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Effect of the Interaction of Graphene Oxide Nanoparticles on a Biological Model Cell Membrane

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ABSTRACT

Understanding the interaction of graphene oxide (GO) with a lipid membrane is important for the development of tissue engineering and to advance graphene-based biology. In an effort to understand the GO-lipid membrane interaction, appropriate characterisation of GO structure was determined by using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and a field emission scanning electron microscope (FESEM). In this study, the lipids 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were used to produce a lipid vesicle with a conventional gentle hydration method and observed under transmission electron microscopy (TEM). Lipid vesicle-GO interactions were also investigated using dynamic light scattering (DLS) (Malvern Zeta) and TEM by focusing on the effect of the surface charge interactions and localisation of GO on the surface of the vesicle membrane. It was observed that the surface charge of the vesicles increased as the GO nanoparticle concentration increased, but for the low saturation lipid the surface charge remained high as the nanoparticle concentration increased. The localisation and positioning of the GO nanoparticles in the lipid vesicles were confirmed with TEM analysis.

Keywords: Graphene oxide (GO), lipid vesicles, interaction lipid-GO, distribution, membrane permeability

INTRODUCTION

Rapid development in nanotechnology has led to the interaction of engineered nanomaterials between humans and the environment [1], such as the interaction between nanoparticles and the biological cell membrane. Use of this technology has led to the emergence of various applications, including biosensor, biotechnology, and tissue engineering that has enabled the extraction of important information, such as biological reactions in the cell as well as the ability to control cellular activities in the biological membrane [2].

In an effort to understand interactions in biological membranes, co-operative functionality of the material used and the cellular lipid membrane is closely involved. Cell membranes are solely the result of various biological reactions, including disease and the transmission of information and substances in and out of the cell [3]. The basic structure of the cell membrane is a lipid bilayer, which is composed of lipids and proteins that play a major role as a solvent to maintain the structure of the membrane itself. The lipid bilayer provides a rich and varied environment for proteins, which includes a highly hydrophobic interior bounded by hydrophilic and/or charged lipid head groups [4].

Artificial lipid membranes composed of lipid bilayers are commonly used as a model system to provide fundamental insights into the nonspecific interactions of cell membranes with nanoparticles. A supported lipid

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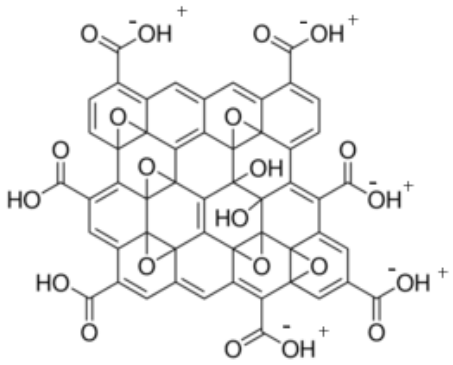

Figure 1. Schematic structure of GO

bilayer is used in this study and this type has been employed as a model in the evaluation of the propensity for silica nanoparticles and quantum dots to attach to membranes [5].

Today, carbon nanotubes (CNTs) and graphene are two of the most studied materials. Graphene has attracted a lot of attention due to its unique properties, such as high surface area, high carrier mobility [6], mechanical, optic, thermal, and magnetic properties [7], which have allowed it to be used as a nano-material ideal for interaction with a biological membrane. Graphene is a single atom thick, in its pristine form, and is composed of a single layer of carbon atoms arranged in a sp^2-bonded aromatic structure. Graphene can offer a superior improvement of the mechanical properties of polymers, due to improved interactions between the sheets and the polymer matrix resulting from the high surface area of the planar graphene sheets compared to CNTs [8].

Recent studies have reported that graphene is not soluble in polar solvents but can form a stable dispersion in organic solvents upon chemical functionalisation, thus providing planar graphene monolayer a better hybridisation with lipid bilayers in cellular membranes [9]. Graphene can also cause phospholipids to exhibit higher mobility, thus allowing the membrane to be uniformly spread across the graphene [10], providing good attachment.

Graphene oxide (GO) is a popular approach to graphene-based nanomaterials due to its low production cost [8]. GO is oxidised from graphene and contains carboxyl, epoxy, and hydroxyl groups on its edge and basal planes [5]. It also possesses hydrophilic functional groups on the surface as well as a hydrophobic graphenic region that enables it to engage in hydrophobic interactions while in water and polar solvents [11]. The schematic structure of GO is shown in **Figure 1**. GO has been applied in few area such as drug delivery vehicles, analytical/sensing devices and GO-based nanocomposite scaffolds for tissue engineering [12].

Recently, the interaction between lipid membranes and GO has been reported, and it is a result of a balance of various interaction forces, including electrostatic and hydrophobic interactions, electrostatic repulsion, and hydrogen bonding [13]. For electrostatic interactions, it has been proven that the negative charges of GO are electrostatically adsorbed to the positive charges on the lipid membrane and also based on the absence of adsorption of GO on negatively charged lipid membranes [14, 15].

Although current studies have provided some insight into the nature of the interaction between graphene/GO and lipid membranes, there are still some questions that remain to be answered. The suitability or appropriate characterisation of graphene/GO for lipid membranes has not been highlighted yet. Even though both types can interact with lipid membranes, the most ideal substance that will give the best compatibility is still unknown. Furthermore, the ability of graphene/GO to alter the structure of the lipid membrane has also not been fully elucidated. The suitability of lipid composition and organisation in the artificial membrane lipid with the graphene/GO is a key parameter for the interaction with the graphene/GO, although this parameter has not yet been fully assessed. Therefore, this work aims to investigate the mechanism of GO-lipid membrane interactions, using supported lipid bilayer as a model membrane to mimic the actual cell membrane.

METHODOLOGY

Preparation of Supported Lipid Bilayer

The preparation of the supported lipid bilayer was performed as previously described [16]. The conventional gentle hydration method was chosen for the preparation of the lipid membrane vesicles. The two types of lipid used in this research were 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) purchased from Avanti Polar Lipids (USA) was dissolved separately in chloroform at a

concentration of 2.5 mg/ml for each lipid and dried in the oven overnight at 45°C to obtain a thin lipid film. The thin lipid film was then prehydrated with 1 ml of distilled water and 0.1 ml of 0.1 M sucrose solution and left at room temperature for 1 h. After that, vesicles were formed as a bulky white suspension in the solution and were extruded through 100 nm polycarbonate membranes (Avanti Polar Lipids) to obtain homogeneous vesicles. This process was repeated for 10 cycles on the hot plate at 40°C to keep the lipid soluble in the solution [17]. The sample of lipid membrane vesicles was then obtained.

Synthesis of Graphene Oxide

GO was synthesised from graphite powder (Sigma-Aldrich, St. Louis, MO, USA) with a modified Hummer's method [18, 19]. Graphite powder (1 g) and sodium nitrate (0.5 g; Sigma-Aldrich) were mixed, followed by the addition of 23 ml of concentrated sulphuric acid (Sigma-Aldrich) under constant stirring. Then, 3 g of $KMnO_4$ was gradually added to the mixed solution after 1 h while keeping the temperature less than 20°C to prevent overheating and explosion. Then, the mixture was stirred at 35°C for 12 h and the resulting solution was diluted with the addition of 500 ml of water under vigorous stirring. The suspension was further treated with 30% H_2O_2 (Sigma-Aldrich) solution (5 ml) to ensure the complete reaction with $KMnO_4$. The slurry was repeatedly washed with water, until the pH of the filtrate was neutral. The GO was then dried with the freeze dryer, finally resulting in a black powder.

Characterisation Techniques of Graphite and Graphene Oxide

The graphite and GO were analysed to determine the characterisation before and after interaction with the lipid bilayer. The instruments used to analyse the characterisation were X-ray powder diffraction (XRD) and Fourier transform infrared (FT-IR). The morphology of graphite and GO was investigated using a field emission scanning microscope (FESEM).

An FT-IR spectrometer (Perkin Elmer Spectrum 400 FT-IR/FT-NIR and Spotlight 400 Imaging System) is an instrument that acquires absorption spectra in the infrared region. It has reported by [20] that FT-IR analysis was performed to provide more information about the process steps, the oxidation of graphite, and reduction of GO. This was done by showing how the functional groups of the materials change as the process progresses.

Since GO is a crystalline lattice structure, XRD analysis was used to investigate structural changes during the reaction process. According to ref.20, every crystalline substance produces a specific pattern, and in a mixture of substances, each produces its pattern independently of each other.

Characterisation Techniques of Lipid Membrane Vesicles

The artificial lipid membrane was analysed to characterise the structure before and after interaction with GO. The instrument used to analyse the samples was a transmission electron microscope (Fei Tecnai G2 Spirit Biotown).

TEM analysis is used both for recording high magnification images of samples, but also for crystallographic studies. The images can be recorded by direct exposure of a photographic emulsion or an image plate inside the vacuum or digitally via a fluorescent screen coupled by a fiber optic plate to a Charged coupled device (CDD) camera.

RESULTS AND DISCUSSION

FTIR and XRD Study on GO

The functional groups of graphite and GO were analyzed using FTIR spectrum based on our results as shown in **Figure 2**. GO spectrum shows the characteristics absorption peaks of several oxygen-containing groups while there is no significant peaks were observed in the graphite [21]. GO spectrum demonstrate there is peak at 3401.0 cm^{-1}, suggesting a strong O-H bond and at peak 1759.0 cm^{-1}, indicating a C=O bond consistent with a previous report [22]. The peak at 1623.3 cm^{-1} also indicated a bond of C=C from sp^2 [23], while the peaks at 1392.9 cm^{-1} and 1078.4 cm^{-1} are due to the vibration bonding of C-OH and C-O respectively [24]. From this, it is clearly shown that the functional group that contains oxygen has been successfully produced onto the surface of graphite during the oxidation process.

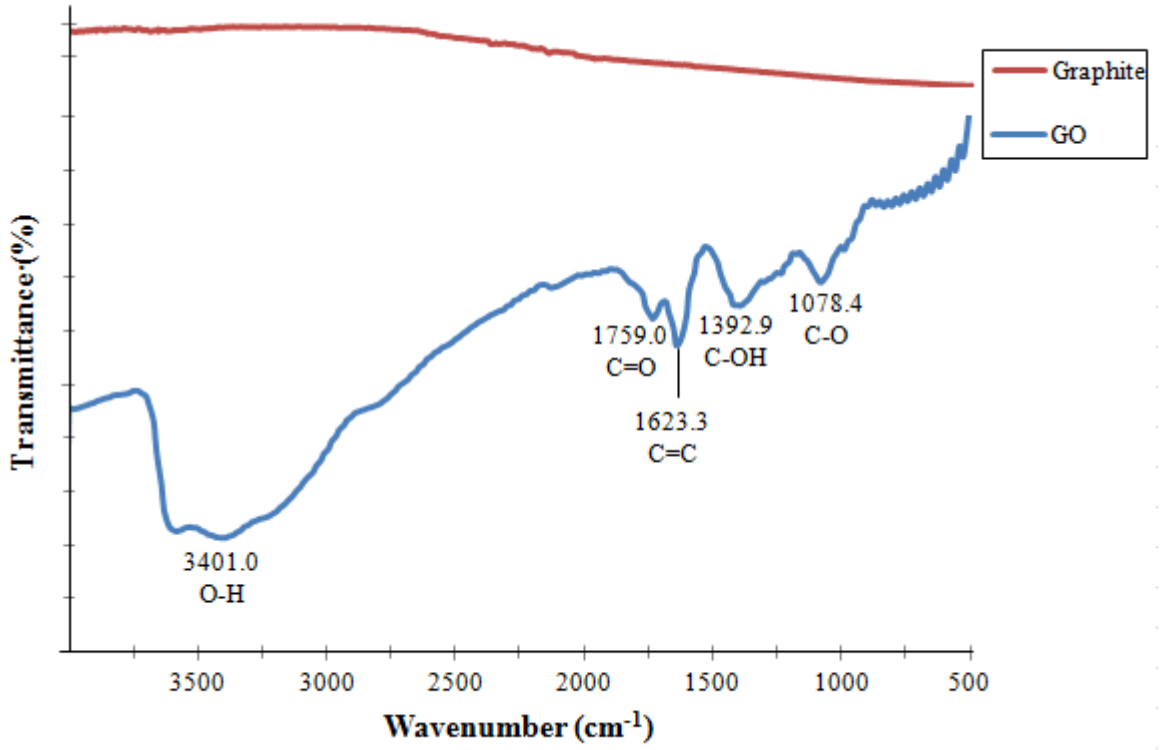

Figure 2. Fourier transform infrared (FT-IR) spectrum of graphene oxide (GO) and graphite

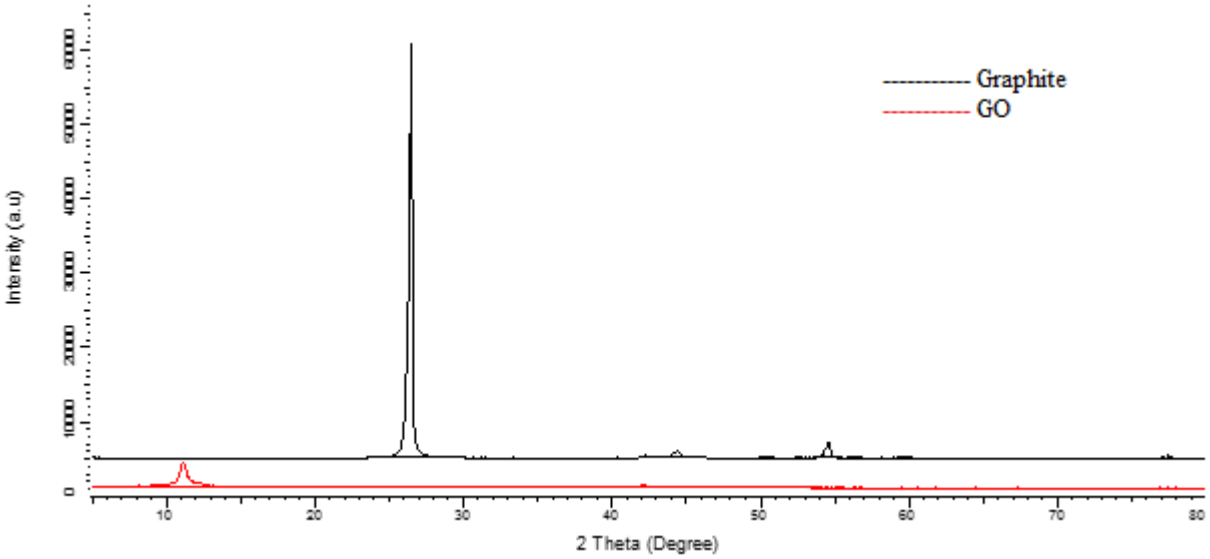

Figure 3. X-ray powder diffraction (XRD) spectrum of graphite and GO

As depicted by XRD diffraction pattern in **Figure 3**, shows a comparison of graphite and GO. Pure graphite displays sharp diffraction peak (001) at 2θ=26.510° with d-spacing of 3.359Å. After completed exfoliation and oxidation of GO using modified Hummer method, the diffraction peak shifted to (002) at 2θ=11.225° with increased d-spacing of 7.877 Å [25].

The Morphology of GO

The morphological structure of GO was observed through FESEM as shown in **Figure 4**. These images show that the structure of GO has a porous network likely the same as a loose sponge (**Figure 4a** and **b**). Similar images

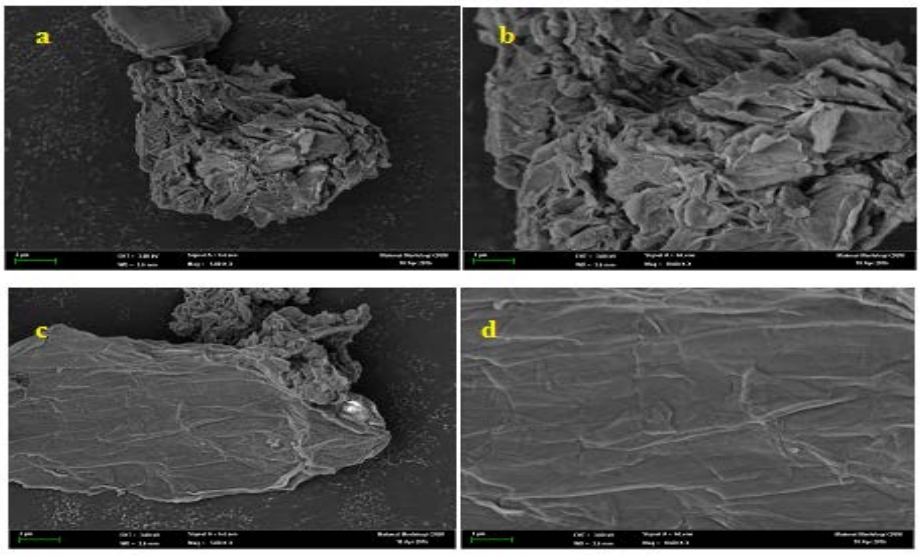

Figure 4. FESEM image of GO by oxidation process. Figure (a) and (b) show the porous network of GO, while (c) and (d) show the thin layer of GO

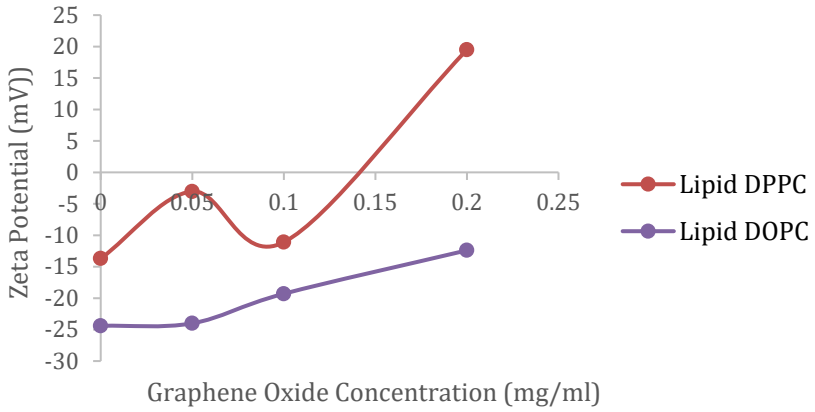

Figure 5. Graph of zeta potential (mV) versus concentration of GO (mg/ml)

have also been reported in a previous study by [26]. **Figure 4 (c)** and **(d)** indicate that the GO layer was successfully produced following oxidation.

Effect on Interaction of GO with Vesicle Lipid

Researchers have reported that there is an electrostatic interaction between GO nanoparticles and lipid membrane vesicles [27]. This interaction has been observed by the distribution of GO nanoparticles on the surface or in lipid membrane vesicles membranes through the surface charge of lipids based on the zeta potential, particle size, and localisation of GO nanoparticles on the lipid vesicle surface [27]. Different concentrations of GO nanoparticles were found to have a significant effect on the different types of membrane lipids based on the zeta potential value. **Figure 5** shows the zeta potential of two types of lipids at different concentrations of GO. DPPC shows an increase in zeta potential from -13.7 to 19.5 mV. This indicated there is a strong interaction between the negatively charge GO nanoparticles and zwitterionic DPPC vesicles, although the value decreases slightly at nanoparticle concentrations of 0.1 mg/ml [28]. The value may decrease due to the addition of sucrose during the preparation of DPPC vesicles as the medium provides a good isotonic condition (in terms of electrostatic stability) for negatively charged nanoparticles [16].

In **Figure 7 (b)**, TEM images show that the GO nanoparticles are attached to the edge of the vesicle walls, resulting in the appearance of dark images surrounding the vesicles compared to normal vesicles without GO nanoparticles in **Figure 7 (a)**. This is due to the high saturation of lipid, which results in an organised and stable, but less permeable, lipid structure that still interacts with GO nanoparticles [16]. **Figure 8** shows the GO

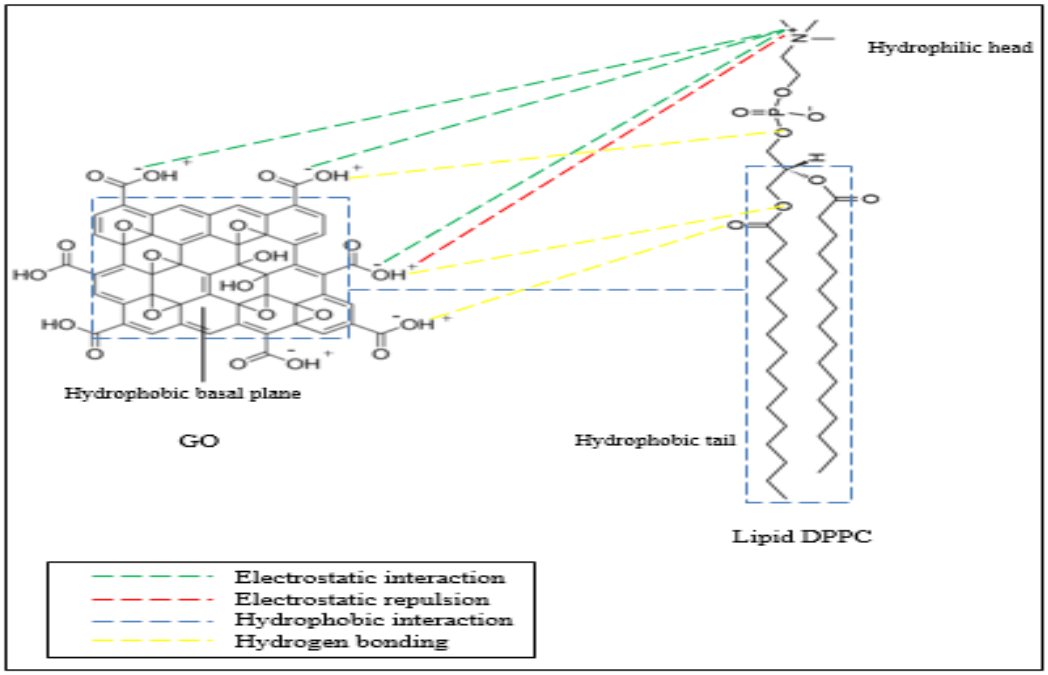

Figure 6. The proposed interaction mechanisms of lipids and GO

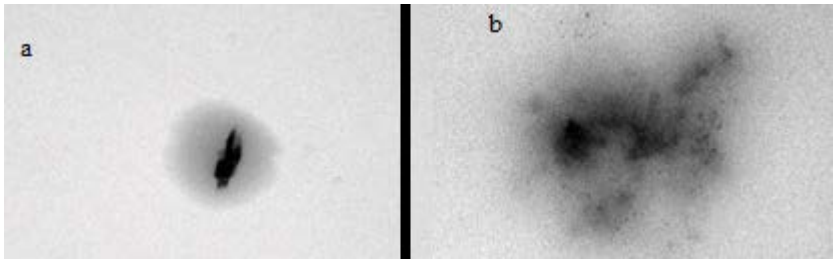

Figure 7. TEM images of (a) DPPC and (b) DPPC vesicles after addition of GO nanoparticles

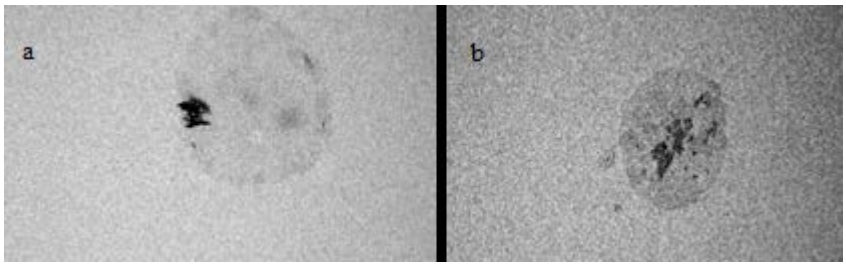

Figure 8. TEM images of (a) DOPC and (b) DOPC vesicles after addition of GO nanoparticles

nanoparticles embedded inside the DOPC vesicles. This indicates that this lipid structure is less permeable compared to the lipid DPPC-GO. However, the zeta potential for DOPC shows an increase from -24.4 to -12.4 mV as the nanoparticle concentration increases. This result may be due to the low level of saturation of the lipid structure, thus making the lipid more permeable as well as increasing the interaction between GO nanoparticles and lipid vesicles. Furthermore, a previous study also found that the interaction of negatively charged GO nanoparticles with a zwitterionic head group is stronger than positively charged nanoparticles [29]. The proposed mechanism of interaction between lipid membrane and GO is shown in **Figure 6**. The effect of nanoparticle concentration on lipid vesicles was also reported by ref. [16], where they used titanium dioxide nanoparticles and reported that zeta potential and lipid size depends on the concentration of nanoparticles.

CONCLUSION

The distribution of the GO nanoparticles was confirmed based on the results obtained from the zeta potential of the lipid vesicles. Our results suggest that the vesicle lipid-GO formed via electrostatic interactions. The interaction is determined by the head group of the lipid and the surface charge of the adsorbing nanoparticles. The saturation of the lipids due to different alkyl chains may also contribute to the lipid vesicle-GO nanoparticle interaction. By dynamic light scattering measurement, it was found that GO nanoparticles increased the zeta potential value of DPPC but at a high concentration of GO, the value decreased slightly. However, since DOPC is less saturated than DPPC, the zeta potential of DOPC remained high as the GO concentration increased. TEM provided confirmation of the localisation of the GO nanoparticles and the structure of the lipid-GO vesicles. These results demonstrated the importance of the use of the zeta potential value especially to understand factors that may contribute to the interaction forces between GO nanoparticles and lipid membranes as well as to predict the stability of the vesicles.

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