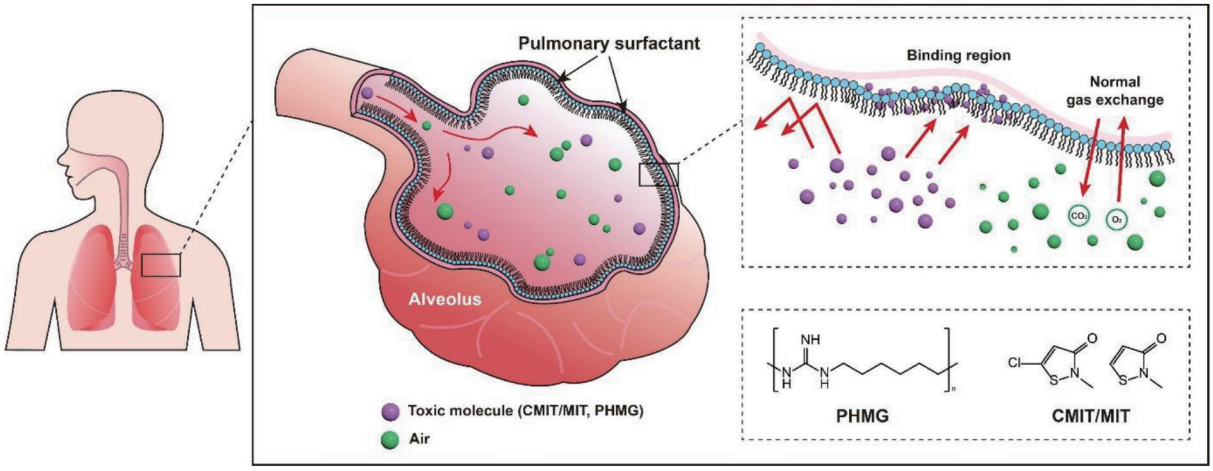

Fig. 1 Schematic images of human pulmonary surfactant and gas exchange mechanisms w/, w/o toxic molecules (PHMG and CMIT/MIT).

2.3 LS monolayer visualization using a fluorescence microscope

LS was mixed with 0.1 wt% of the fluorescent Texas-Red-labeled-DHPE to monitor the lipid domain morphology of the LS monolayer to provide fluorescence contrast. Large fluorescent molecules cannot fit into the condensed phases of LS monolayers; instead, they segregate into a disordered phase, causing the liquid-expanded (LE) and liquid-condensed (LC) stages to appear bright and dark, respectively[27]. Fluorescence images of the LS monolayer with fluorescence contrast caused by the phase difference of the lipids were recorded using a fluorescence microscope (Andor). Fluorescence microscopy was performed in the upright direction in the Langmuir trough (with no temperature control setup), and the surface tension gradient due to compression was minimized by forming an isolated region using a circular reservoir.

3 Results and Discussion

3.1 π-A isotherm measurement

To investigate the influence of PHMG and CMIT/MIT on the mechanical properties of the LS monolayer, π-A isotherms were measured using a Langmuir trough (Fig. 2) in the presence of these chemicals in the sub-phase. Figure 2a shows the steps of π-A isotherm measurements, including deposition, toxin injection, and compression. After depositing the LS monolayer, PHMG, and CMIT/MIT were injected into the sub-phase at a targeted final concentration (0.001～0.1 wt% dissolved in water). Upon analyzing the π-A isotherm of the bare LS monolayer, the typical plateau regime representing the LE – LC phase coexistence was not pronounced, in contrast to the pure DPPC lipid monolayers[16, 28]. A kink region not present in the pure DPPC monolayer was observed at around 50 mN/m because POPG (collapse pressure, π ～48 mN/m) contained in LS can be squeezed out from the interface[29].

Figure 2b shows the π-A isotherms of the LS monolayer at different PHMG concentrations. The π-A isotherms of the LS monolayer exhibited a significant increase (upward

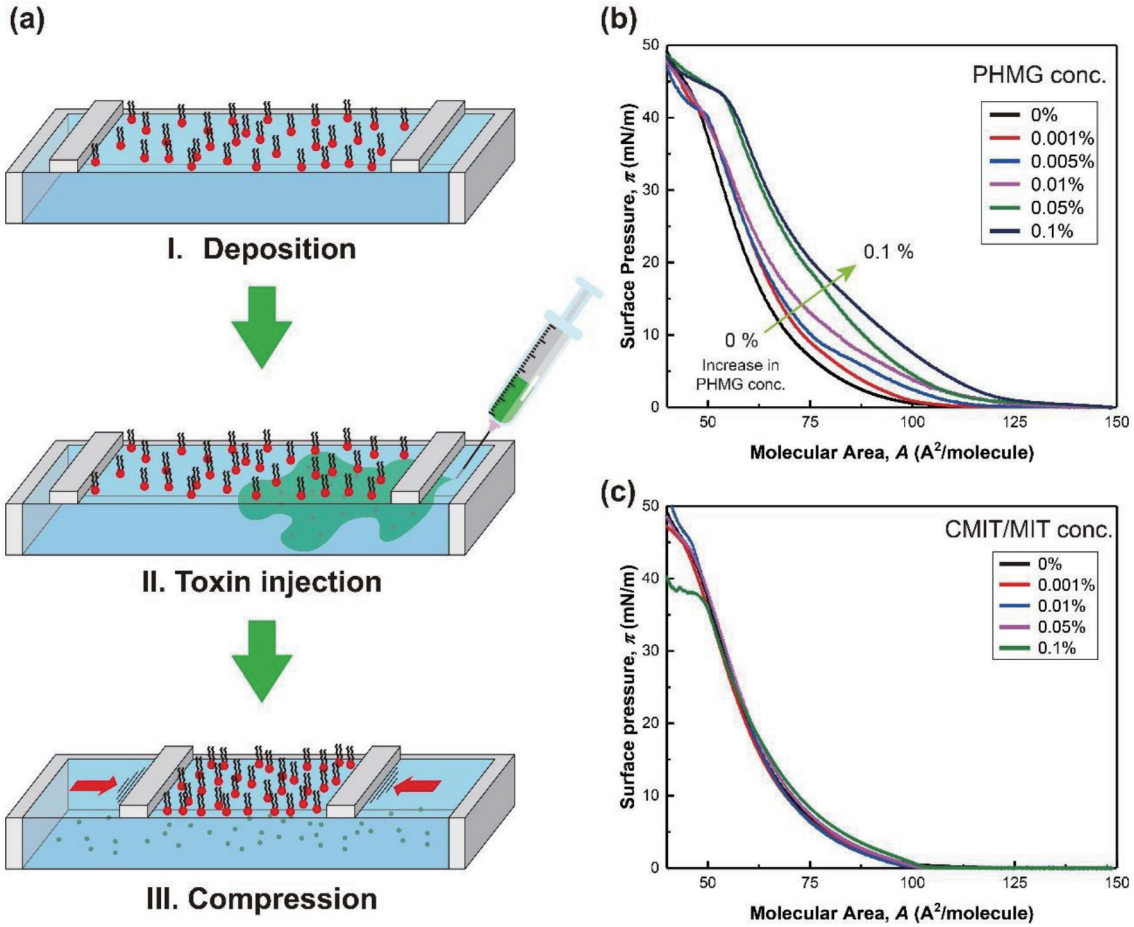

Fig. 2 (a) Schematic images of experimental steps for π–A isotherm measurement using Langmuir trough. The π–A isotherms of LS monolayer as a function of sub-phase (b) PHMG and (c) CMIT/MIT concentration.

shift) with increasing PHMG concentrations. This indicates that PHMG is bound to LS, thereby influencing the mechanical properties of the LS monolayer. With an increase in the injected PHMG concentration, the liftoff area, where the surface pressure started to increase during compression, increased from ~100 Å^2/molecule to ~150 Å^2/molecule. The surface pressure in the same area also significantly increased at all stages before the collapse. For example, at $A = 75$ Å^2/molecule, $\pi = 6.97$ and 20.38 mN/m at 0 and 0.1 wt%, respectively. Furthermore, PHMG concentrations above 0.05 wt% showed significant changes in the kink region. In contrast, kink showed at the same pressure and area for concentrations between 0 and 0.01 wt%; concentrations higher than 0.05 wt% resulted in kink showing at relatively higher pressures and broader areas (~43 mM/m at 57 Å^2/molecule). This tendency seems to cause a kink more clearly, owing to the tendency of the fluidizing component present in the interface to be squeezed out when toxic components are present. In addition, as the concentration of PHMG increased, more PHMG appeared to exist at the interface, shifting π-*A* isotherm to the right.

The procedure was repeated to examine the adsorption of CMIT/MIT onto the LS monolayer (Fig. 2c). Unlike PHMG, there were no significant differences in π-*A* isotherms upon injection of CMIT/MIT up to 0.05 wt%, whereas 0.1 wt% of CMIT/MIT leads to a lowering in a kink pressure from ~44 mN/m to ~37 mN/m, caused by the squeeze out of the fluidizing component. These results indicate no noticeable adsorption of CMIT/MIT on the LS monolayer below a concentration of ~0.1 wt%.

3.2 Fluorescent microscopy analysis of LS monolayer lipid domains

Fluorescence microscopy analysis is one of the most effective instruments for visually verifying the phase transition of lipid monolayers and changes in lipid domains during dynamic compression at the air/water interface[30–34]. The phase transition of the LS monolayer, which was affected by compression, was visually observed under varying concentrations of PHMG and CMIT/MIT. Upon compression of LS at the air/water interface, the phase separation of the LS monolayer can be visualized as a lipid-disordered (L_d) phase and lipid-ordered (L_o, also known as the lipid domain) phase (Fig. 3a)[35]. This arises from molecular area and mobility differences resulting from the structural differences between unsaturated and saturated lipid molecules[36, 37]. It has been previously reported that upon binding to another molecule, the shape and size distribution of lipid domains can change[34, 38, 39]. We suspected that if PHMG and/or CMIT/MIT attach to the LS, the lipid domain should have a different morphology than pure LS, which could be strong evidence for the binding phenomena.

The presence of PHMG in the sub-phase resulted in a distinct formation of the L_o phase, observed at the concentration of 0.01 wt% or higher in contrast to the pure LS monolayer where only the L_d phase exists at 0 mN/m (~149 Å^2/molecule). Based on these observations, PHMG appeared to facilitate the nucleation of the condensed phase in the LS monolayer. Subsequently, when the pure LS monolayer film was gradually compressed at a constant rate, the apparent contrast at the boundary of the domains sharply diminished at a surface pressure >25 mN/m (~57 Å^2/molecule), indicating the mixing of the two different phases. However, this transition was observed at 0.01 wt% of PHMG at a much lower surface pressure >15 mN/m (~72 Å^2/molecule). The transition pressure was even lower at higher PHMG concentration, which was decreased to ~5 mN/m (~107 Å^2/molecule) at 0.1 wt% PHMG (Fig. 3b). This decrease in the mixing pressure with an increase in the PHMG concentration signifies the adsorption of PHMG onto the LS monolayer, reducing stability (Fig. 2b). Therefore, as reported previously, the surface properties and rheological changes induced by PHMG exposure could potentially trigger alveolar instability and atelectasis[40].

In the case of CMIT/MIT, regardless of the presence of CMIT/MIT in the sub-phase, the L_o phase did not distinctly form at 0 mN/m pressure, and only the L_d phase was observed (Fig. 3c). Considering that there were no significant differences in the domain morphologies with and without CMIT/MIT, we can conclude that CMIT/MIT may not bind to the model LS monolayer and does not promote the nucleation of the condensed phase. The pure LS monolayer and the 0.1 wt% CMIT/MIT condition exhibited phase transitions at pressures exceeding 10 mN/m (~72 Å^2/molecule). The different phase transition pressure from pure LS in Fig. 3b is anticipated to be influenced by temperature due to the experimental setup (Figure 3b was measured at T~20℃, and Figure 3c was measured at T~18℃). Nevertheless, CMIT/MIT does not adsorb significantly onto the LS monolayer, making it difficult to alter the integrity of the LS monolayer.

4 Conclusion

In summary, we measured the changes in π-*A* isotherms and lipid raft morphology of LS monolayers upon PHMG or CMIT/MIT injection, using a Fluorescence Microscope-equipped Langmuir trough to understand the adsorption characteristics of CMIT/MIT on a pulmonary surfactant.

1. No significant difference between π-*A* isotherm of LS monolayers is measured for relatively lower concentrations of CMIT/MIT, whereas high (~0.1 wt%) CMIT/MIT concentration induced slight changes in π-*A* isotherm. In the presence of PHMG, a significant increase in the surface pressure (at a specific molecular area), and a change in the kink pressure caused

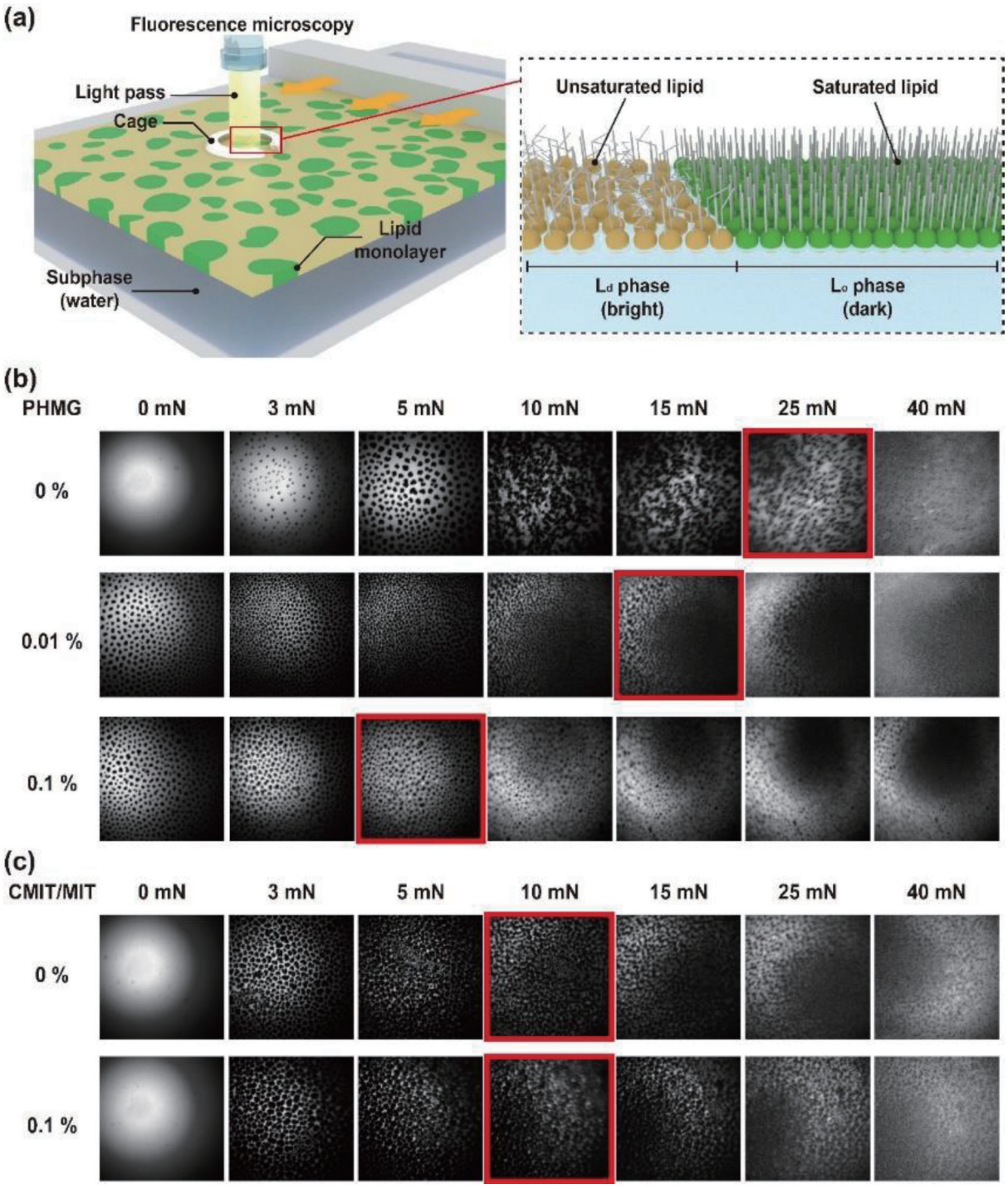

Fig. 3 (a) Schematic images of experimental steps for fluorescence microscopy analysis connected to Langmuir trough (L_d = dark yellow; L_o phase = green). Fluorescence microscopy images (200 × 200 μm) of LS monolayer lipid domains during compression, showing the L_d phase (bright) and L_o phase (dark) in the presence of (b) PHMG or (c) CMIT/MIT. The images in which the phase transition occurred were marked with red squares.

by the toxic components of the interface were measured.

2. Compared with the pure LS monolayer, no significant changes in the lipid domain morphology and mixing pressure were observed in the presence of CMIT/MIT. PHMG seemed to bind to the LS monolayer strongly, accelerating Lo phase growth, and decreasing the mixing pressure.

This *in vitro* study revealed for the first time that the two substances currently attracting attention as toxic substances have significantly different effects on the adsorption and interfacial properties of the model LS monolayer. Moreover, this study is significant for investigating the adsorption capability of toxins to pulmonary surfactants, distinct from the conventional focus on cellular toxicity. Therefore, it can also serve as foundational information for subsequent research related to the characterization and evaluation of the toxicity of CMIT/MIT and PHMG, including *in vitro* and *in vivo* toxicity validation. In addition, the animal-free *in vitro* experiments conducted in this study can be used as a new methodology for evaluating the binding of toxins to targeted lipid layers.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

J.P. and J.K. performed the research and analyzed the data. J.P., J.K., and D.W.L. wrote the manuscript. K.K. and S.Q.C. reviewed the manuscript. D.W.L. supervised this project. All authors have given approval to the final version of the manuscript.

References

1) Lee, J.D.; Kim, H.Y.; Kang, K.; Jeong, H.G.; Song, M.-K. *et al.* Integration of transcriptomics, proteomics and metabolomics identifies biomarkers for pulmonary injury by polyhexamethylene guanidine phosphate (PHMG-p), a humidifier disinfectant, in rats. *Arch. Toxicol.* **94**, 887-909 (2020). doi: 10.1007/s00204-020-02657-x
2) Rosin, M.; Welk, A.; Bernhardt, O.; Ruhnau, M.; Pitten, F.A. *et al.* Effect of a polyhexamethylene biguanide mouthrinse on bacterial counts and plaque. *J. Clin. Periodontol.* **28**, 1121-1126 (2001). doi: 10.1111/j.1600-051X.2001.281206.x
3) Rasmussen, K.; Chemin, P.; Haastrup, P. Regulatory requirements for biocides on the market in the European Union according to Directive 98/8/EC1The authors and the European Commission disclaim all liability with regard to any decisions, which may be made by readers on the basis of information included in this article. Data given and statements made in this report have no official or legal value 1. *J. Hazard. Mater.* **67**, 237-251 (1999). doi: 10.1016/S0304-3894(99)00042-4
4) Chapman, J.S. Biocide resistance mechanisms. *Int. Biodeterior. Biodegrad.* **51**, 133-138 (2003). doi: 10.1016/S0964-8305(02)00097-5
5) Ha, Y.; Kwon, J.-H. Effects of lipid membrane composition on the distribution of biocidal guanidine oligomer with solid supported lipid membranes. *RSC Adv.* **10**, 22343-22351 (2020). doi: 10.1039/D0RA03108A
6) Zhang, Y.; Jiang, J.; Chen, Y. Synthesis and antimicrobial activity of polymeric guanidine and biguanidine salts. *Polymer* **40**, 6189-6198 (1999). doi: 10.1016/S0032-3861(98)00828-3
7) Broxton, P.; Woodcock, P.M.; Gilbert, P. A study of the antibacterial activity of some polyhexamethylene biguanides towards Escherichia coli ATCC 8739. *J. Appl. Bacteriol.* **54**, 345-353 (1983). doi: 10.1111/j.1365-2672.1983.tb02627.x
8) Ikeda, T.; Tazuke, S.; Watanabe, M. Interaction of biologically active molecules with phospholipid membranes: I. Fluorescence depolarization studies on the effect of polymeric biocide bearing biguanide groups in the main chain. *Biochim. Biophys. Acta - Biomembranes* **735**, 380-386 (1983). doi: 10.1016/0005-2736(83)90152-9
9) Zabelinskii, S.A.; Brovtsyna, N.B.; Chebotareva, M.A.; Gorbunova, O.B.; Krivchenko, A.I. Comparative investigation of lipid and fatty acid composition of fish gills and mammalian lungs. A model of the membrane lipid component areas. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **111**, 127-140 (1995). doi: 10.1016/0305-0491(94)00210-L
10) Lee, J.; Choi, S.-J.; Jeong, J.-S.; Kim, S.Y.; Lee, S.-H. *et al.* A humidifier disinfectant biocide, polyhexamethylene guanidine phosphate, inhalation exposure during pregnancy induced toxicities in rats. *J. Hazard. Mater.* **404**, 124007 (2021). doi: 10.1016/j.jhazmat.2020.124007
11) Sang-Bum, H.; Hwa Jung, K.; Jin Won, H.; Kyung-Hyun, D.; Se Jin, J. *et al.* A cluster of lung injury associated with home humidifier use: Clinical, radiological and pathological description of a new syndrome. *Thorax* **69**, 694 (2014). doi: 10.1136/thoraxjnl-2013-204135
12) Ostapenko, Y.N.; Brusin, K.M.; Zobnin, Y.V.; Shchupak,

A.Y.; Vishnevetskiy, M.K. *et al.* Acute cholestatic liver injury caused by polyhexamethyleneguanidine hydrochloride admixed to ethyl alcohol. *Clin. Toxicol.* **49**, 471-477(2011). doi: 10.3109/15563650.2011.592837
13) Kim, H.; Ji, K. Exposure to humidifier disinfectants induces developmental effects and disrupts thyroid endocrine systems in zebrafish larvae. *Ecotoxicol. Environ. Saf.* **184**, 109663(2019). doi: 10.1016/j.ecoenv.2019.109663
14) Oh, H.; Kim, C.Y.; Ryu, B.; Kim, U.; Kim, J. *et al.* Respiratory toxicity of polyhexamethylene guanidine phosphate exposure in zebrafish. *Zebrafish* **15**, 460-472(2018). doi: 10.1089/zeb.2018.1571
15) Li, X.; Zhang, J.; Du, C.; Jiang, Y.; Zhang, W. *et al.* Polyhexamethylene guanidine aerosol triggers pulmonary fibrosis concomitant with elevated surface tension via inhibiting pulmonary surfactant. *J. Hazard. Mater.* **420**, 126642(2021). doi: 10.1016/j.jhazmat.2021.126642
16) Lim, C.; Park, S.; Park, J.; Ko, J.; Lee, D.W. *et al.* Probing nanomechanical interaction at the interface between biological membrane and potentially toxic chemical. *J. Hazard. Mater.* **353**, 271-279(2018). doi: 10.1016/j.jhazmat.2018.04.017
17) Yonhap News Agency "Ex-SK Chem chief, 33 others indicted over humidifier sterilizer scandal" July 23 2019(https://en.yna.co.kr/view/AEN20190723003600315)
18) The Korea Times "Harmful substances in SK humidifier disinfectant" June 18 2020(https://www.koreatimes.co.kr/www/tech/2023/09/419_291452.html)
19) Business Korea "Aekyung and SK Chemicals Fined for Toxic Humidifier Disinfectants" Oct 27 2022(https://www.businesskorea.co.kr/news/articleView.html?idxno=102961)
20) Song, M.-K.; Eun Park, J.; Ryu, S.-H.; Baek, Y.-W.; Kim, Y.-H. *et al.* Biodistribution and respiratory toxicity of chloromethylisothiazolinone/methylisothiazolinone following intranasal and intratracheal administration. *Environ. Int.* **170**, 107643(2022). doi: 10.1016/j.envint.2022.107643
21) Lee, J.; Lee, H.; Jang, S.; Hong, S.-H.; Kim, W.J. *et al.* CMIT/MIT induce apoptosis and inflammation in alveolar epithelial cells through p38/JNK/ERK1/2 signaling pathway. *Mol. Cell. Toxicol.* **15**, 41-48(2019). doi: 10.1007/s13273-019-0005-0
22) Kim, D.; Shin, Y.; Kim, E.-H.; Lee, Y.; Kim, S. *et al.* Functional and dynamic mitochondrial damage by chloromethylisothiazolinone/methylisothiazolinone (CMIT/MIT) mixture in brain endothelial cell lines and rat cerebrovascular endothelium. *Toxicol. Lett.* **366**, 45-57(2022). doi: 10.1016/j.toxlet.2022.06.010
23) Go, H.-N.; Lee, S.-H.; Cho, H.-J.; Ahn, J.-R.; Kang, M.-J. *et al.* Effects of chloromethylisothiazolinone/methylisothiazolinone (CMIT/MIT) on Th2/Th17-related immune modulation in an atopic dermatitis mouse model. *Sci. Rep.* **10**, 4099(2020). doi: 10.1038/s41598-020-60966-8
24) Kim, M.K.; Kim, K.-B.; Lee, J.Y.; Kwack, S.J.; Kwon, Y.C. *et al.* Risk assessment of 5-chloro-2-methylisothiazol-3(2H)-one/2-methylisothiazol-3(2H)-one (CMIT/MIT) used as a preservative in cosmetics. *Toxicol. Res.* **35**, 103-117(2019). doi: 10.5487/TR.2019.35.2.103
25) Ma, G.; Allen, H.C. New insights into lung surfactant monolayers using vibrational sum frequency generation spectroscopy. *Photochem. Photobiol.* **82**, 1517-1529(2006). doi: 10.1111/j.1751-1097.2006.tb09807.x
26) Ding, J.; Doudevski, I.; Warriner, H.E.; Alig, T.; Zasadzinski, J.A. *et al.* Nanostructure changes in lung surfactant monolayers induced by interactions between palmitoyloleoylphosphatidylglycerol and surfactant protein B. *Langmuir* **19**, 1539-1550(2003). doi: 10.1021/la0261794
27) Ma, G.; Allen, H.C. DPPC Langmuir monolayer at the air-water interface: Probing the tail and head groups by vibrational sum frequency generation spectroscopy. *Langmuir* **22**, 5341-5349(2006). doi: 10.1021/la0535227
28) Kim, K.; Choi, S.Q.; Zasadzinski, J.A.; Squires, T.M. Interfacial microrheology of DPPC monolayers at the air–water interface. *Soft Matter* **7**, 7782-7789(2011). doi: 10.1039/C1SM05383C
29) Bringezu, F.; Ding, J.; Brezesinski, G.; Zasadzinski, J.A. Changes in model lung surfactant monolayers induced by palmitic acid. *Langmuir* **17**, 4641-4648(2001). doi: 10.1021/la0103158
30) Wüstneck, R.; Perez-Gil, J.; Wüstneck, N.; Cruz, A.; Fainerman, V.B. *et al.* Interfacial properties of pulmonary surfactant layers. *Adv. Colloid Interface Sci.* **117**, 33-58(2005). doi: 10.1016/j.cis.2005.05.001
31) Zasadzinski, J.A.; Ding, J.; Warriner, H.E.; Bringezu, F.; Waring, A.J. The physics and physiology of lung surfactants. *Curr. Opin. Colloid Interface Sci.* **6**, 506-513(2001). doi: 10.1016/S1359-0294(01)00124-8
32) Veldhuizen, R.; Nag, K.; Orgeig, S.; Possmayer, F. The role of lipids in pulmonary surfactant. *Biochim. Biophys. Acta Mol. Basis Dis.* **1408**, 90-108(1998). doi: 10.1016/S0925-4439(98)00061-1
33) Lee, D.W.; Min, Y.; Dhar, P.; Ramachandran, A.; Isr. aelachvili, J.N. *et al.* Relating domain size distribution to line tension and molecular dipole density in model cytoplasmic myelin lipid monolayers. *Proc. Natl. Acad. Sci. USA* **108**, 9425-9430(2011). doi: 10.1073/pnas.1106368108
34) Dhar, P.; Eck, E.; Israelachvili, Jacob N.; Lee, Dong W.; Min, Y. *et al.* Lipid-protein interactions alter line tensions and domain size distributions in lung surfactant monolayers. *Biophys. J.* **102**, 56-65(2012). doi: 10.

1016/j.bpj.2011.11.4007
35) Baumgart, T.; Hunt, G.; Farkas, E.R.; Webb, W.W.; Feigenson, G.W. Fluorescence probe partitioning between Lo/Ld phases in lipid membranes. *Biochim. Biophys. Acta Biomembr.* **1768**, 2182-2194(2007). doi: 10.1016/j.bbamem.2007.05.012
36) Blanco, O.; Cruz, A.; Ospina, O.L.; López-Rodriguez, E.; Vázquez, L. *et al.* Interfacial behavior and structural properties of a clinical lung surfactant from porcine source. *Biochim. Biophys. Acta Biomembr.* **1818**, 2756-2766(2012). doi: 10.1016/j.bbamem.2012.06.023
37) Nag, K.; Perez-Gil, J.; Ruano, M.L.F.; Worthman, L.A.D.; Stewart, J. *et al.* Phase transitions in films of lung surfactant at the air-water interface. *Biophys. J.* **74**, 2983-2995(1998). 10.1016/S0006-3495(98)78005-1
38) Piknova, B.; Schram, V.; Hall, S. Pulmonary surfactant: Phase behavior and function. *Curr. Opin. Struct. Biol.* **12**, 487-494(2002). doi: 10.1016/S0959-440X(02)00352-4
39) Lee, D.W.; Banquy, X.; Kristiansen, K.; Kaufman, Y.; Boggs, J.M. *et al.* Lipid domains control myelin basic protein adsorption and membrane interactions between model myelin lipid bilayers. *Proc. Natl. Acad. Sci. USA* **111**, E768-E775(2014). doi: 10.1073/pnas.1401165111
40) Malakhova, Y.N.; Stupnikov, A.A.; Chekusova, V.P.; Kuznetsov, N.M.; Belousov, S.I. Rheological behavior of polydimethylsiloxane Langmuir layers at the air-water interface. *BioNanoScience* **10**, 403-408(2020). doi: 10.1007/s12668-020-00728-y