Salt-induced Adsorption and Rupture of Liposomes on Microplastics

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Abstract

Microplastics have attracted considerable attention because of concerns regarding their environmental risks to living systems. The interaction between lipid bilayer and microplastics is important for examining the potential harm to biological membranes in the presence of microplastics. In addition, membrane coatings may change the surface and colloidal properties of microplastics. Herein, phosphatidylcholine (PC) lipids, whose headgroup is most common in cell membranes, were used as model lipids. The adsorption and rupture of PC liposomes on microplastics were systematically studied. We found that divalent metal ions, such as Mg^{2+} and Ca^{2+}, facilitate liposome adsorption onto microplastics and induce 40-55% liposome leakage at 2.5 mM. In contrast, to achieve a similar effect, 300 mM Na^{+} was required. Adsorption and rupture followed the same metal concentration requirement, suggesting that liposome adsorption was the rate-limiting step. After adsorption with liposomes, microplastics became more hydrophilic and could be better dispersed in water. Similar behavior was observed for all five types of tested microplastics, including PP, PE, PVC, PET, and PS. Leakage also occurred in ocean water. This study provides fundamental insights into the interactions between liposomes and microplastics and has implications for the colloidal and transport properties of microplastics.

Introduction

Plastics are widely used because of their ease of manufacturing, low cost, and stable chemical properties. The extensive production and use of plastics have resulted in their considerable release into the environment. The released plastic products can be decomposed into microplastic. Microplastics are widely found in oceans, rivers, and soil.[1] These microplastics can further enter the food chain and pose a threat to humans and other organisms.[2] Therefore, it is significant to understand their environmental risks.[3]

Compared with bulk plastics, microplastics have larger specific surface areas and microscale surface features that may interact with metal ions,[4-6] proteins,[7, 8] DNA,[9-11] and microbes differently.[12, 13] They also have interesting colloidal properties.[14, 15] So far, extensive studies have been performed on the adsorption of DNA and proteins to microplastics. For DNA adsorption, divalent metal ions played important roles,[10] especially transition metal ions.[16]

Lipid bilayers are the basic component of cell membranes, and liposomes have been used as model systems to understand the behavior of biological membranes.[17, 18] Many previous studies were carried out to interface liposomes with various nanomaterials such as silica nanoparticles,[19, 20] gold nanoparticles,[21-23] and graphene oxide.[24-26] For polymeric materials, hydrogel beads were mixed with various liposomes, and electrostatic interactions appeared to be important to direct the interactions.[27] Fusion of liposomes with hydrogels was observed for oppositely charged liposomes and hydrogels. However, for zwitterionic liposomes, simple adsorption was observed.[27] Numerous studies have indicated the effect of microplastics to human health.[28, 29] In a recent study, adverse effects of microplastics on cellular morphology and proliferation were observed in human embryonic kidney cells and hepatocellular liver cells.[30] While most previous studies focused on the effect of microplastics on enzyme activities, gene expression, oxidative stress and cell proliferation, few studies examined the interaction between lipid membranes and microplastics.[31-33]

Among the different types of lipids, phosphatidylcholine (PC) lipids are the most common in the cell membranes and pulmonary surfactants.[34] In this work, we systematically studied the adsorption and fusion of PC liposomes with microplastics. Both fluid DOPC and gel-phase DPPC liposomes were tested. We found that the interactions were insensitive to lipid phase transition

temperature (T_c) or the type of microplastics. Instead, divalent metal ions can promote liposome adsorption and leakage, whereas monovalent sodium achieved the same effect at a much higher concentration. The colloidal properties and dispersion of lipid-coated microplastics were also studied.

Materials and Methods

Chemicals. All phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL). Zinc chloride, disodium calcein, Triton X-100, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich. Magnesium chloride, calcium chloride, and sodium chloride were purchased from Mandel Scientific (Guelph, ON, Canada). Milli-Q water was used to prepare all buffers, solutions, and suspensions.

Preparation of Liposomes. Liposomes were prepared using the standard extrusion method as described previously.[21] 2.5 mg of DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) and DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) were dissolved in chloroform, respectively. For rhodamine (Rh)-labeled liposomes, 1 wt % Rh-PE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl)) (ammonium salt) was incorporated. After evaporating chloroform by blowing with N_2, the samples were dried overnight in a vacuum oven at room temperature to fully remove residual chloroform. The dried lipid films were stored at −20 °C in a N_2 atmosphere prior to use. To prepare liposomes, the lipid films were hydrated with 0.5 mL buffer A (10 mM HEPES, pH 7.6 with 100 mM NaCl) yielding a lipid concentration of 5 mg mL^{-1}. The resulting cloudy suspension was extruded 21 times through two stacked polycarbonate membranes with a pore size of 50 nm giving clear liposome suspension. To encapsulate calcein, the lipid films were hydrated with 100 mM calcein overnight followed by extrusion. For DPPC liposome, hydration and extrusion were performed at 60 °C. The free calcein was removed by passing 35 μL of the sample through a PD-10 column using buffer A for elution. The first 600 μL of the fluorescent fraction was collected.

ζ-Potential and Dynamic Light Scattering. The ζ-potential and hydrodynamic size of the liposomes were measured on a Malvern Zetasizer Nano ZS 90 with a He–Ne laser (633 nm) at 90° optics at 25°C with a lipid concentration of 50 μg mL^{-1} at pH 7.4 (10 mM HEPES, 100 mM NaCl).

Preparation of Microplastics. Multiple commonly used plastic items were collected including a plastic container composed of a centrifuge tube composed of polypropylene (PP), a plastic container composed of polyethylene (PE), a plastic spoon composed of PS, a plastic sheet composed of polyvinylchloride (PVC), and a plastic water bottle composed of polyethylene terephthalate (PET). The composing material of the plastics was verified by Raman spectroscopy, after which they were cut into smaller pieces and washed with ethanol. The plastic pieces were then shredded via mechanical force using a stainless-steel grater to acquire microplastics. The microplastics were collected and dried at room temperature overnight using a vacuum dryer. A Raman spectrometer (DeltaNu, Advantage 785) with a 785 nm laser and a 10 s integration time was utilized to acquire Raman spectra and identify the composing materials of the plastic items. Microscopic images of the microplastics were collected using a Nikon Eclipse Ti–S inverted microscope and a laser scanning confocal fluorescence microscope (LSM510Meta, Carl Zeiss Inc., Thornwood, NY).

Liposome Leakage Studies. To monitor liposome leakage with microplastics, 5 μL of calcein-loaded liposomes, 600 μL of 10 mM HEPES buffer (pH 7.4), and 12 mg of microplastics were added to a quartz cuvette. The concentration of metal chloride was adjusted by adding 6 μL of metal chloride stock solution. Finally, 5 μL of 5% Triton X-100 was added to fully rupture the liposomes. Samples were occasionally stirred during the measurement because plastics were floating or sinking in the cuvette. The percentage of leakage was calculated using the formula $(F_{\text{Released}} - F_{\text{minimum}}) / (F_{\text{Total}} - F_{\text{minimum}}) \times 100\%$. Calcein was excited at 485 nm, and the emission was monitored at 525 nm.

Adsorption of Microplastics with Rh-Labeled Liposomes. To study liposome adsorption, 600 μL microplastics (2 mg mL^{-1}) with 10 mM HEPES (pH 7.4) were mixed in a quartz cuvette with Rh-labeled liposomes (5 mg mL^{-1}) in the presence of metal ions. Samples were occasionally stirred. Finally, the microplastics were separated from the solution. The plastics were dispersed in 600 μL of 10 mM HEPES (pH 7.4), and then 6 μL 5% Triton X-100 was added to fully release liposomes from adsorbed lipids. The fluorescence intensity enhancement of the supernatant was measured to quantify the desorbed lipids. The fluorescence spectra were collected on a Varian Eclipse fluorometer with excitation at 560 nm and emission at 592 nm.

Results and Discussion

Characterization of liposomes and microplastics

In this study, DOPC lipid was used because of its low phase transition temperature (T_c) of -17°C, which allows it to exist in the fluid phase at room temperature. For mechanistic studies, gel-phase DPPC lipid was also used for comparison. The structure of a DOPC lipid is shown in Figure 1A. The headgroup of DOPC contains a negatively charged phosphate and cationic choline, resulting in an overall neutral charge under the physiological condition. The DOPC liposomes used in the experiments were prepared by extrusion and had a hydrodynamic size of approximately 50 nm (Figure 1B). The ζ-potential of the DOPC liposomes was slightly negative (-5.4±2.2 mV) at pH 7.4 (Figure 1C, the first bar), which is consistent with the literature.[22]

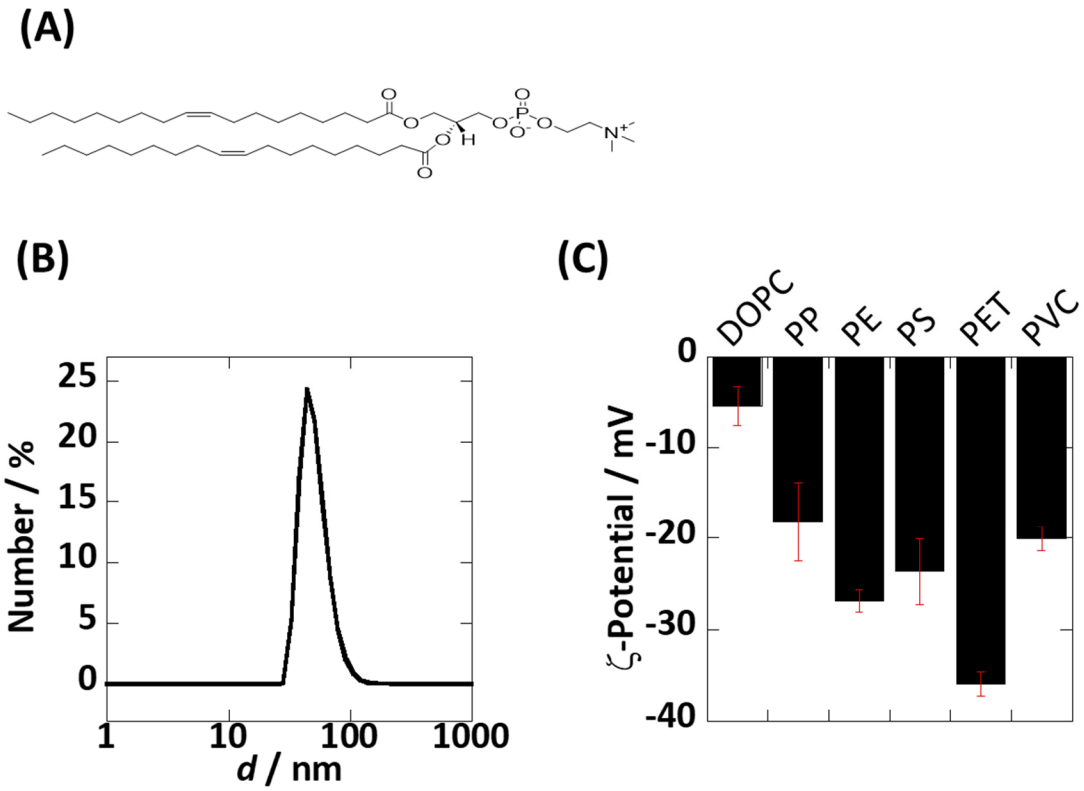


Figure 1. (A) The structure of a DOPC lipid. (B) A size distribution of the DOPC liposomes at pH 7.4 (10 mM HEPES, 100 mM NaCl). (C) ζ-Potential of the DOPC liposomes and the five microplastic samples.

Five household plastic objects, including a centrifuge tube, a container, a spoon, a water bottle, and a plastic sheet, were collected and analyzed using Raman spectroscopy to identify their constituent materials as PE, PP, PS, PVC, and PET, respectively (Figure S1A).[10] Microplastic samples were prepared by mechanically shredding these plastic items. The size and morphology of the prepared microplastics were then observed using an optical microscope (Figure S1B). While the samples exhibited a broad distribution of shapes and sizes (ranging from 20 to 800 μm), they can all be called microplastics since they are all below 1 mm. At pH 7.4, all these five microplastic samples were negatively charged, and they were more negatively charged compared to the DOPC liposomes (Figure 1C). As such, we can expect electrostatic repulsion or at least a lack of electrostatic attraction between DOPC liposomes and these microplastics.

Salt-induced liposome leakage

An important property of liposomes is their membrane integrity, as charged molecules cannot cross bilayer membranes. To assess whether microplastics can leak liposomes, a dye leakage assay was performed. We encapsulated 100 mM calcein dye in DOPC liposomes, resulting in self-quenched fluorescence.[35] Disruption of the liposome membrane causes the release of calcein into the solution, leading to an enhancement of fluorescence. The addition of Triton X-100 fully ruptures the liposomes and releases all calcein, allowing for the calculation of the percentage of leakage induced by microplastics.

We first studied PP microplastics for its effect on the integrity of calcein-loaded DOPC liposomes. The black curve in Figure 2A shows the kinetics of leakage without additional salt. The background fluorescence was monitored at 525 nm for 5 min, during which no change was observed, indicating that the liposomes were stable. At 5 min, a final concentration of 2 mg mL^{-1} PP microplastics was added, and no increase in fluorescence was observed. This suggests that there is no strong interaction between the liposomes and PP microplastics, which agrees with an electrostatic repulsion supported by slightly negative surface charges as determined by ζ-potential measurements. To screen charge repulsion, a final of 10 mM Mg^{2+}, an environmentally abundant metal ion, was added to the mixture of DOPC and PP microplastics at 10 min. An immediate enhancement in fluorescence was observed, indicating compromised membrane integrity. The fluorescence continued to increase until around 60 min, and the percentage of leakage reached ~90% from the final fluorescence intensity obtained by adding Triton X-100 at 70 min. Therefore, with

10 mM Mg^{2+}, the PP plastics almost fully leaked the liposomes likely due to the rupture of liposomes on plastic surfaces. We hypothesized that the remaining 10% non-leaked fluorescence was from non-adsorbed liposomes. To test this hypothesis, the same experiment was performed by reducing the concentration of PP microplastics by half, which indeed reduced the amount of leakage to ~50% (Figure 2B). This experiment supported that leakage was dependent on the concentration of microplastics, and thus the surface capacity of microplastics. In addition, this experiment also indicated that mechanical friction or shear forces caused by the microplastics in the stirred mixture was not the reason for the leakage since the Mg^{2+}-free samples were treated in the same way.

The effect of Mg^{2+} on leakage without PP microplastics was also tested (red curve in Figure 2A). At 5 min, a final concentration of 10 mM Mg^{2+} was added, and no leakage was observed. The fluorescence even decreased slightly, which is attributable to metal-induced quenching of free calcein dye. At 10 min, PP microplastics were added, which immediately caused a fluorescence enhancement. The kinetics of the leakage reaction followed a similar trend regardless of the order of adding PP microplastics and Mg^{2+}. Thus, both Mg^{2+} and PP microplastics were required to leak the DOPC liposomes. Our previous isothermal titration calorimetry measurements showed that the binding of divalent metal ions to PC lipids is weak.[22] Additionally, divalent metal ions alone did not affect the hydrodynamic diameter or leakage of liposomes.[22] However, individual weak interactions may be amplified by the abundance of binding sites on metal-adsorbed microplastics, which can induce adsorption and subsequent leakage of liposomes.

The effect of Mg^{2+} concentration on leakage was then studied (Figure 2C). While 2.5 mM Mg^{2+} could cause around ~40% of leakage, no increase in fluorescence was observed in the presence of 1 mM Mg^{2+}. The critical concentration of Mg^{2+} to induce leakage was estimated to be less than 2.5 mM in this experiment.

The effects of different metal ions on liposome leakage on PP microplastics were also compared. Figures 2D and 2E show that both Ca^{2+} and Na^{+} increased fluorescence of liposomes when present Ca^{2+} in low mM, while a much higher concentration (~200 mM) was required for Na^{+}. Figure 2F summarizes the leakage rates calculated from the slope of the initial 10 min in the leakage kinetics. Ca^{2+} is slightly more efficient than Mg^{2+} in facilitating liposome rupture on the PP microplastics, which can be explained by better bonding affinity of Ca^{2+} for phosphate of lipids

than that of Mg^{2+}.[36, 37] Considering the Debye lengths (k^{-1}) of the divalent ions (e.g., k^{-1}≈1.62 nm for 2.5 mM Mg^{2+} and Ca^{2+} in 10 mM HEPES) and the monovalent ions (k^{-1}≈0.47 nm for 200 mM Na^{+}), the divalent metals have additional roles beyond simple charge screening. It is possible that they can act as a salt bridge to directly interact with negatively charged groups on both the microplastics and liposomes.

Phase transition temperature independent leakage

Leakage was then investigated for gel-phase DPPC liposomes, which have a T_c of 41°C. The structure of a DPPC lipid is shown in Figure S2A. DPPC has the same headgroup as DOPC, and thus ζ-potential of the DPPC was also slightly negative (-5.5±2.0 mV). The hydrodynamic size of DPPC was approximately 50 nm (Figure S2B). The leakage test of DPPC liposomes was performed by varying Mg^{2+} concentration as shown in Figure S2C. The Mg^{2+}-dependent leakage was observed in DPPC with a similar Mg^{2+} concentration requirement to that in DOPC. As compared in Figure S2D, the leakage rates of DPPC were slightly higher than those of DOPC. These results suggest that both fluid and gel-phase liposomes can be similarly leaked by PP microplastics in the presence of Mg^{2+}. This observation was very different from what was previously reported for other materials. For example, to rupture liposomes on glass or silica surfaces, fluid phase PC liposomes are more favored, and this process is also strongly affected by metal ions.[38, 39] In addition, when citrate-capped gold nanoparticles are mixed with PC liposomes, leakage was observed with DOPC but not DPPC.[21]

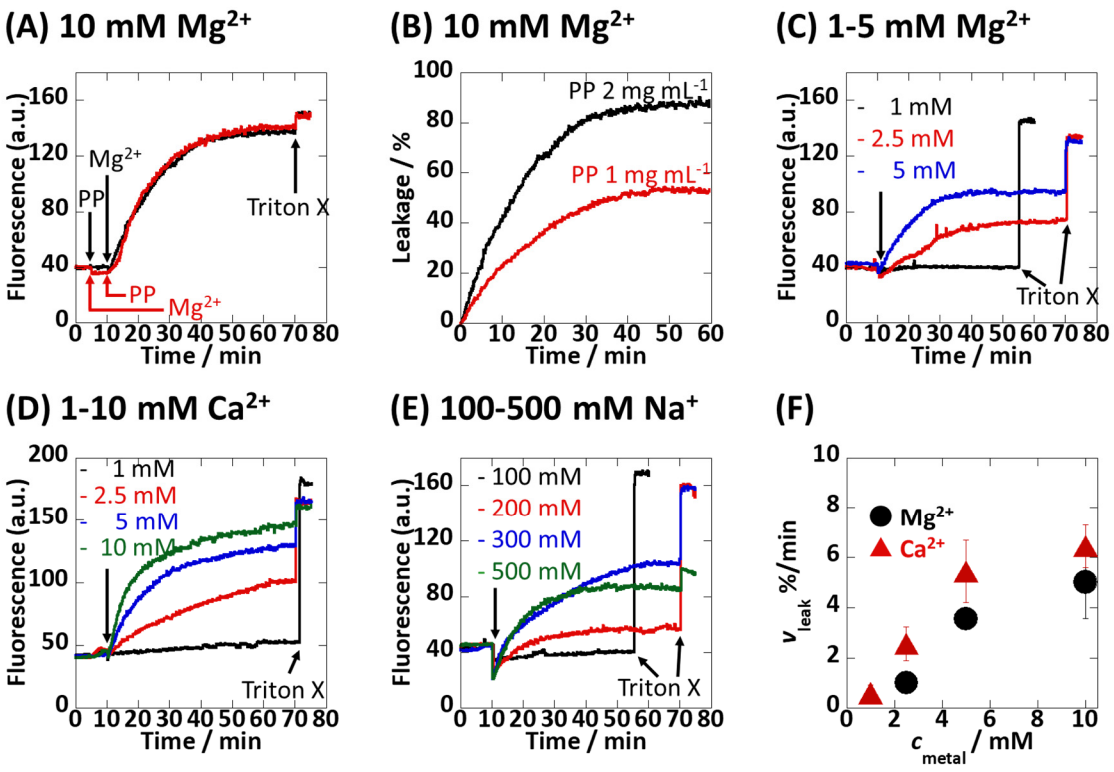


Figure 2. (A) Leakage kinetics of calcein-loaded DOPC upon adding PP microplastics and 10 mM Mg^{2+} at different times. (B) Effect of PP microplastics concentration on leakage in 10 mM Mg^{2+}. (C) Effect of Mg^{2+} concentration on leakage. Effects of different metal ions, including (D) Ca^{2+} and (E) Na^{+}, on leakage. (F) Leakage rates (v_{leak} %/min) calculated from the slope of the initial 10 minutes in panels A, C and D. The final concentration of PP microplastics was 2 mg mL^{-1} in each experiment. Experiments were repeated 3 times.

Adsorption of liposomes by microplastics

Since PP microplastics can induce leakage of DOPC liposomes, liposomes must first adsorb onto them. Thus, the adsorption of liposomes onto PP microplastics was studied. To quantitatively analyze liposome adsorption, we incorporated 1 wt% rhodamine-labeled PE lipids (Rh-PE) in our DOPC liposomes and incubated them with PP microplastics by varying the concentration of Mg^{2+}. After incubation, microplastics were separated from the solution and were dispersed in 500 µL of 10 mM HEPES (pH 7.4). Finally, Triton X-100 was added to wash the adsorbed lipids off from the microplastics, and the fluorescence intensity enhancement of the supernatant was measured to

calculate the percentage of adsorption. The adsorption of liposomes onto PP microplastics was quantitatively analyzed by varying the concentration of Mg^{2+} (Figure 3A). In the absence of Mg^{2+}, PP microplastics hardly adsorbed liposomes, which can explain the lack of leakage. The adsorbed liposomes increased as the concentration of Mg^{2+} increased. Therefore, the requirement of Mg^{2+} for leakage was consistent with the need of it for adsorption.

To further understand the relationship between the adsorption and leakage of DOPC liposomes, the kinetics of adsorption onto PP microplastics was analyzed in the presence of 5 mM Mg^{2+} (Figure 3B). The adsorption kinetics almost fully overlapped with the leakage kinetics. The similarity of the adsorption kinetics and leakage indicates that adsorption was the rate-limiting step, and leakage was immediate upon adsorption.

To gain more quantitative insights into the adsorption of liposomes onto microplastics, Langmuir isotherms were measured in the presence of 5 mM Mg^{2+} as shown in Figure 3C. By fitting the data to the Langmuir isotherm $\theta = aKC/(1 + KC)$, where K is the Langmuir constant, a is the adsorption capacity, C is the liposome concentration, and θ is the fraction of adsorbed liposome, we determined that a is 2.6 μg and K = 0.16 μg^{-1} mL. At an infinitely high liposome concentration, each gram of the microplastic can adsorb 1.3×10^{-3} gram of the liposome. Due to the small specific surface area of microplastics and their irregular shapes, we could not measure or calculate their surface area. Based on the Langmuir adsorption isotherm, the liposomes or lipids were adsorbed as a monolayer or a bilayer (but not multilayers). The effect of Mg^{2+} concentration on the adsorption capacity of liposome onto PP microplastics was compared by adding a saturated amount of liposome (100 μg mL^{-1}). Interestingly, the plateau levels are proportionally changed depending on the Mg^{2+} concentration ranges of 0-5 mM (Figure S3).

Liposome adsorption onto PP microplastics was visually examined using fluorescent microscopy. Figure 3D shows the optical and fluorescent microscopic images of PP microplastics in the presence of 25 μg mL^{-1} Rh-PE. Under the fluorescence mode, no distinct features were observed without Mg^{2+}, indicating that the liposomes were only present in the solution and did not adsorb onto the microplastics in this condition. By contrast, clear fluorescent microscopic features co-localized with the microplastics were observed in the presence of 10 mM Mg^{2+}, confirming the adsorption of the liposomes.

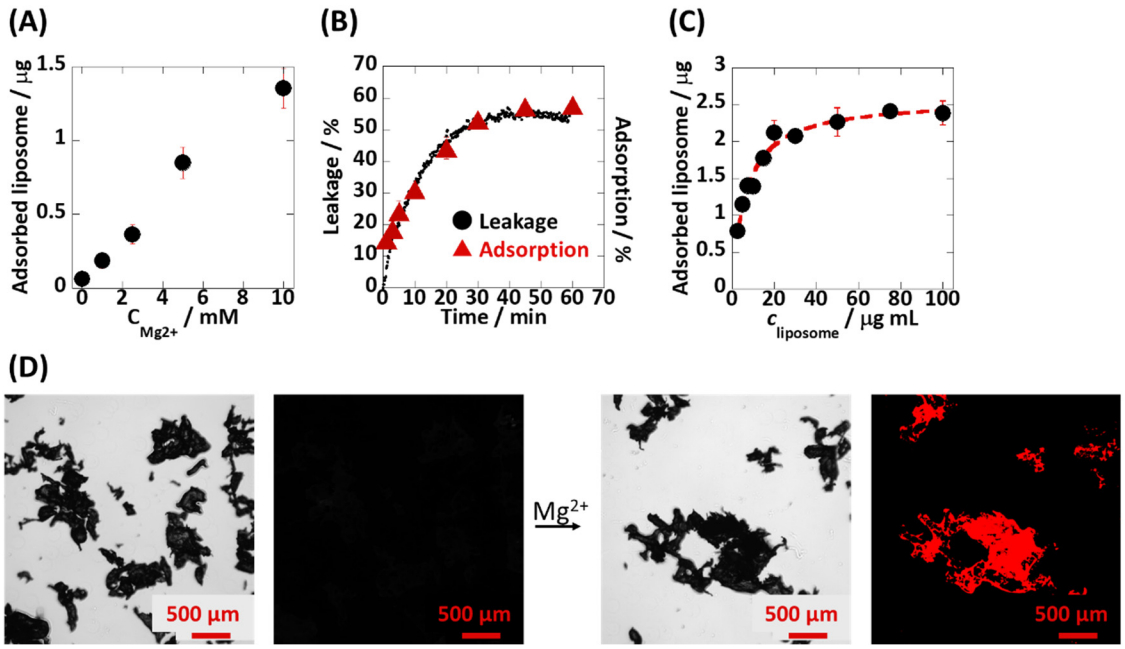


Figure 3. (A) Effect of Mg^{2+} concentration on the adsorption of Rh-labeled DOPC liposomes onto the PP microplastics. (B) The kinetics of Rh-PE adsorption onto PP microplastics. Adsorption was calculated from the ratio of added and adsorbed liposomes. Leakage of DOPC liposomes in Figure 2C is depicted in black dots for comparison. (C) Liposome adsorption isotherm. For (A)-(C), concentrations of Rh-PE, Mg^{2+}, and PP microplastics were 2.5 µg mL^{-1}, 5 mM and 2 mg mL^{-1}, respectively, and experiments were repeated 3 times. (D) Optical and fluorescent microscopic images of PP microplastics in the presence of 25 µg mL^{-1} Rh-PE. Final concentration of Mg^{2+} was 10 mM.

Adsorption and leakage of liposomes by different microplastics

The above experiments were performed all with the PP microplastics. To understand the effects of different microplastics, a Mg^{2+}-dependent leakage study was conducted for PE, PVC, PET and PS as well. Figure 4A and 4B show the leakage kinetics of calcein-loaded DOPC with microplastics in the presence of Mg^{2+}. In all plastics, no leakage was observed in the presence of 1 mM Mg^{2+} (Figure 4A). With 2.5 mM Mg^{2+}, a similar rate of leakage was observed for all these microplastics, regardless of their chemical composition (Figure 4B). The effect of Mg^{2+} on liposome adsorption onto different microplastics was also studied. The affinity of liposomes to all microplastics in the

presence of Mg^{2+} followed the similar trend (Figure 4C). These data agreed with the leakage experiments, where the types of plastics did not significantly affect. Thus, no specific interaction was occurred from different functional groups and same adsorption mechanism were the same in all plastics in this study.

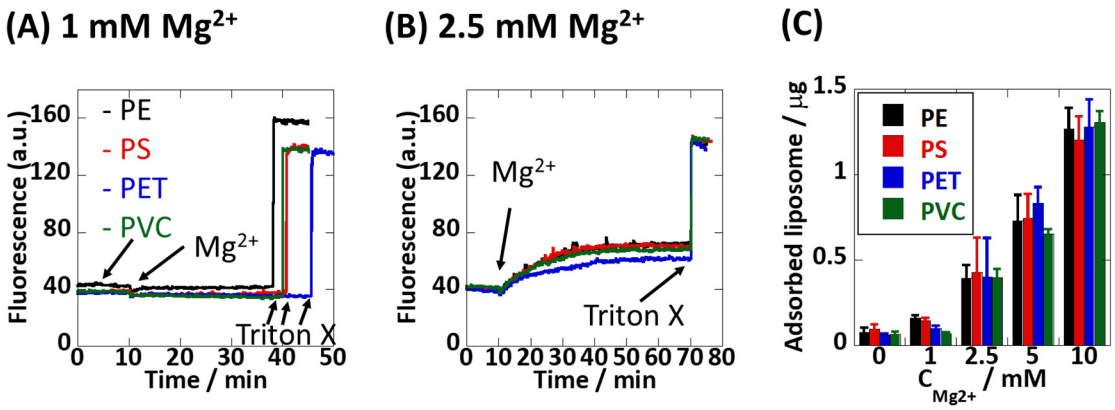


Figure 4. Leakage and adsorption with different microplastics. Leakage kinetics of calcein-loaded DOPC with microplastics in the presence of (A) 1 mM Mg^{2+}, and (B) 2.5 mM Mg^{2+}. (C) Effect of Mg^{2+} concentration on adsorption of Rh-labeled DOPC on microplastics. The final concentration of microplastics was 2 mg mL^{-1} in each experiment. Experiments were repeated 3 times for each.

Colloidal property of microplastics

PP microplastics float on the surface of water because of their lower density compared to that of water. Figure 5 illustrates the floating behavior of 20 mg PP microplastics dispersed in 1 mL water. Immediately after being dispersed in the water, the microplastics were located at the top of the water surface, avoiding contact with the water and barely floating (Figure 5A). The addition of 5 mM Mg^{2+} did not significantly affect floating behavior (Figure 5B). In contrast, the microplastics with liposomes floated more densely and partially sank near the surface of the water, indicating increased hydrophilicity (Figure 5C). Floating behavior was then quantitatively investigated by varying the concentration of liposomes. As shown in Figure S4, hydrophilicity gradually increased with increase in the liposome concentration. Around 40 μg mL^{-1} liposome was required to observe the above floating behavior. Based on the Langmuir isotherm (Figure 3C), 20 mg plastics should adsorb ~22 μg liposomes in the presence of 40 μg mL^{-1} DOPC liposomes, while the saturated

adsorption capacity was 26 μg for 20 mg PP microplastics. Therefore, ~85% of the saturated adsorption capacity was required to disperse microplastics on the water surface.

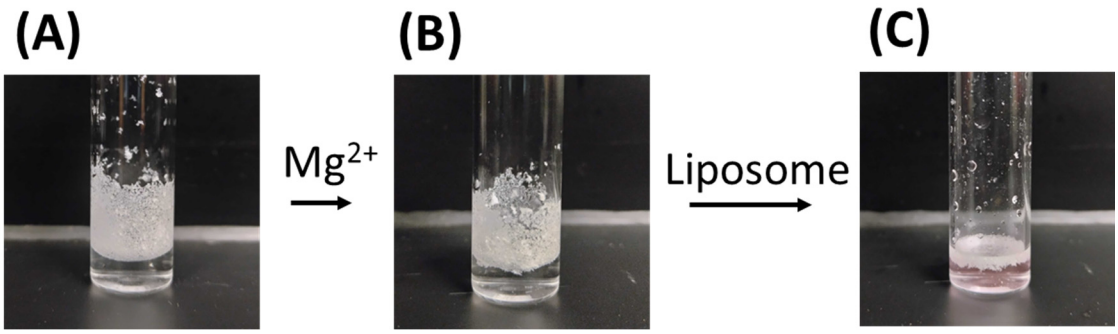


Figure 5. Photographs illustrating the floating behavior of (A) PP microplastics, (B) PP microplastics in the presence of 5 mM Mg^{2+}, and (C) PP microplastics in the presence of 5 mM Mg^{2+} and 100 μg mL^{-1} Rh-PE. 20 mg of PP microplastics was added to 1 mL of 10 mM HEPES (PH 7.4).

Models of interactions

Based on all these data, there are three possible models of interaction as shown in Figure 6. First, liposomes were adsorb as a lipid vesicle as shown in Figure 6A, but at the point of adsorption, local leakage occurred. However, based on the similar kinetics between leakage and adsorption, we do not believe this is the correct mechanism. For this mechanism to occur, we expect that the leakage kinetics to be slower than the adsorption kinetics. The second mechanism shown in Figure 6B is the formation of supported bilayers. After liposome adsorption, the liposomes were deformed to maximize the contact with the microplastic surface, which eventually ruptured the liposomes to form supported lipid bilayers. Fleury and Baulin also observed stretching of lipid membranes by microplastics.[31] The hydrophobic plastic surface thus becomes hydrophilic lipid headgroup surfaces, which can explain the improved affinity to water surface. It can also explain the similar adsorption and leakage kinetics and adsorbed liposomes immediately ruptured. Finally, a third possibility is the formation of supported monolayers, where the hydrophobic tails of lipids directly touch the plastic surface (Figure 6C). The formation of a lipid monolayer can also increase

hydrophilicity if the hydrophobic tail of the released lipid faces the hydrophobic plastic surfaces and the hydrophilic headgroup directs towards the solution.

To examine the type of interaction mechanism, microplastics with liposomes were washed with 10 mM HEPES (pH 7.4), HEPES containing 5 mM Mg^{2+}, 50% ethanol containing 5 mM Mg^{2+}, and Triton X-100, respectively. As shown in Figure 7A, liposomes were completely released after washing with Triton X-100, while most of the liposomes were retained after washing with HEPES containing Mg^{2+}. In contrast, partial adsorption was retained after washing with 50% ethanol containing Mg^{2+}. Ethanol may wash away adsorption based on hydrophobic interactions, and the remaining lipids might be from those shown in Figure 7D. If a lipid bilayer model is reasonable, adsorption requires Mg^{2+}. However, the adsorbed lipids largely remained even after washing in HEPES without Mg^{2+}. As such, supported monolayers might also be formed on hydrophobic plastic surfaces. Therefore, overall, we believe that the final structure of the lipid layer can be a mixture of supported mono- and bilayers as illustrated in Figure 7B.

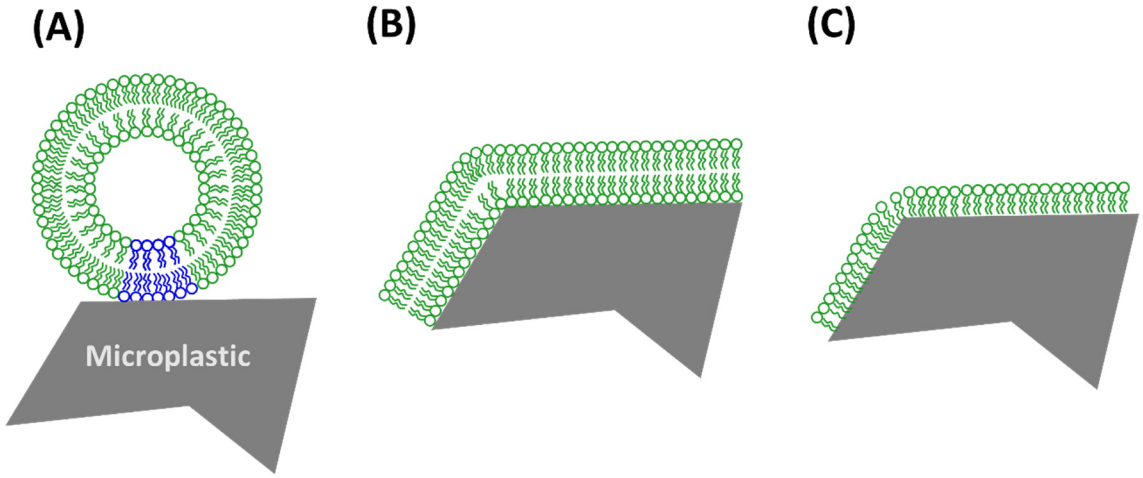


Figure 6. A cartoon showing the process of adsorption and rupture promoted by metal ions: (A) local leakage, the formation of (B) supported bilayers and (C) supported monolayers.

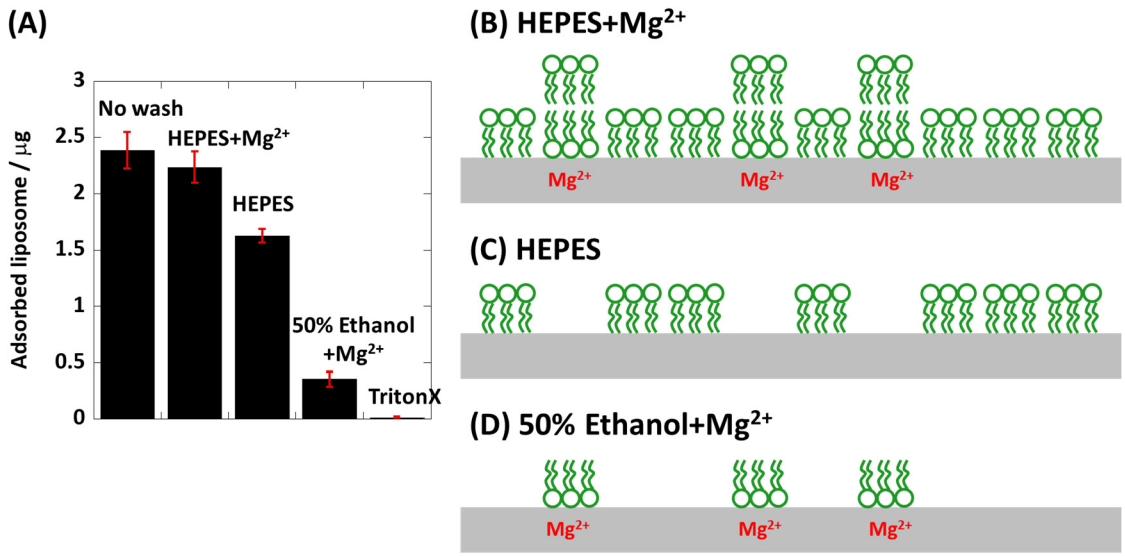


Figure 7. (A) The Rh-PE adsorption on PP microplastics water after washing with 10 mM HEPES, 10 mM HEPES containing 5 mM Mg^{2+}, 50% ethanol containing 5 mM Mg^{2+}, and Triton X-100. 100 μg mL^{-1} liposomes and 5 mM Mg^{2+} were incubated with 2 mg mL^{-1} PP microplastics dispersed in 10 mM HEPES. PH of 10 mM HEPES solutions were 7.4. The concentration of Triton X-100 was 5%. PP microplastics were washed 3 times for each experiment. (B)-(D) Cartoons showing the liposome adsorption after washing with (B) 10 mM HEPES containing 5 mM Mg^{2+}, (C) 10 mM HEPES, and (D) 50% ethanol containing 5 mM Mg^{2+}.

Leakage test in real sample

Since a large fraction of microplastics end up in the oceans and ocean water has a high salt content, we thus tested the interaction between liposomes and microplastics in Atlantic Ocean water sampled in New Waterford, Nova Scotia. Microplastic pollution in the ocean is a major environmental concern due to its potential adverse effects on living systems. Seawater contains a variety of metal ions, such as Na^{+} (sub-molar), Mg^{2+}, and Ca^{2+} (low mM),[40] which can cause microplastics to adsorb and rupture liposomes. Figure S5 shows the results of the liposome leakage test using PP microplastics in seawater. For the first 5 min, no change was observed, indicating that the liposomes were stable even in seawater. At 5 min, a final concentration of 2 mg mL^{-1} PP was added, resulting in an immediate enhancement in fluorescence. This indicated that microplastics in seawater can adsorb and rupture liposomes. While leakage of liposomes indicates

potential perturbation of cell membrane, it does not necessarily mean complete cell membrane disruption. Nevertheless, this observation suggests that microplastics in environmental waters can adsorb onto cell membranes and potentially affect their properties.

Discussion

Adsorption of liposome can take place in the presence of Mg^{2+}. Although based on the structure of the liposome and microplastics, there is no charge attraction or repulsion, both have slightly negative surface charge based on our ζ-potential measurement. What we are certain is that there is no electrostatic attraction. A major role of metal ions is to screen charge repulsion since a high concentration of monovalent Na^{+} could induce rupture. In addition, divalent Mg^{2+} and Ca^{2+} induce leakage in relatively lower concentrations since they can bridge the headgroup of liposomes and the surface of microplastics. In addition, the Mg^{2+}-dependent adsorption and rupture have a similar metal concentration requirement, suggesting that rupture can occur after adsorption, and there is no additional energy barrier that required higher metal concentrations. Salt and pH-dependent fusion of liposomes with silica surfaces has been systematically studied by Cremer and Boxer, and the authors concluded that the fusion process was governed by van der Waals and electrostatic interactions.[41] At neutral pH, rupture of PC liposomes on silica is favored by a high salt concentration. In addition, PC liposomes need to be in their fluid phase to rupture on silica forming supported bilayers.[42] This is different from our reported microplastics, which can rupture DPPC liposomes at room temperature. This reminds us of a previous study of interactions of DPPC liposomes with graphene oxide (GO), and leakage was observed only with reduced GO and pristine graphene, but not with highly oxidized GO.[25] A common feature of microplastics and graphene is probably hydrophobicity.

Natural membranes have a much more complex composition than the DOPC lipids used here. There are not only more types of lipids but also transmembrane proteins, and for some bacterial cells, there are even cell walls. The studies performed here served only as a reduced model system to gain fundamental insights. It indicates a strong affinity between PC headgroups and plastic surfaces. This study has added another type of material that can adsorb PC liposomes.[43] Due to the affinity between lipids and microplastics, such adsorption may change the colloidal

property of microplastics and thus their transportation.[14] Finally, for liposome research, it is common to use plastic tubes as containers, and this study suggests that at low liposome concentrations, adsorption and rupture of liposomes may need to be considered.

Conclusions

In summary, this study systematically investigated the leakage and adsorption of DOPC liposomes onto microplastics in the presence of metal ions. While neither metal ions nor microplastics alone induced the leakage of liposomes, metal ions were found to facilitate the adsorption and rupture of liposomes on microplastics. Divalent metal ions, including Mg^{2+} and Ca^{2+}, were more efficient than monovalent Na^{+} in promoting the adsorption of liposomes onto microplastics by bridging them. The leakage test and adsorption studies followed similar trends regardless of the type of plastic components used in this study. This indicates that interaction between plastics and liposomes is a combination of nonspecific screening of charge repulsion and more specific metal-bridged interactions. Similar observations on the adsorption and leakage of liposomes were also observed in the seawater sample.

PC lipids are the most common in cell membranes. While DOPC is not a natural lipid, the similar behavior of DOPC and DPPC liposomes indicated that lipid phase is less important. Future studies could expand on this work by investigating the chemistry of other liposomes with different headgroups, such as phosphorylethanolamine (PE) and phosphoserine (PS) lipids. These groups have different metal preferences, with phosphate preferring hard metals, while PE and PS bind better to soft metals.[44-47] By combining different lipids, a better understanding of metal-mediated adsorption and rupture of lipid membranes on microplastics may be achieved. This work provides a fundamental understanding of the adsorption of environmental microplastics onto lipids and facilitates future studies on the environmental risks associated with microplastics.

Supporting Information

The characterization of the five microplastic samples, the characterization and leakage test of DPPC, the effect of Mg^{2+} concentration on the adsorption capacity of liposome onto PP

microplastics, the effects of liposome concentration on the floating behavior of PP microplastics, and the leakage test in real sample.

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