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Review

The milk fat globule membrane as an ingredient: why, how, when?

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Abstract – This paper presents a personal view on the potential applications of the milk fat globule membrane as an ingredient in the processed foods area. Several factors are of importance for this dissertation: the biological origin of the membrane, the voluminous literature on its individual components and their relationship with health and wellness, the biological role of milk in nutrition to mammals and the innovation on scientific tools being applied in many fields of chemistry and biology. We hope to give a glimpse of the reasons on why it is a good idea to use more efficiently the components of the milk fat globule membrane. In addition we consider current advances in the fractionation of milk components to propose how this ingredient can be produced. However, we leave the timing question, when?, open for discussion in the different scientific and technological fields.

milk fat globule membrane (MFGM) / isolation / analytical tools / biological activities

摘要 – 乳脂肪球膜在食品工业中的应用。本文基于个人的观点对乳脂肪球膜作为一种食品成分在食品加工领域中的潜在用途进行了论述。作者从以下几个重要方面进行了论述, 如膜的生物来源, 关于单一化合物的文献报道以及这些化合物与人类健康之间的关系, 乳对哺乳动物在营养方面的生物学作用, 以及通过化学和生物技术手段对这些化合物的改性等。希望通过本文的介绍能够对有效地使用乳脂肪球膜中的化合物给出一些有用的建议和理由。加之, 考虑到目前对乳研究的热点主要是对乳成分的分离及其生产; 然而, 如何实现这一目标, 则给科技工作者们留下了一个深远的话题。

乳脂肪球膜 (MFGM) / 分离 / 分析手段 / 生物活性

Résumé – Application de la membrane du globule gras du lait comme ingrédient : perspectives actuelles et futures. Cet article présente nos vues personnelles sur les applications potentielles de la membrane du globule gras du lait comme ingrédient dans le domaine de la transformation alimentaire. Notre réflexion s'articule sur les éléments suivants : l'origine biologique de la membrane du globule gras, la vaste littérature couvrant ses divers composants et leur impact sur la santé et le bien-être, le rôle nutritionnel du lait chez les mammifères et le recours à des outils analytiques novateurs associés aux domaines de la chimie et de la biologie. Nous espérons que ce survol permettra de convaincre le lecteur de l'importance d'optimiser l'utilisation des divers composants de la membrane du globule gras du lait. Cet article propose également un aperçu des plus récents développements techniques qui permettront de fractionner et de produire commercialement ces composants. Nul doute que la réalisation de ces défis continuera de stimuler les milieux scientifiques et technologiques.

membrane du globule gras du lait / séparation / analyse / activité biologique

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1. INTRODUCTION

This paper presents some relevant information on the potential of the milk fat globule membrane (MFGM) as an ingredient in our food supply. In today's world where milk fat has an image problem due to the load of saturated fat and the role of triglycerides in the modern American diet, it is important to emphasize that there are very important components in milk that have not been fully considered. Those are the minor lipids, glycolipids and glycoproteins that compose the MFGM. Since the industrialization of butter, these components have taken a second seat to primary commodity ingredients such as skim milk powder and whey protein concentrates. The lipids contained in buttermilk powder are not used to their full potential in the processed foods industry. In foods, these lipids exhibit superior emulsifying properties, and help significantly in thickening formulations due to water distribution properties, and also aid where foaming is important such as in ice cream. However, the popular current view of buttermilk is of a milk-derived powder with an important liability; it goes rancid in about 6 months due to lipid oxidation.

This view overlooks the fact that buttermilk contains the MFGM components that in today's world can give the lipids of milk some better public relations image. The glycoproteins and lipids it contains may have been contributors to wellness in traditional diets even if we have not been fully aware of this. The fact that MFGM is rich in important minor lipids and glycoproteins found only in specific animal tissues (such as brain) which makes it valuable in our diets. Some of these lipids are sphingomyelin (SP), phosphatydylserine (PS) and phosphatidylcholine (PC), together considered milk-derived lecithin, and other minor lipids (such as gangliosides and cerebrosides); all are necessary in a healthy diet.

In this paper we will try to bring MFGM and its potential relevance in the context of a new challenge confronting the food scientist and technologist of today. Demand of consumers for better and healthier foods while at the same time maintaining safety, demands that the technologist of today recognize the natural biological role of the food components, isolate the important factors that impart the functional and nutritional benefit while at the same time considering the matrix of the food, and finally design a process that preserves the targeted biological and food function of the ingredient. True advancement in the development of the foods of the future may rest on the success that we have in addressing this challenge.

This idea is not new and has been presented before [61], where these ideas join the above mentioned challenge and suggests meeting it with the new tools available to scientist. Such tools include proteomics and genomics, where a large repository of information can be used to find out relationships among the components of a complex organism, and novel analytical methods that can be applied to complex systems. Milk is a good food in which to apply such concepts and in particular the MFGM. Its composition and its interactions with other components of milk and foods can be a starting point.

2. ORIGIN AND FUNCTION OF THE MFGM

The MFGM is a surface-active membrane surrounding each of the milk fat globules (MFG) allowing them to remain dispersed in milk. The MFG core is essentially composed of triacylglycerides (TG), while the MFGM envelope is a true polar lipids bilayer with proteins, enzymes, neutral lipids and other trace components [11]. The composition of the milk fat constituents and their distribution between the

Table I. General composition of the milk lipids and their distribution between the milk fat globule (MFG) and the milk fat globule membrane (MFGM) (adapted from Walstra et al. [60], Jensen [27], Michalski and Januel [34]).

Lipid class	Total fat ($g \cdot kg^{-1}$)	Fraction content in MFG (%)	Fraction content in MFGM (%)
Neutral glycerides			
Triacylglycerol	38.3–39.3	100	
Diacylglycerol	0.11–0.90	≈ 90	≈ 10
Mono-acylglycerol	0.01–0.17	Traces	Traces
Free fatty acids	0.04–0.18	60	≈ 10
Phospholipids	0.08–0.44	–	65
Cerebroside	0.4	–	70
Gangliosides	0.004	–	≈ 70
Sterols		80	10
Cholesterol	0.12–0.18		
Cholesteryl ester	⩽ 0.008		
Carotenoids + Vitamin A	0.0008	≈ 95	≈ 5

MFG core and the MFGM is given in Table I. The MFGM structure could be considered, to some extent, as the fingerprints of the milk fat biosynthesis in the mammary epithelial cells. Thus, a brief overview on the milk fat synthesis is essential to get a better understanding of the MFGM structure. However, due to the complexity of the MFG secretion process, some of the reports of the latest advances on the topic are strongly suggested to the reader [11, 24, 29–31]. To summarize, milk fat secretion starts in the epithelial cell's endoplasmic reticulum as micro lipid droplets (MLD). Upon their release into the cytoplasm, these MLD coalesce into larger droplets called the cytoplasmic lipid droplets (CLDs). While the CLDs migrate toward the apical pole of the epithelial cell's membrane, various components, such as phospholipids, glycosphingolipids, cholesterol and proteins, coat their surface. This interface is the inner layer of the MFGM. The peripheral layer of the MFGM is formed during the excretion of the CLDs out of the epithelial cells by a process generally referred as the milk fat globule "budding". The CLDs are gradually surrounded by the cell membrane before being expelled into the alveolar lumen. Upon closure of the cell membrane, some components of the cytoplasm could be entrenched between the inner and the peripheral membrane layer forming "crescents" on the globule surface (under the microscope, the cytoplasmic material resembles a moon crescent, Fig. 1). The MFGM peripheral membrane is a true polar lipids bilayer that includes an amalgam of glycoproteins, enzymes and phosphoproteins. To complete the picture, an electron dense layer is located at the inner face of the peripheral membrane. This layer is composed of proteins such as xanthine oxidase (XO), butyrophilin (BTN), and adipophilin (ADPH) among many others.

Because of its cellular membrane origin, the MFGM is the richest source of phospholipids, glycolipids, gangliosides, and glycoprotein in milk. The properties of the MFGM components have been recently

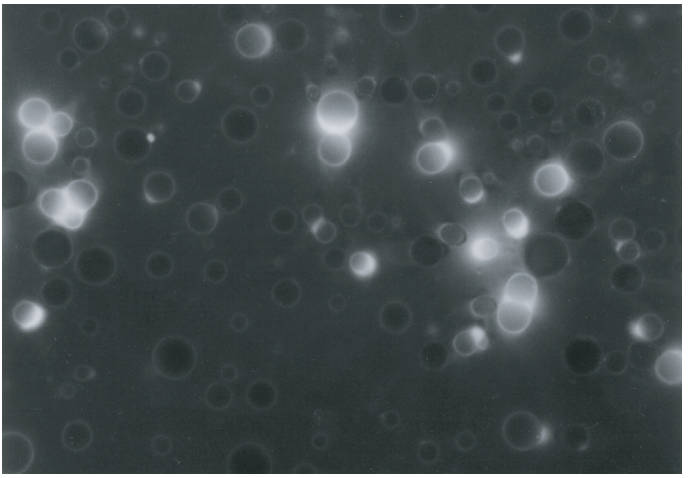


Figure 1. Milk fat globule crescents fluorescently stained with acrydine orange. (Picture from Huston and Patton [25], personal communication).

investigated and a number of health benefits have been reported (Tab. II). These MFGM biological activities have triggered thoughts of its potential as a nutraceutical in food applications [58]. However, this potential has yet to be fulfilled and some structural considerations of the MFGM in regards of the suggested health benefits need to be assessed.

3. STRUCTURAL CONSIDERATIONS OF THE MFGM

The surface of the MFGM, considering the above description of its origin, is anything but homogeneous. Perhaps a view on the comparison of the MFGM of different milks can give us better clues as to their biological roles. Based on some descriptive work [62] where a remarkable intra- and site-specific variability of MFGM glycoproteins was detected, a suggestion was made that they play a role in the intestine of the new born. Observing the interactions of the globules in Figure 1 [25], it is important to notice that the globules are interacting among themselves through sections of the membrane that is presumably in its native state. That is, since the presence of the crescents indicate that the cytoplasm between the membrane and the triglyceride globule forces the membrane has to be in its bilayer form. This observation begs the question, would the interaction among membranes of the intestine and the MFGM follow such a specific interaction? If so we can imagine an interaction between membranes designed for nutrition delivery.

Biologist and chemist may give us some information on the structure and the binding function of the MFGM, and hopefully in a near future we will find out more on the role of this component in nutrition. However, in the present paper, we shall examine where this membrane resides, and what efforts are being made to enrich this component and make it into a dairy ingredient we could use in the food industry.

4. MFGM ISOLATION

The most important source of MFGM material is buttermilk, the by-product of butter making. While churning cream, the

Table II. Reported health benefits claims of different MFGM components (adapted from Riccio [44], Spitsberg [58], Pan et al. [42], Fong et al. [17], Michalski and Januel [34]).

Proteins	**Molecular mass ($kg \cdot mol^{-1}$)**	**Reported health benefits**
Mucin 1 (MUC1)	160	Antiviral action/Anti Rotavirus
Mucin 15 (MUC15 or PAS III)	94–100	Antiviral action
Butyrophilin (BTN)	66	Suppression of multiple sclerosis
Xanthine oxidase (XO)	155	Bactericidal agent
Cluster of differentiation (CD36 or PAS IV))	78	Glycoproteins that act as receptors due to high sugar content
Fatty acid binding protein (FABP)	15	Cell growth inhibitor Anti-cancer factor
BRCA1 and BRCA2	210	Inhibition of breast cancer
Lactadherin (PAS 6/7)	43–59	Role in epithelialization, cell polarization, cell movement and rearrangement, neurite outgrowth, synaptic activity in the central nervous system, protection against viral infection in the gut
Adipophilin (ADPH)	52	Milk synthesis
Other components		
β-Glucoronidase inhibitor		Inhibition of colon cancer
Helicobacter pylori inhibitor		Prevention of gastric diseases
Cholesterolemia-lowering factor		Anti-cholesterol activity
Vitamin E and carotenoids		Anti-oxydants
Phospholipids		Inhibition of colon cancer, anti-cholesterol activity,
Shingomyelin		Suppression of gastrointestinal pathogens Anti-Alzeimer, anti-depressant, anti-stress
Phosphoproteins		Source of organic phosphorus and Ca-phosphate

membrane of the milk fat globule is disrupted and MFGM fragments are released in the buttermilk accompanied with the rest of the water-soluble fraction, such as the proteins, lactose and minerals. To give an idea of the importance of this by-product, from the 610 million kg of butter produced annually in the US, 35.4 million kg of buttermilk are condensed or evaporated [1]. These buttermilk ingredients have been commercially used essentially for their good emulsifying properties in a wide range of products [8, 9, 28, 57]. However, the potential of the MFGM components in regard to their health benefits is not fully realized or used in these commodity products. Moreover, the numerous processing steps sustained by the buttermilk could impair the MFGM nutritional and biological value. Thus, the challenge that faces food scientists is to develop proper techniques that allow MFGM isolation or enrichment while maintaining its biological value.

So far, the most feasible approach investigated to fractionate or concentrate MFGM from buttermilk has been the use of tangential cross-flow membrane filtration. Microfiltration (MF) is well suited for the fractionation of buttermilk since its pore size range corresponds approximately to the MFGM fragments size ($\geqslant 0.1\,\mu m$ [38]). Many efforts have been directed in the improvement of the

retention of MFGM fragments during the MF of buttermilk by adjusting the process parameters such as temperature, membrane pore size, and the addition of a diafiltration step [5, 37, 59]. However, those who have been working with the direct MF of buttermilk have face a similar problem, namely the presence of caseins. In fact, in their micellar forms, the caseins are comparable in size with the MFGM fragments and conjointly retained during MF. Different pretreatments have been applied to the buttermilk to overcome this problem. For example, Sachdeva and Buchheim [50] used acid or rennet coagulation to remove the casein prior to filtration. Other authors have investigated the ability of different techniques to dissociate the casein micelles in order to improve their permeation during MF by either enzyme cleavage [45] or sodium citrate [10]. Morin et al. [40] have tested a wash cream process [7] to produce a casein depleted buttermilk to improve the MF separation performances. The use of different MFGM sources has also been examined. Rombaut et al. [46] have evaluated the feasibility of MF of buttermilk sera, a MFGM rich by-product of the anhydrous milk fat production, after casein micelles destabilization. The same group of authors also have adapted the thermolcalcic aggregation process developed for the delipidation of cheese or acid whey [15, 16] for the precipitation of MFGM fragments in acid buttermilk cheese whey [48]. They also investigated the recovery of the precipitated MFGM fragments by means of MF [47]. The use of whey buttermilk, the by-product from the churning of the casein depleted whey cream, has also been investigated [39]. We also developed new approaches to further separate buttermilk components by using biosilicates to remove proteins and non-polar lipids from buttermilk [19]. Furthermore, a technique using supercritical CO_2 extraction on MF buttermilk powder has shown its ability to separate the MFGM polar lipids from the non-polar lipids [5]. Another research group designed a MF process of whole milk that allowed the selective separation of the MFG in their native forms according to their diameters [20, 21, 35]. This process has been showed to increase the MFGM content in dairy products by collecting selectively the smaller globules richer in MFGM [20, 35].

As seen above, several research groups are actively working on the improvement of the separation process of MFGM. These recent advancements open the route for new applications of the MFGM materials. However, there is an increasing need for more refined techniques to further separate the different MFGM proteins or lipids. This will allow the realization of the individual properties of the MFGM components to respond to the growing need in new ingredients for very specific application.

5. MFGM AND LACTIC ACID BACTERIA BINDING

In this and the following sections we would like to propose a view on where the potential applications of the MFGM properties and biological activities are most likely to occur given some of the advances in the scientific and technological fields applied to food. Figure 2 represents a good graphic example of lactic acid bacteria binding to milk fat globules.

There are many similarities as to the components found on the MFGM and the intestinal cells. Carbohydrate chains, proteins, glycoproteins (e.g. Mucins), enzymes and phospholipids are present in both systems yet little has been researched as to what of these components plays a role in probiotics' success in dairy. We advance that in order to understand how probiotic bacteria transfer from the dairy product to the intestinal lining, the manner and mechanisms in which bacteria bind

to different substrates must be understood. First we can consider the lactic acid bacteria (LAB). The main region they present to bind to surfaces is their calyx, of which two components are of great importance, exopolysaccharides, and surface proteins that for simplicity we will equate to the S-layer proteins, located at the surface of the bacterial cell. The bacterial cell surface carbohydrate binding proteins and the mechanism in which bacteria bind with the S-layer could be equivalent with the interaction between polysaccharides and lectins [51, 52]. Also it could be suggested that the carbohydrates provide some localized protection against proteases through steric hindrance or by creating a sheltered microenvironment [14]. The amount of S-layer protein present varies between *Lactobacillus* strains but when present is the most dominant cellular protein [54]. Researchers have found that in some *Lactobacillus* strains, the S-layer is responsible for the cells ability to adhere to a substance for upon removal of the S-layer, the binding ability of the bacterium is greatly compromised [18, 49, 56].

On the other hand, considering the gastrointestinal tract there are three types of membrane-linked glycoconjugates that are thought to play key roles in the binding ability of probiotics: glycoproteins, proteoglycans and glycolipids. Bacterial adhesion is initially based on nonspecific physical interactions between two surfaces, which then enable specific interactions between adhesions (usually proteins) and complementary receptors [32]. Glycoproteins have been localized to a variety of extracellular matrices. They are large, complex, multidomain molecules with numerous biological activities, one of the most important of which involves their adherence to a variety of cell types through cell surface membrane receptors.

Given this complexity, it is important to study the factors that are important for the interaction between LAB, intestine and MFGM. To this effect our group has been studying these interactions. Many studies have focused on the protein binding properties while the binding to lipids has been poorly studied. We focused on developing an assay that gives a quantitative measurement of lactic acid bacteria's affinity to bind to various lipids found in dairy foods [13]. We found two types of lipid binding: non-specific binding to triglycerides (non-polar lipids), in which the lipid concentration was the significant variable, and strain specific binding to phospholipids (polar lipids), where regardless of composition, each strain showed specific binding affinity. More importantly, these results show the specificity of binding as the direct result of the degree of processing of the dairy product. Those powders with lower triglycerides or undergoing supercritical fluid extraction showed an increase in binding to phospholipids. These results will help in the design and formulation of dairy foods containing probiotic strains thus optimizing the bacteria's beneficial effects on health [13].

6. ANTIVIRAL PROPERTIES OF THE MFGM

Here is another example of an area where MFGM will very likely have a contribution in the near future. There is evidence today that the protection of milk to viral infections is through MFGM components. However, the exact mechanism seems to be complex and not due to a single component. Pan et al. [42] have published an extensive review on potential mechanism of lactoferrin as a powerful inhibitor of viral infectivity. Most of the research in this area has been made in vitro, and more evidence is needed for measuring this activity in vivo. There have been also many studies in which immunoglobulin in milk (natural and induced) have been tested for antiviral activity [2, 6, 43].

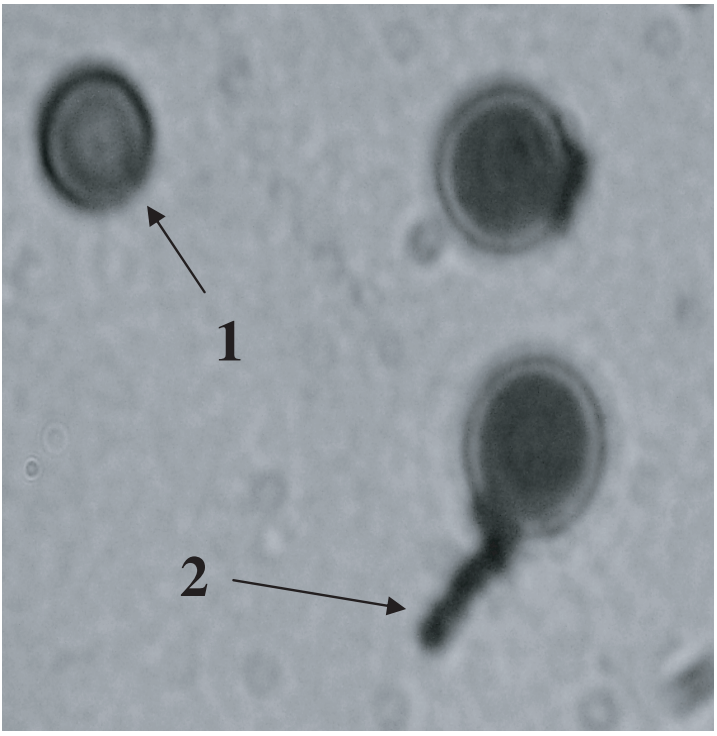


Figure 2. Microscope image of milk fat and lactic acid bacteria at 100× oil immersion with a depression slide. 1. Fat droplet and 2. lactic acid bacteria. (Picture from Elizondo-Bachiero [12]).

Asensi et al. [2] analyzed anti-rotavirus antibodies in human milk in order to determine their isotypes and neutralizing activity on rotavirus strains representing different viral serotypes. Interestingly, they concluded that anti-rotavirus antibodies are only partly responsible for the neutralizing activity detected in milk and serum [2]. Their result suggests that other components possessing suppressive activity against rotavirus must also be present. MFGM proteins are also well studied and in particular lactadherin has been found recently as a main component in the anti-rotaviral activity [6, 33]. We further speculate, that not only the individual proteins and carbohydrates are players in this protective action against viral infections, but due to some of the similar results of LAB binding to lipids, it seems logical to us to add the important role that lipids in the membranes play in the presentation of other biologically active membrane proteins to the "milieu" in which viral interactions take place; whether it is in the food or the intestine.

7. NEW TOOLS TO MONITOR BIOLOGICAL ACTIVITY OF MFGM IN FOODS

7.1. Atomic force microscopy (AFM)

AFM is the ideal technique to provide a means of visualizing, mapping and measuring monolayer domain formation,

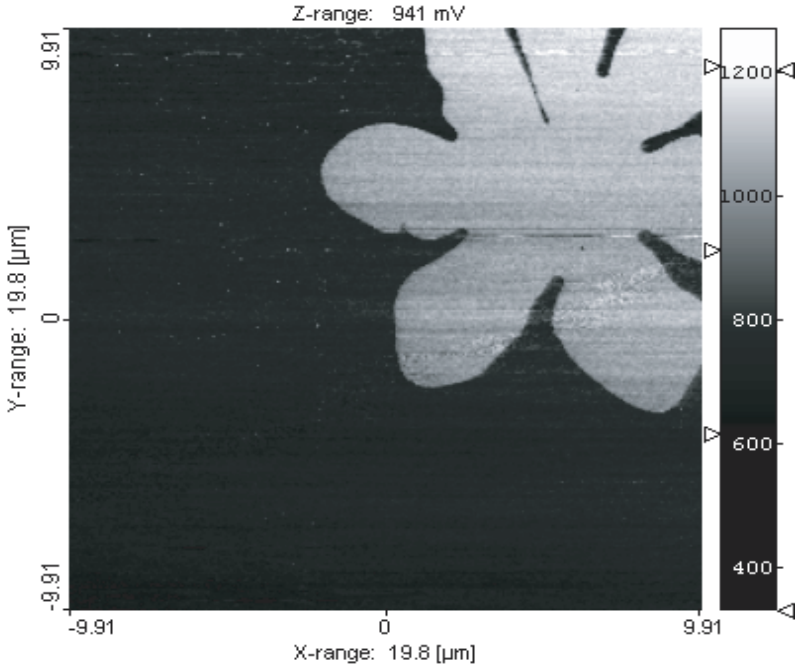


Figure 3. AFM phase image of a monolayer film prepared by depositing the MFGM components isolated from buttermilk powder using solid phase extraction (SPE) onto a mica surface at a film pressure of 40 $mN \cdot m^{-1}$ and a temperature of 15 °C.

binding events, and other membrane-membrane interactions. Evidence is growing that biological membranes contain lipid microdomains or "rafts" that may be involved in processes such as cellular signaling and protein trafficking [22, 36, 41, 53, 55, 63]. An AFM operates by measuring attractive or repulsive forces between a tip and the sample [23]. In its repulsive "contact" mode, the instrument lightly touches a tip at the end of a leaf spring or "cantilever" to the sample. A laser beam is reflected from the back of the cantilever and detected by a split photodiode. As the tip raster scans over the sample, the vertical deflection of the cantilever, and thus the repulsive force, is measured by the change in direction of the reflected laser beam. In "constant force" mode, the signal from the photodiode is used to adjust the height of the cantilever, allowing the AFM tip to move over the surface while applying a constant force. Thus, in contact mode the AFM can be used to produce a height or topography image of the surface as well as measure forces.

Of special interest is the observation by Saslowsky and coworkers [53] that rafts formed in bilayers composed of dioleoylphosphatidylcholine and sphingomyelin are detectable as raised features by AFM. These authors were able to compare the extents of protrusion of the rafts in monolayers and bilayers to show that the raft is continuous across both layers of the bilayer. These studies demonstrate that AFM can be used to distinguish between different species on a membrane surface. In an applied experiment using milk

lipids isolated from buttermilk, we have confirmed that domains can be detected even in monolayers, using AFM (Fig. 3). In Figure 3, we clearly see a different domain or "raft" formed on a Langmuir trough, in a film at a pressure of 40 $mN \cdot m^{-1}$ and at a temperature of 15 °C. We therefore think that the use of AFM as a powerful tool for the study of raft structure and properties.

7.2. Laser tweezers in measuring properties of the MFGM

Laser tweezers, sometimes called optical tweezers, is a technique built upon the principle that small particles/objects can be trapped in the waist of a strongly focused laser beam. The optical trap results from the fact that the objects that are trapped in the focus of the laser beam experience a restoring force if they try to leave the high intensity volume. The technique has its routs in the early seventies in the work by Ashkin [3] concerning trapping of micrometer-sized objects using two focused counter propagation laser beams. It took however until the mid-eighties before the optical tweezers based on one focused laser beam was realized [4]. Since then the optical tweezers technique has found its application in a number of fields, across all sciences. The optical tweezers system has been used for direct manipulation of a variety of micrometer-sized objects and for force measurement in the pN region.

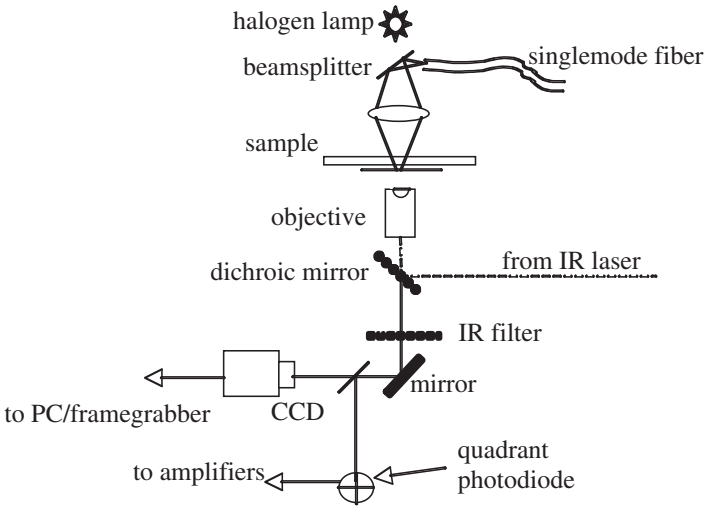
The radiation pressure from a focused laser beam is able to trap small particles. In the biological sciences, these instruments have been used to apply forces in the pN-range and to measure displacements in the nm range of objects ranging in size from 10 nm to over 100 μm. A laser beam is focused by a high-quality microscope objective to a spot in the specimen plane. This spot creates an "optical trap" which is able to hold a small particle at its center. The forces felt by this particle consist of the light scattering and gradient forces due to the interaction of the particle with the light (Fig. 4A).

Most frequently, optical tweezers are built by modifying a standard optical microscope. These instruments have evolved from simple tools to manipulate micron-sized objects to sophisticated devices under computer-control that can measure displacements and forces with high precision and accuracy. In our research group we have started a program using laser tweezers to measure objectively binding forces between bacteria and MFGM. This is best explained graphically in Figure 4B [26]. Objective measurements of the forces involved in binding through membrane-to-membrane interactions are of central importance for studies on the role of each of the MFGM components. Using this approach is easy to imagine a systematic examination of the role in interactions of the different components, proteins, glycoproteins and lipids among themselves and with their surroundings. The versatility of the technique allows for interrogation of interactions with bacteria or intestinal components under a variety of conditions. Furthermore, we hope to be able to use this procedure to elucidate the necessary combination of components involved in the efficient binding and related biological action of the MFGM, and that way generate better nutrition delivery systems.

CONCLUDING REMARKS

The challenge for the food technologist and scientist of the future will focus on the new demands on our foods. They will continue to be the source of nutrition but also of wellness, fitted to the individual life style and all of this delivered with outmost safety. To meet this challenge new tools are being used to generate data on our food components and their interactions. Ideally,

4A



4B

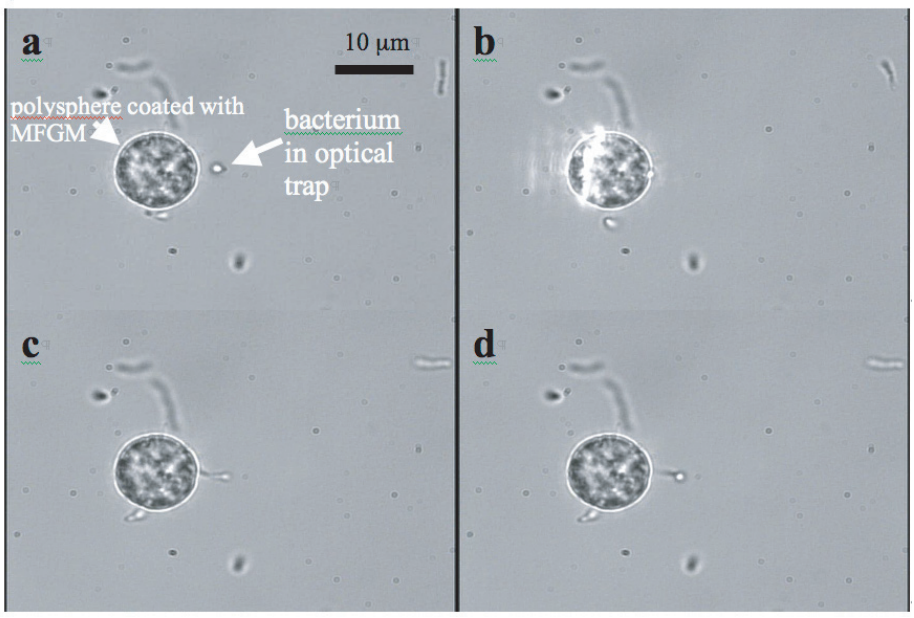


Figure 4. (A) Schematic of optical tweezers system. (B) Frames from a binding experiment using Laser tweezers. (a) Shows the MFGM coated sphere and the bacterium in the trap. The bacteria, which are long, tend to point straight up in the traps when unattached and appear circular. (b) Shows the bacterium just as we push it against the sphere. (c) Shows the bacterium being pulled with one end of it attached to the sphere. In (d) the bacterium has just detached.

the application of our knowledge will be correlated to the complexity of the systems we can handle scientifically. We think that the MFGM is a good model in which to start testing these new concepts. This paper is a personal attempt to exemplify the information available for innovation in the area of dairy ingredients.

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