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Infrared and fluorescence spectroscopic studies of a phospholipid bilayer supported by a soft cationic hydrogel scaffold

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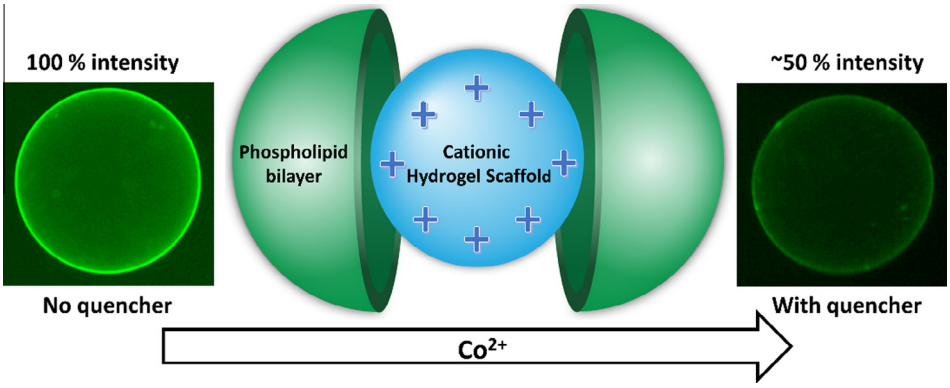
GRAPHICAL ABSTRACT



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ABSTRACT

Polarized attenuated total reflection (ATR-IR) spectroscopy and fluorescence microscopy techniques were used to characterize a 1,2-diphytanoyl-*sn-glycero*-3-phosphocholine (DPhPC) membrane supported on porous, cationic hydrogel beads. Fluorescence microscopy images showed that the DPhPC coated the external surface of the hydrogel scaffold. In addition, a fluorescence assay of the emission intensity of the Tb^{3+}/dipicolinic acid complex demonstrated that the DPhPC coating acted as a barrier to Tb^{3+} efflux from the scaffolded vesicle and successfully sealed the porous hydrogel bead. Fluorescence quenching and ATR-IR spectroscopic measurements revealed that the lipid coating has a bilayer structure. The phytanoyl chains were found to exhibit significant trans-gauche isomerization, characteristic of the fluid liquid phase. However, no lipid lateral mobility was observed by fluorescence recovery after photobleaching (FRAP) measurements. The phosphocholine headgroup was found to be well hydrated and oriented such that the cationic choline group tucked in behind the anionic phosphate group, consistent with an electrostatic attraction between the cationic scaffold and zwitterionic lipid. The absence of lipid lateral mobility may be due to the strength of this attraction.

1. Introduction

Supported vesicle systems have generated much interest because of their desirable properties including structural stability, biocompatibility, modifiable surface properties, and an enclosed internal volume [1–5]. These properties make supported vesicles attractive candidates for use in biotechnological applications such as therapeutic drug delivery and as biomimetic membrane platforms [1]. Supported vesicles exhibiting good structural stability have been developed on a wide range of materials, including silica, polymer gels, polyelectrolytes, and numerous nanoparticles; and

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have ranged between tens of nanometers to tens of micrometers in diameter [1,6–25]. The size of the support dictates vesicle size, and significantly impacts their potential applications. Large architectures (~1 μm) are primarily used for studying cell membrane properties, while smaller architectures (~100 nm) are targeted towards biomedical applications [1]. Developing supported vesicles suitable for characterization by surface analytical and electrochemical techniques can provide valuable information about these systems. This work describes the development of a giant supported vesicle accessible to both surface analytical and electrochemical methods, for use as a model membrane. Specifically, the model is designed to be studied simultaneously by attenuated total reflection infrared (ATR-IR) spectroscopy and electrophysiological patch-clamping, which necessitates a scaffold diameter ⩾100 μm [26]. We have investigated similar systems for this purpose as detailed in reference [26,27].

In addition to serving as a model membrane, the spherical geometry, and porosity of the hydrogel scaffold in conjunction with the barrier properties of a lipid bilayer can allow for the encapsulation of appropriate molecules. Ref. [26] detailed the permeability characteristics of scaffolded vesicles with a 70:30 DMPC:cholesterol coating. These studies revealed a maximum permeability at the main phase transition temperature of DMPC [26]. In that study, large (~100 μm diameter) hydrophilic hydrogel beads were used as a structural scaffold for a lipid bilayer [26]. The large scaffold allows for the measurement of lipid orientation by ATR-IR spectroscopy. To improve membrane durability and its barrier properties a subsequent study examined the properties of the lipid membrane supported on a hydrophobically modified hydrogel scaffold. It was found that the durability and barrier properties of these lipid coatings were greatly improved [27].

In Refs. [26,27] the Sephadex® hydrogel particles were coated by fusion and rupture of unilamellar lipid vesicles producing scaffolded vesicles. The hydrogel particles were coated with lipids, however we did not know if the coating consisted of a single bilayer or a multibilayer system. An objective of the present work is to demonstrate that the vesicle fusion procedure provides a single bilayer coating to the hydrogel particle. To achieve this goal, design changes to both the hydrogel scaffold and lipid membrane were made. The spherical scaffolds used in this work are Sephadex® A50 hydrogel beads. Sephadex® A50 is an anion exchanger consisting of cross-linked dextran functionalized with positively charged diethyl(2-hydroxypropyl) quaternary aminoethyl groups. The functional groups are attached to the glucose units of the dextran [28]. These cationic functional groups provide the hydrogel with a positive charge, which has been shown to promote adhesion and formation of supported lipid bilayers, providing an attractive electrostatic interaction between the support and bilayer [29–33]. Electrostatic attractive interactions have been used in various charged polyelectrolyte and microgel systems to promote stable lipid bilayer coating on spherical particles [22–25,34–38]. However, these systems do not exceed ~10 μm in diameter, making them unsuitable for investigation by ATR-IR spectroscopic and patch-clamp techniques.

The average diameter of the hydrated Sephadex® A50 beads is ~ 225 μm, which is about twice that of the scaffolds used in our previous studies. Sephadex® A50 is less cross-linked than Sephadex® G10 and thus it is a more flexible, porous hydrogel. Using a porous hydrogel as the scaffold is intended to provide the inner leaflet of the bilayer with access to an aqueous-like environment. An internal aqueous environment is important for promoting the insertion of membrane proteins into the host lipid matrix, and in investigations of membrane transport processes. The synthetic phospholipid 1,2-diphytanoyl-*sn-glycero*-3-phosphocholine (DPhPC) was used for the construction of the biomimetic membrane. The phytanoyl chains of DPhPC are 16 carbons long with branched methyl groups. DPhPC has been shown to form high quality, stable model membranes and has been used as a matrix for the reconstitution of membrane proteins [39–46]. This stability has been attributed to the interdigitation of the branched methyl groups of the hydrophobic tails [47]. The phosphocholine headgroup has been shown to have an affinity to positively charged supports [14,31]. DPhPC does not exhibit a phase transition between −120 and +120 °C, and therefore avoids the permeability spike associated with phospholipid main phase transitions [48]. This improvement to the system design will aid further controlled-release studies on scaffolded vesicles.

The scaffolded vesicles have been characterized using fluorescence microscopy and spectroscopy, fluorescence quenching, fluorescence recovery after photobleaching (FRAP) and ATR-IR spectroscopy. Fluorescence microscopy was used to evaluate the coating of the hydrogel scaffold and stability of the lipid membrane. A phytanoyl lipid labeled with a fluorescent tag was used as the fluorescent probe. A fluorescence spectroscopy Tb^{3+}/dipicolinic acid assay was implemented to monitor the temporal efflux of Tb^{3+} from the lipid coated hydrogel in order to further corroborate the presence of a complete coating. Fluorescence quenching experiments were performed in order to obtain structural information for the phospholipid coating. By using a positively charged hydrogel and positively charged fluorescence quencher we would be able to demonstrate that the hydrogel scaffolds can be coated by a single lipid bilayer. The mobility of fluorescently tagged phytanoyl phospholipid within the membrane was probed by FRAP experiments. Lipid mobility is a measure of membrane fluidity, an important feature of biological membranes. ATR-IR spectroscopy, a surface sensitive infrared spectroscopic technique, was used to examine the order and orientation of the hydrophobic phytanoyl chains and polar headgroup. The accessibility of the scaffolded vesicles to ATR-IR spectroscopy is important because these measurements can provide valuable insight into the membrane structure. Furthermore, when studying controlled-release from scaffolded vesicles, it may be possible to correlate the controlled-release properties of the system with the membrane structure. Using this strategy we will provide a complete description of the structure and properties of the lipid coating on a hydrogel particle. These results are significant because they could be used to develop strategies for the controlled-release of the internal contents of scaffolded vesicles, with potential applications in fields such as drug and nutraceutical delivery

2. Experimental section

2.1. Sample preparation

2.1.1. Phospholipid vesicle preparation

Dry lipid films were prepared by combining DPhPC and 1,2-diphytanoyl-*sn-glycero*-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-DPhPE) chloroform solutions in a test tube, at a 99:1 molar ratio. The chloroform solvent was slowly evaporated under a stream of argon while vortexing. After evaporation, the test tubes were stored in a desiccator under vacuum for at least 24 h prior to use. To make vesicles, a Milli-Q water was added to the dry lipid film such that a 5 mg/mL solution was obtained. The mixture was sonicated for 5 min at 45 °C.

2.1.2. Scaffolded vesicle preparation

To prepare scaffolded vesicles, phospholipid vesicles and hydrated Sephadex® A50 were incubated at 45 °C for 1 h with constant vortexing. The system was then allowed to incubate further at room temperature for 24 h. The scaffolded vesicles were then rinsed thoroughly prior to use.