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Invited review: Milk phospholipid vesicles, their colloidal properties, and potential as delivery vehicles for bioactive molecules

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ABSTRACT

The milk fat globule membrane (MFGM) is a unique colloidal assembly of phospholipids and proteins, with numerous potential applications as functional ingredient. The phospholipid components of the MFGM are gaining interest as they are a useful matrix for use as a constituent of delivery systems such as liposomes. Liposomes formulated with milk phospholipids are becoming an alternative to other sources of phospholipids such as soybean or egg yolk. However, incorporation of phospholipids fractionated from the milk fat globule membrane in dairy products requires an in-depth understanding of the functional properties of phospholipids. In particular, it is critical to understand which factors play a role in their stability and bioefficacy as delivery systems. Moreover, chemical and physical modifications of phospholipid liposomes occurring during digestion and the fate of the encapsulated compounds are very important to understand. This review discusses recent findings on the structure and functionality of MFGM, the bioactivity of the phospholipids fraction, their utilization as delivery systems, and their stability through gastrointestinal transit.
Key words: milk fat globule membrane, polar lipid, liposome, bioactivity, gastrointestinal tract

INTRODUCTION

Consumer demand for foods with benefits beyond nutrition is continuously growing and has led to an increase in research into design of food matrices that can deliver biological functionality with consumption. In this context, several studies have been conducted to better evaluate the potential use of minor components in milk as functional ingredients.

Milk is an aqueous solution that contains mainly fat, proteins, lactose, and minerals; it is an exceptional source of energy for breast-fed infants, providing essential nutrients and bioactive compounds. Milk contains about 3 to 5% fat, which exists in milk fat globules (Jensen, 2002). The stability of this oil-in-water emulsion is maintained by a thin lipid layer, the milk fat globule membrane (**MFGM**), that surrounds the fat globules, preventing them from coalescing freely. Milk fat globules are unique colloidal structures (0.1–20 μm in diameter), naturally and exclusively found in milk. The MFGM results from the secretion of milk fat globules from epithelial cells of the mammary gland, and its structure has been extensively studied (see, for example, Lopez, 2011). A proteomics study of MFGM revealed that the composition of bovine MFGM includes 69 to 73% lipid and 22 to 24% protein. The protein accounts only for 1 to 4% of total milk protein but includes several proteins exclusive to the MFGM with recognized biological functionality (Cavaletto et al., 2008).

The milk fat globules have a polydisperse size distribution, and this polydispersity is conserved among species and may be of biological significance (Michalski et al., 2005a). The smaller the size of the globule, the greater the surface area available for interactions with other molecules or microorganisms in the gut. An in-depth study of milk fat globules in human milk demonstrated that, in addition to large milk fat globules, there is a large population of nanodroplets, with an average diameter of 25 nm, composed of proteins and lipids, called lactosomes (Argov et al., 2008). In contrast to the large milk fat globules, lactosomes do not contain a substantial triglyceride core and are not a source of energy for the neonate. Rather, it is thought that their surface components have important biological functionality; for example, immunomodulatory functions (Argov-Argaman et al., 2010). These particles have a similar density to plasma high-density lipoproteins, and can be separated from other milk fat globules by ultracentrifugation. Lipidomic and proteomic analyses on these natural nanostructures suggest that they derive from a different secretory or biosynthetic pathway than milk fat globules (Argov-Argaman et al., 2010).

Fragments of MFGM can be found in dairy products, with buttermilk and butter serum (the byproducts from

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the churning of cream for butter making) containing the highest concentrations (Dewettinck et al., 2008; Vanderghem et al., 2010). During butter making, the milk fat globules are destabilized and disrupted, causing the release and agglomeration of milk fat and the selective concentration of the MFGM fractions and most of the water-soluble material into the aqueous phase, known as sweet buttermilk. This fraction also contains a substantial amount of protein derived from skim milk, and sodium citrate can be used to dissociate the caseins and allow for fractionation of MFGM material from buttermilk (Corredig et al., 2003). Other alternatives for the extraction of the MFGM from buttermilk include the use of ultrafiltration followed by supercritical fluid extraction to obtain an ingredient especially enriched in phospholipids (Costa et al., 2010).

The MFGM act as natural emulsifiers in milk. The fraction isolated from buttermilk can be utilized as a functional ingredient to stabilize oil-in-water emulsions. The functional properties of such isolates depend on their processing history (Corredig and Dalgleish, 1998). The phospholipid fractions are of particular interest because they have shown to have biological functionality in addition to their processing functionality (Snow et al., 2010; Snow et al., 2011; Zanabria et al., 2014a,b). Recently, milk phospholipids isolated from MFGM fractions became commercially available and new possibilities have opened based on the ability of phospholipid liposomes to entrap bioactive compounds. Numerous publications have focused on the characterization of liposomes obtained using phospholipids derived from the MFGM (see, for example, Thompson et al., 2006a; Thompson and Singh, 2006; Dewettinck et al., 2008; Farhang et al., 2012; Farhang and Corredig, 2012).

This work will review the current understanding on the MFGM fractions, their composition, and beneficial properties. Particular focus is given to the utilization of milk phospholipids (isolated from MFGM fractions) as constituents of delivery systems, as well as the fate of encapsulated compounds during gastrointestinal transit.

MILK FAT GLOBULE MEMBRANE AS A COLLOIDAL STRUCTURE

Milk is a natural oil-in-water emulsion, with an oil phase comprising mainly fat globules with diameters of about 0.1 to 10 μm. These fat globules are protected from enzymatic degradation and coalescence by the MFGM, which acts as a natural emulsifier and encapsulates the nonpolar triglyceride core. The complexity of MFGM architecture ensures stable dispersion of triglycerides in the aqueous solution of milk. Polar lipids, proteins, and glycoproteins present in the membrane induce electrostatic and steric repulsion, preventing coalescence and aggregation of the fat globules (Lopez, 2011).

Composition and Structure of the MFGM

The major components of the MFGM are polar lipids with a unique and complex composition (Evers et al., 2008; Lopez, 2011). However, polar lipids are considered minor components of milk, as they comprise only 0.1 to 2% of total milk lipids (Månsson, 2008). The use of polar milk lipids as functional ingredients is gaining attention due to the increased evidence of their nutritional and health benefits upon consumption (Singh, 2006). The MFGM has been described as having a trilayer structure, with a thickness of 10 to 50 nm (Lopez, 2011). The surface-active inner monolayer, which surrounds the triglyceride core, is composed of polar lipids deriving from the endoplasmic reticulum. The central layer appears denser by electron microscopy and it contains mainly proteins (Mather and Keenan, 1998). Finally, the outer layer is a bilayer membrane of polar lipids. Loosely attached and transmembrane proteins, as well as cholesterol molecules, are present in the external layer (Gallier et al., 2010). Glycoproteins are also present on the surface, with carbohydrate domains oriented into solution (Lopez et al., 2010).

The most abundant polar lipids described in MFGM are glycerophospholipids, including phosphatidylcholine (**PC**, 35–36% of total polar lipids), phosphatidylethanolamine (**PE**, 27–30%), phosphatidylinositol (**PI**, 5–11%), and sphingolipids, mainly sphingomyelin (**SM**, 25%; Lopez et al., 2008). Glycerophospholipids and SM are classified as phospholipids due to the presence of a phosphate group in the polar head. Glycerol forms the backbone of glycerophospholipids, esterified with 2 fatty acids and a phosphate group with organic residues (choline, ethanolamine, or inositol, among others), whereas sphingolipids have sphingosine as backbone with a fatty acid and a phosphocholine head group. Other sources of phospholipids, such as egg or soy, differ in composition, with PC being the main phospholipid present in egg, and a mixture of PC, PE, and PI being described for soy (Burling and Graverholt, 2008; Farhang and Corredig, 2012).

Regarding the concentration of polar lipids in milk, a study has reported a content 28% higher in cow milk than in buffalo milk. This difference is associated with the smaller size distribution of the fat globules in cow milk and, consequently, their greater surface area compared with buffalo milk (Ménard et al., 2010). In addition, the fat globule size affects polar lipid composition: PI was found in higher concentration in fat globules with an average diameter of 2 μm, whereas PE concen-