Lipid coating technology: A potential solution to address the problem of sticky containers and vanishing drugs

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Abstract
Pharmaceutical drugs and vaccines require the use of material containers for protection, storage, and transportation. Glass and plastic materials are widely used for packaging, and a longstanding challenge in the field is the nonspecific adsorption of pharmaceutical drugs to container walls – the so-called “sticky containers, vanishing drugs” problem – that effectively reduces the active drug concentration and can cause drug denaturation. This challenge has been frequently discussed in the case of the anticancer drug, paclitaxel, and the ongoing coronavirus disease 2019 (COVID-19) pandemic has brought renewed attention to this material science challenge in light of the need to scale up COVID-19 vaccine production and to secure sufficient quantities of packaging containers. To reduce nonspecific adsorption on inner container walls, various strategies based on silicinization and thin polymer films have been explored, while it would be advantageous to develop mass-manufacturable, natural material solutions, especially ones involving pharmaceutical grade excipients. Inspired by how lipid nanoparticles have revolutionized the vaccine field, in this perspective, we discuss the prospects for developing lipid bilayer coatings to prevent nonspecific adsorption of pharmaceutical drugs and vaccines and how recent advances in lipid bilayer coating fabrication technologies are poised to accelerate progress in the field. We critically discuss recent examples of how lipid bilayer coatings can prevent nonspecific sticking of proteins and vaccines to relevant material surfaces and examine future translational prospects.

KEYWORDS
antifouling, pharmaceuticals, phospholipids, supported lipid bilayer, vaccines
1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is one of the most serious global health challenges in modern history and has not only accelerated the development of next-generation vaccines based on messenger ribonucleic acid (mRNA) technology and liposomal delivery systems, but also brought renewed attention to the issue of global supply chains and packaging materials for storage and distribution. Chief among the material considerations is the selection of primary packaging materials in which to store vaccines and pharmaceutical drugs. Ideal materials would be physically stable and protect the vaccine or drug formulation from degradation, prevent contamination, and minimize interactions between the formulation and inner walls of the packaging material.

Among the different material options, borosilicate glass is widely used to manufacture glass vials but is facing a global shortage due to heightened demand. Plastic vials are also being considered, especially ones with an inner silica (SiO₂) coating to minimize nonspecific adsorption events, and there are also various surface functionalization strategies to coat inner vial walls with organosilicate or polymer coatings. Even so, there are still longstanding challenges to prevent the nonspecific adsorption of pharmaceutical drugs to container walls, which has been referred to as the “sticky containers, vanishing drugs” problem. Indeed, pharmaceutical drugs can adsorb to the container walls, which reduces the effective drug concentration. In addition, for certain drug classes, the drug molecules can undergo denaturation in the adsorbed state and, depending on whether the adsorption is irreversible or reversible, the denatured drug molecules can leech and pose adverse immunological risks. While siliconization and thin polymer films are being explored to coat glass vial walls and minimize nonspecific adsorption, it would be advantageous to develop mass-manufacturable, natural material solutions, especially ones that utilize pharmaceutical grade excipients.

In this perspective, we propose that supported lipid bilayer (SLB) coatings are a promising strategy to precoat container walls and to prevent nonspecific adsorption of pharmaceutical drugs and vaccines. The rapid development and deployment of mRNA vaccines based on liposomal drug delivery systems has highlighted the potential of lipid nanotechnologies to solve a wide range of global health challenges. Importantly, these advances have also reinvigorated interest in lipids as pharmaceutically accepted carriers and excipients and led to increased global manufacturing capabilities for pharmaceutical-grade lipid materials. Coupled with recent advances in SLB fabrication technologies, there is excellent potential to mass-manufacture lipid bilayer coatings for medical packaging, with compelling advantages over inorganic and polymer alternatives.

2 | LIPID BILAYER FABRICATION TECHNOLOGIES

SLBs are ultrathin, two-dimensional lipid bilayer coatings that can self-assemble on various types of surfaces, including borosilicate glass and silica, and consist of a single bilayer of phospholipid molecules. The effectiveness of SLB coatings to inhibit nonspecific adsorption of various classes of biological macromolecules and nanoparticles, such as proteins, virus particles, and cells, is well-established, and zwitterionic-phospholipid-based SLB coatings have demonstrated superior performance to albumin- and polymer-based coatings. However, conventional methods of SLB coating fabrication are technically challenging and hardly scalable, which has limited widespread acceptance of SLB coatings for practical applications.

One of the most widely used methods to fabricate SLBs involves the adsorption and spontaneous rupture of lipid vesicles onto a solid surface – a process called “vesicle fusion” (Figure 1). On certain surfaces, vesicles can adsorb and either remain intact or rupture to form an SLB depending on the vesicle-substrate interaction strength. The specific outcome is highly dependent on the surface type, especially atomic composition, along with vesicle properties (e.g., size, lipid composition, lipid concentration) and environmental conditions (e.g., osmotic pressure, solution pH, temperature, divalent cations). In practice, the vesicle fusion method only works on silica-based materials, has limited production scalability, and is mainly used in scientific laboratories working on membrane-related topics. Moreover, specialized equipment is required to produce lipid vesicles with suitable properties for vesicle fusion, thus creating high know-how and cost barriers that limit mass manufacturing possibilities.

In light of these shortcomings, there have been extensive efforts by our groups and others to develop more scalable SLB fabrication methods, including the solvent-assisted lipid bilayer (SALB) and bicelle methods (Figure 2). Both of these methods and their competitive merits are briefly introduced below:

The SALB method involves simply dissolving phospholipid molecules in a water-miscible organic solvent, typically isopropyl alcohol, and phospholipids tend to self-assemble into inverted micelles or stay as monomers in organic solvent. Then, the lipid solution is incubated with a solid surface, followed by a solvent-exchange step...
FIGURE 1  Vesicle fusion method for SLB fabrication. Step-by-step illustration of the vesicle fusion process. Lipid vesicles with well-defined properties are first produced using specialized equipment and then added to a solid surface, upon which they adsorb and can spontaneously rupture to form an SLB if vesicle-substrate interactions are sufficiently strong. Using the vesicle fusion method, successful SLB fabrication is mainly limited to silica-based materials. Adapted with permission from ref. 43. Copyright 2020, American Chemical Society

FIGURE 2  Emerging material science strategies for SLB fabrication. Step-by-step illustration of the SALB and bicelle methods. Top row: The SALB method involves dissolving lipids in a water-miscible organic solvent, depositing the lipid solution on a solid surface, and exchanging organic solvent with aqueous solvent. Bottom row: The bicelle method utilizes bicellar nanostructures composed of long-chain (yellow) and short-chain (red) phospholipids, which adsorb, rupture, and fuse to form an SLB composed of long-chain phospholipids. Adapted with permission from ref. 43. Copyright 2020, American Chemical Society

to replace the organic solvent with an aqueous solvent. This latter process causes deposited phospholipids to eventually form a lamellar-phase nanostructure on the solid surface, resulting in an SLB coating. Compared to vesicle fusion, the SALB method is advantageous because it does not require vesicle preparation and can work with otherwise-intractable surfaces such as gold. However, the SALB method can be sensitive to the lipid concentration and solvent-exchange flow conditions, and hence optimization is warranted on a case-by-case basis.

While the SALB method has compelling features, there is also interest in developing more robust fabrication methods that do not require organic solvent and are relatively insensitive to the operating parameters used in the SLB fabrication process. Among the different options, the bicelle method has attracted widespread interest because it requires minimal freeze-thaw-vortex processing to form bicellar nanostructures composed of long- and short-chain phospholipids, requires much lower bulk lipid concentrations to form SLBs compared to other methods, and works in fully aqueous conditions and across a wide range of environmental conditions.

While long-chain phospholipids are abundantly available, one initially limiting aspect of the bicelle method was the need to include short-chain phospholipids, which are far less common and relatively expensive; a commonly used one is 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC). However, recent progress has demonstrated the potential to use bicellar nanostructures composed of long-chain phospholipids and low cost, widely available free fatty acids or monoglycerides to effectively and affordably fabricate SLBs. Hence, bicelles are
an attractive tool to mass-manufacture SLB coatings on account of robust fabrication, simple processing, and low-cost materials.

3 | APPLICATION EXAMPLES

We next discuss how SLB coatings can inhibit the nonspecific adsorption of proteins and other biological materials by revisiting findings from the scientific literature and comparing protein adsorption results obtained on bare and SLB-coated silica surfaces. Indeed, silica surfaces have often been suggested as a low-adsorption substrate to minimize protein adsorption19,21 while literature findings show that SLB coatings can significantly enhance antifouling performance.26–29 The use of surface-sensitive measurement techniques to track protein adsorption kinetics and corresponding adlayer properties is a popular experimental strategy that has been used by our groups and others.59–71 In Figure 3, we present quartz crystal microbalance-dissipation (QCM-D) data that are compiled from our laboratories and demonstrate the utility of SLB coatings to inhibit protein adsorption on silica surfaces.

The QCM-D technique tracks time-resolved resonance frequency (ΔF) and energy dissipation (ΔD) shifts when proteins adsorb onto bare or SLB-coated silica surfaces, and these measurement signals reflect adlayer mass and viscoelastic properties, respectively.72–73 Representative QCM-D ΔF signals are presented for various proteins – serum albumins from different species, lysozyme, immunoglobulin G antibody, and fibrinogen – at different bulk concentrations adsorbing onto bare and SLB-coated silica surfaces (Figure 3A). The results show that proteins can adsorb to varying degrees on bare silica surfaces while protein adsorption onto SLB-coated silica surfaces is effectively nullified.

The QCM-D ΔF signal at saturation can also be further analyzed to determine the adsorbed protein mass,74 and the corresponding data are presented in Figure 3B. For example, bovine serum albumin (BSA) is a widely studied model protein and >200 ng/cm² BSA adsorbs onto bare silica surfaces while <5 ng/cm² BSA adsorbs onto SLB-coated silica surfaces. These results demonstrate that SLB coatings can inhibit nonspecific protein adsorption on silica surfaces.

In a more practical context, these findings also have implications for preventing nonspecific protein adsorption to glass vials. For example, when 0.1 mg/ml BSA protein in an aqueous solution is incubated in a glass vial, the solution-phase protein concentration changes over time due to nonspecific protein adsorption on the glass walls (Figure 4A). There was a negligible change in the bulk protein concentration after 1-min incubation but an ~36% decrease after 10-h incubation. In addition, when a 0.1 mg/ml BSA protein solution was successively transferred between 10 glass vials, there was a decrease in the bulk protein concentration after each transfer step (2-min incubation per vial) (Figure 4B).

After 10 transfer steps, the bulk protein concentration decreased by ~37%. In marked contrast, when the same transfer process was applied to SLB-coated glass vials, there was negligible change in the bulk protein
concentration even after 10 transfer steps (Figure 4C). As such, SLB coatings have excellent potential to coat vial surfaces and minimize nonspecific protein adsorption.

In addition to minimizing nonspecific adsorption of purified proteins such as BSA, SLB coatings can also inhibit the adsorption of medical products such as vaccines. An example is the SKYCellflu Quadrivalent seasonal vaccine for multiple influenza A and B virus strains, which is an inactivated split virus vaccine and is typically made from virus particles cultured from cells that are then disrupted using a detergent.75–76 While this vaccine mainly contains viral antigen proteins (0.12 mg/ml), it also possibly contains other subviral components (i.e., viral nucleic acids and proteins) as well as cell culture-related impurities (e.g., host cell proteins and glycosaminoglycans).77–78 Representative QCM-D ΔF signals are presented for nonspecific vaccine-related material adsorption onto bare and SLB-coated silica surfaces (Figure 5A). While >600 ng/cm² vaccine-related mass adsorbs onto bare silica surfaces, <20 ng/cm² vaccine-related mass adsorbs onto SLB-coated silica surfaces (Figure 5B). This finding demonstrates that the SLB coating can inhibit nonspecific adsorption of the wide range of biomacromolecules found in the vaccine composition.

4 | CONCLUSION AND OUTLOOK

While SLB coatings have long attracted interest for potential antifouling applications, recent progress in the development of streamlined and manufacturing-compatible SLB fabrication technologies along with heightened interest in medical packaging innovations due to the ongoing COVID-19 pandemic are creating new opportunities for SLB coatings to become mainstream technology solutions to overcome the longstanding problem of "sticky
containers, vanishing drugs.” Indeed, SLB coatings have several practical advantages over existing inorganic and polymer functionalization strategies, in terms of natural material sourcing, low cost, production ease, biocompatibility, and high performance. From a nanoscience perspective, the lipid coating technology is also an important example of the nanoarchitectonics concept79–80 and demonstrates how molecular self-assembly driven by physical interactions at the nanoscale can give rise to ordered lipid assemblies with high functional performance.

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CONFLICT OF INTEREST
Nam-Joon Cho and Joshua A. Jackman are inventors on patents and patent applications related to supported lipid bilayer coatings, including US patent number 10,427,124, U.S. patent number 10,787,470, and PCT patent number US2019/047518. Nam-Joon Cho is a founder of, Ki Yeol Yoo and Seung Hwa Lee are employees of, and Joshua A. Jackman is a scientific advisor to LUCA Health and LUCA ACell Inc., which are developing lipid-related diagnostic and therapeutic technologies. The other authors declare no competing interests.

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